

The Binary Simplistic Heterogeneity- an unrealistic model for risk assessment and phytoremediation.

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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. This is because uptake models used for risk assessment and remediation of contaminated land assume homogeneity of soil and growth medium. However, homogeneous models are not realistic in field scenarios. The effect of simplistic binary heterogeneity on plant uptake was investigated in a greenhouse pot trial using two Brassica species. Plant uptake in the simplistic binary treatment was compared with a homogeneous treatment. This 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 the homogeneous treatment. Biomass was higher by 10 to 50% in *Brassica juncea* and 20 to 40% lower for *B. napus* in the binary treatment, when compared to the homogeneous and control treatments. This report demonstrated that the presence and extent of *in situ* heterogeneity of Pb in soil plays an important role in metal uptake by plants. It also showed that the homogeneous and simplistic binary model of heterogeneity do not give reliable estimates of plant growth and metal uptake in realistic field conditions. This work has implications for improving the efficiency of phytoremediation of Pb contaminated land and reliability of risk assessment models of human exposure to contaminants.

[Ogunlade- Anibasa, Grace Oyiza, Elizabeth .A. John. **The Binary Simplistic Heterogeneity- an unrealistic model for risk assessment and phytoremediation** *J Am Sci* 2023;19(10):1-15]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). <http://www.jofamericanscience.org>. 01. doi:[10.7537/marsjas191023.01](https://doi.org/10.7537/marsjas191023.01).

Keywords: Binary, Simplistic, Heterogeneity, Risk assessment, Phytoremediation

1. Introduction

Materials in the terrestrial environment are rarely homogeneously 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).

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 ; Lechowicz 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 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; Manciualea 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 Manciualea 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).

This paper discusses the 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); Wijesinghe *et al.*, (2001); Haines, (2002). Root proliferation of *T. caerulea* to Zn patches has been reported (Schwartz *et al.*, 1999b, Whiting *et al.*, 2000; Haines, 2002).

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 *Pteris 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 several 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 can 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; Wijesinghe *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 because of altered resource acquisition, allocation patterns and changes in total biomass.

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). It is one of the known accumulators of Pb and Zn (Bennett *et al.*, 2003; Anjum *et al.*, 2012). It has been reported as accumulating 9580 mg/kg of Pb in roots and 3580 mg/kg in shoots (Meyers *et al.*, 2008) 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).

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 to produce animal feed, vegetable oil for human consumption and biodiesel (Suh *et al.*, 1988). 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.

This study examined and compared 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. (ii) examined root responses of the selected plant species to Pb in the homogeneous

and the binary heterogeneous treatments of the growth medium.

MATERIALS AND METHODS

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.

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 1.

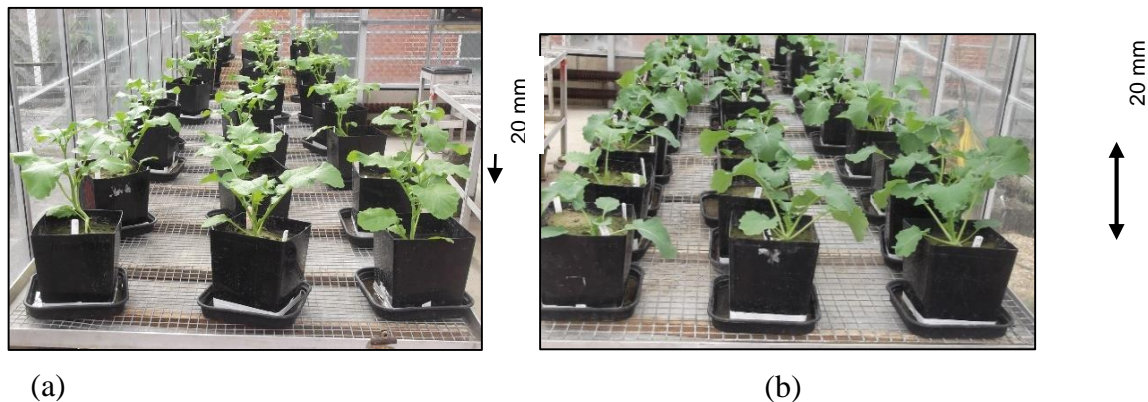


Figure 1: Randomized block design showing (a) *B. juncea* - left (Scale bar: 9 mm represents 20 mm) and (b) *B. napus* --right (Scale bar: 7 mm represents 20 mm). Arrows represent scale bars.

POT TRIAL PREPARATIONS

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 by Anibasa, 2016 , 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 2).

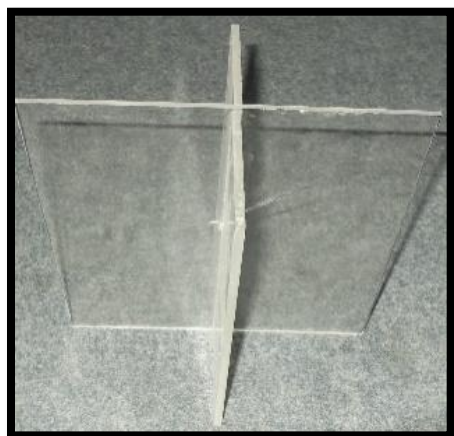


Figure 2: Binary pot divider used in the simplistic binary experiment (4-way 40 mm x 80 mm x 170 mm).

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) was calculated. 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.

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 3 a and b) while for the binary treatment, a nominal concentration of 2000 mg/kg (DW) Pb was introduced into two opposite quadrants of the pot (Figure 3 c). Plants were grown for six weeks under natural light (photoperiod of 16 hours) in a greenhouse at a

temperature of 20±5° C. Power analysis used values for the variances of shoot Pb concentration (mg/kg) of both species taken from an earlier 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.

Dried and milled herbage samples were analysed for Pb. The growth medium was also analysed for its actual Pb concentration and reported in Table 1 below. Certified reference materials (NIST standard reference materials- NIST 2709a, 2710a, 2711a and house reference materials HRM 31), duplicate samples and reagent blanks were used for quality control.

Table 1: Actual Pb concentration of growth media in the binary pot trial

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, height, number of true leaves, number of dead leaves, was recorded at initial transplant, in the third, fourth, fifth week, and 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. 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.

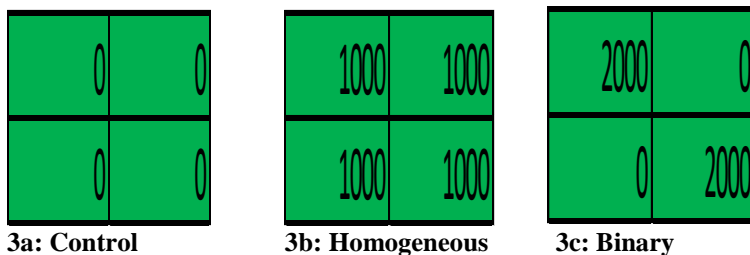


Figure 1: Diagrammatic representation of the experimental design, values in mg/kg (mean nominally 1000 mg/kg for both treatments).

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 4a to 4b and 5a to 5b). 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).



Figure 4.: *Brassica juncea* {4a} 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), {4b}: *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.b. Arrows represent scale bars for each figure and information highlighted in blue.

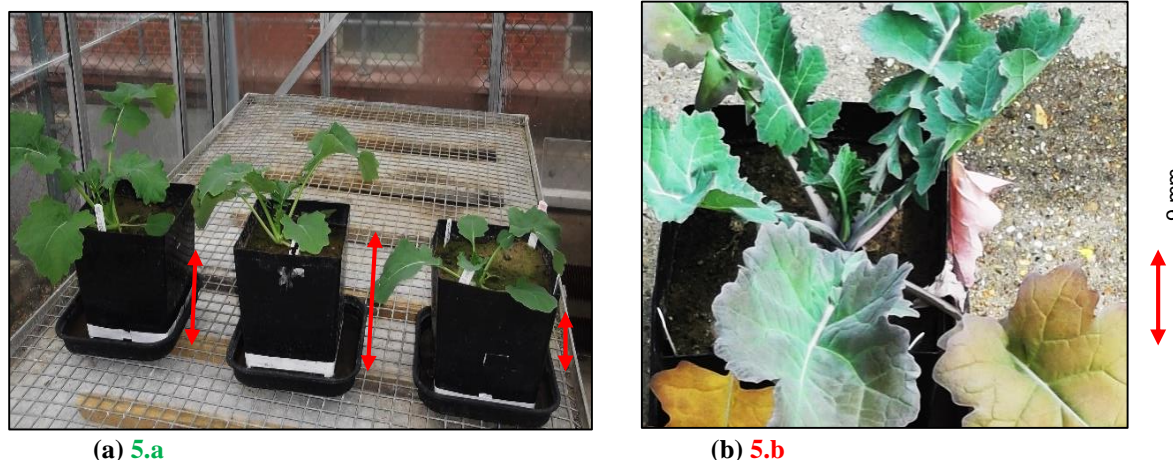


Figure 5.: *Brassica napus* {5.a} 36 days after planting seedlings showing decreased height in simplistic binary (right pot- Scale bar: 9 mm represents 20 mm) compared against both the homogeneous (central pot- Scale bar: 13 mm represents 20 mm) and to the control (Left pot: Scale bar: 9 mm represents 10 mm), {5.b}: *B. napus* in the binary treatment at harvest (56 days) showing chlorosis and wilting of leaves (Scale bar: 9 mm represents 20 mm).

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 { $F_{3, 26}=23.97; 64.11; 32.38$, $P<0.05$ } Further comparison with the Tukey HSD post-hoc test confirmed this significance (Figure 6). This

same trend was observed for the individual shoot and root dry biomass values, as shown in Figure.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.

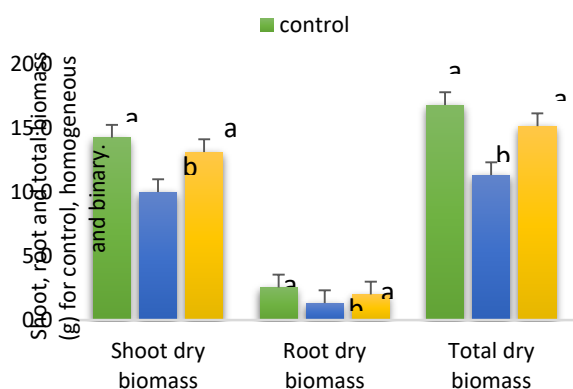


Figure 6: Mean shoot, root and total biomass (DW) between treatments of *B. juncea*. Means that share the same letters for each variable are not significantly different, as judged by the Tukey post-hoc test). Error bars represent 1 standard error on the mean for ten replicates (n=10).

Biomass results for *Brassica napus*.

Early visible response to treatments was observed for *B. napus* after 28 days growth (Figure 7). Plants in the binary treatment were stunted with severe chlorosis (Figure 5b) 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 { $F_{3, 26} = 48.97; 27.71; 64.78, P < 0.05$ }. Further comparison with Tukey HSD post-hoc test also confirmed significant differences in the above and below ground biomass between treatments (Figure 7). 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.

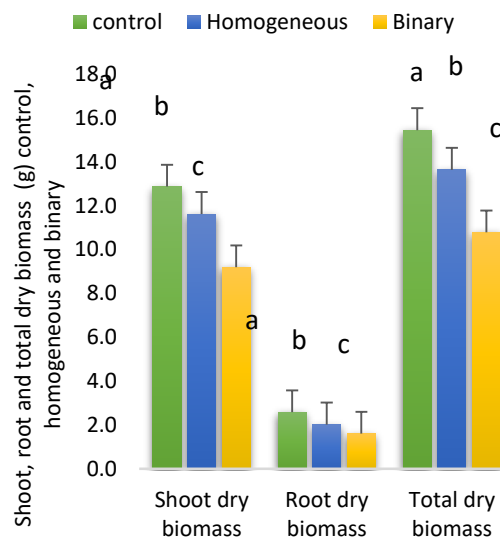


Figure 7: Mean shoot, root and total biomass DW between treatments of *B. napus*. Means that do not share letters for each variable are significantly different, as judged by the Tukey post-hoc test). Error bars represent 1 standard error on the mean (SEM), for ten replicates (n=10).

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 8. 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 { $P=0.011 > 0.05$ } in root-shoot biomass of *B. napus* between treatments, whilst the differences were statistically significant { $P=0.000 < 0.05$ } (for *B. juncea*).

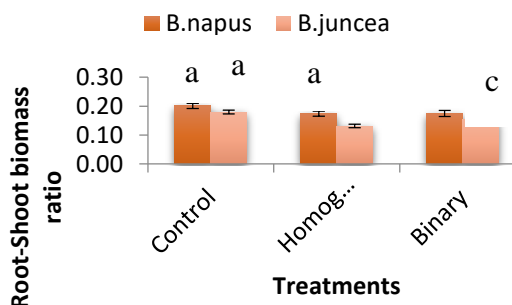


Figure 8: Mean root-shoot biomass DW between treatments of *B. napus* and *B. juncea*. Means that do not share letters for each species are significantly different, as judged by the Tukey post-hoc test). Error bars represent 1 standard error on the mean (SEM), for ten replicates (n=10).

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 9). 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 10. 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 11a and 11b). A tap root was observed in *B. napus* (Figure 11b), whilst *B. juncea* lacked tap root (Figure 11a), but had a network of fibrous roots.

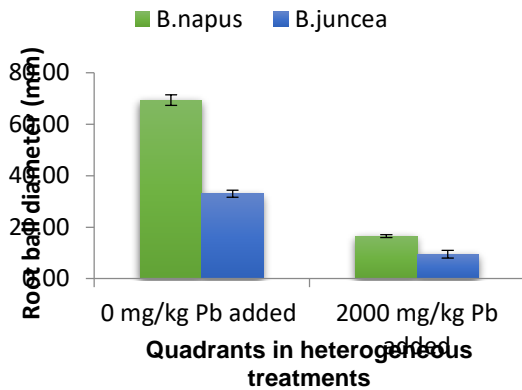


Figure 9: Root ball diameter between binary patches of *B. napus* and *B. juncea*. Error bars represent 1 standard error on the mean where n=10.

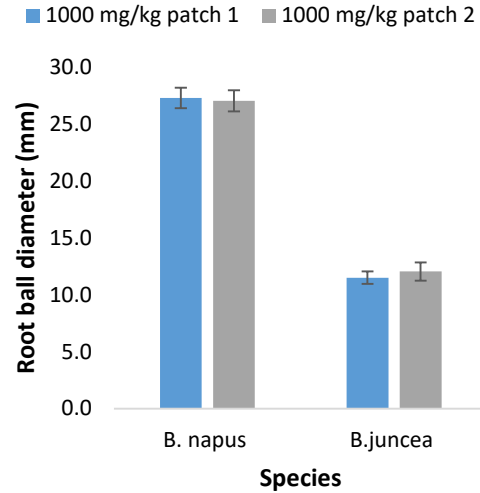


Figure 10: Comparison of the root ball diameter in homogeneous quadrants of *B. napus* and *B. juncea*. Error bars represent 1 standard error on the mean where n=10.

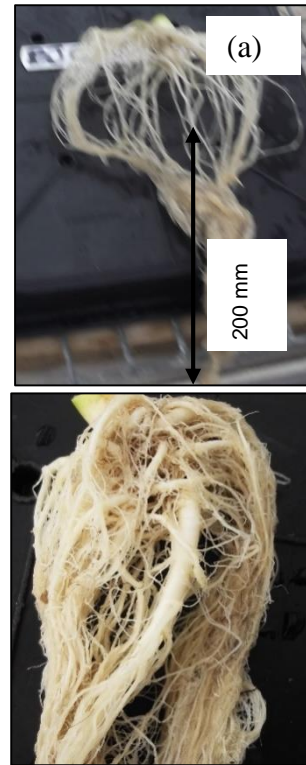
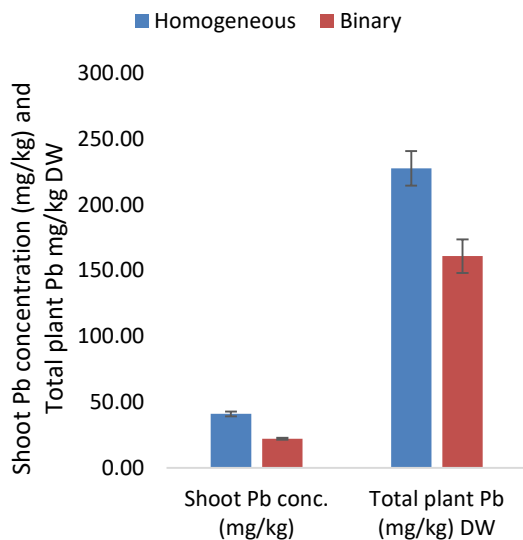


Figure 11 Roots of (a) *B. juncea* with no tap root (left- 2.9 mm represents 200 mm) and that of (b) *B. napus* showing a central tap root (right- 2 mm represents 100 mm)

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 12).

Similarly, shoot and root Pb concentration in the homogeneous treatment were twice and 57% higher than those of the binary treatment respectively (Figures 12 and 13). 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); Manciualea and Ramsey (2006); Thomas, (2010) and



Moradi *et al.*, (2009).

Figure 12: 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).

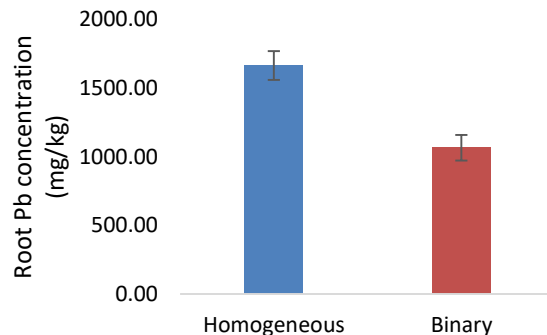


Figure 13: Mean root Pb concentration mg/kg between treatments of *B. juncea*. Error bars represent 1 standard error on the mean (SEM), for ten replicates (n=10).

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 14), as opposed to the case of *B. juncea* which had reduced uptake and increased biomass.

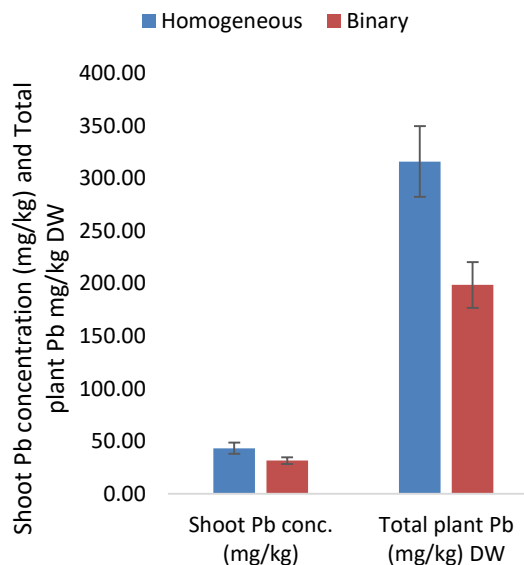


Figure 14: Mean shoot Pb (mg/kg) and total plant Pb concentration {mg/kg DW} between treatments of *B. napus*. Error bars represent 1 standard error on the mean (SEM), for ten replicates (n=10).

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 14 and 15 below).

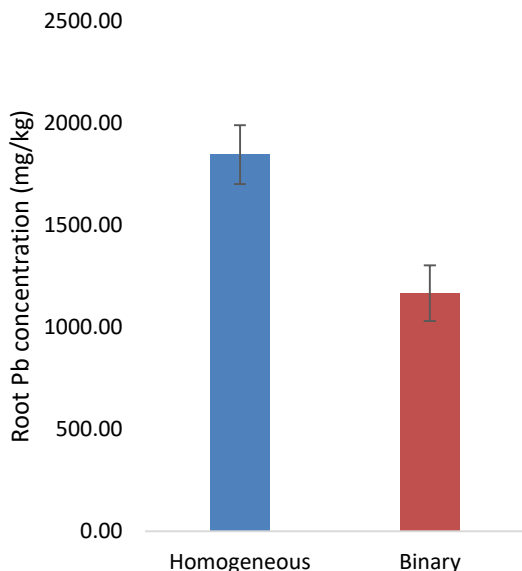


Figure 15: Mean root Pb concentration mg/kg between treatments of *B. napus*. Error bars represent 1 standard error on the mean (SEM), for ten replicates (n=10).

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 16). 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.

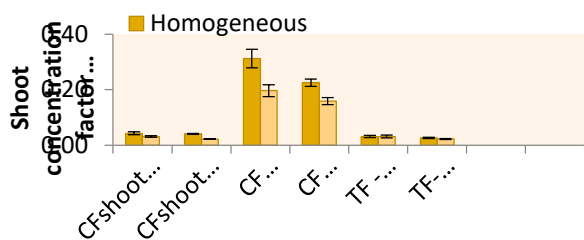


Figure 16: Mean total concentration factor (CF_{total}), shoot concentration factor (CF_{shoot}) and translocation factor (TF) of *B. napus* and *B. juncea* in homogeneous and binary treatments. Error bars represent 1 standard error on the mean where n=10.

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.

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 17 to 18. The advantage of expressing uptake in (μg) is that it provides better estimate of uptake for phytoremediation purposes as it take into consideration the biomass while concentration in mg/kg t is useful f in estimation of human exposure for risk assessment or risk assessment 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 17). There was no statistically significant difference ($P = 0.185$; $0.988 > 0.05$) in shoot Pb mass between species. 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*.

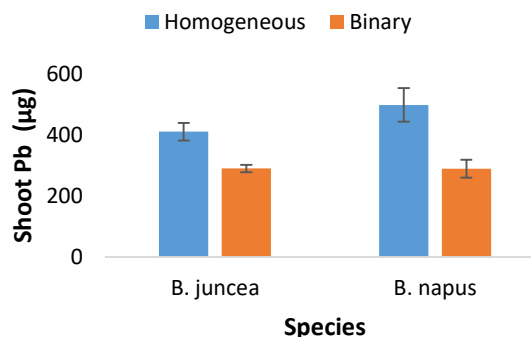


Figure 17: Mean shoot Pb mass (μg) for *B. juncea* and *B. napus* in homogeneous and binary treatments. Error bars represent 1 standard error on the mean where n=10.

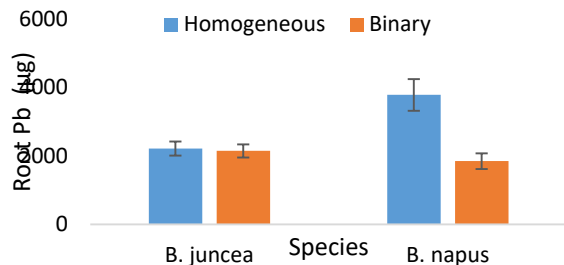


Figure 18: Mean root Pb mass (µg) for *B. juncea* and *B. napus* in homogeneous and binary treatments. Error bars represent 1 standard error on the mean where n=10.

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 18). 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 ($P = 0.334 > 0.05$). 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*. 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.

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); Manciuola 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. *Brassica 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 suggests 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 suggests 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.

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). 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% (Anibasa, 2016). 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.

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*. The root biomass for the different quadrants were not taken, but this was an improvement implemented in another pot trial (Solomon-Wisdom *et al.*, 2015). 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 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.

Another experiment simulating a more realistic heterogeneity model confirmed this finding (Solomon-Wisdom *et al.*, 2015). It is highly unlikely that contaminant spatial heterogeneity in the field will have this simplistic distribution.

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 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).

CONCLUSION

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. It also showed that binary simplistic heterogeneity is unrealistic for estimation of plant uptake for risk assessment and phytoremediation as the soil in nature do not exist in the binary mode of contaminant distribution. Risk assessment and phytoremediation models based on homogeneous and binary simplistic designs are unlikely to be true assessment of human exposure to contaminants and potentially toxic elements.

ACKNOWLEDGMENT

The authors thanked the School of Life Sciences of the University of Sussex in UK for the use of the laboratory and facilities and to the United States Department of Agriculture (USDA) for supplying the plant seeds gratis.

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