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Accumulation of Pb and Cu in different parts of a groundnut plant (*Arachis hypogaea*) under laboratory conditions

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Abstract

This study focused on the accumulations of Pb and Cu in different parts of *Arachis hypogaea* exposured to each metal separately under laboratory conditions. Three replications and a randomized complete block design were used in this study. About 3.5 kg of air-dried soil was placed into a 17 × 18 cm poly bag, using a soil mixture (loam soil : organic fertilizer : sand) in a 3:2:1 ratio for the different treatments. For the plants exposed to Cu and Pb, approximately 100 ml of solution was applied twice, during weeks 4 and 5 of the flowering stage. The plants were harvested 90 days after germination. Soil samples were also collected to determine the concentrations of Cu and Pb. The accumulation pattern observed was roots > seeds (with hulls) > stems > leaves for both Cu and Pb treated plants, and the control plants exhibited a similar

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distribution of metals across plant parts. These findings highlight the potential of *A. hypogaea* as a biomonitor in future research.

Keywords:
A. hypogaea, trace metals, laboratory experimental studies, environmental pollution.

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1.0 Introduction

The accumulation of heavy metals in soils and plants has emerged as a critical global environmental issue due to its implications for food security and human health. According to Zu (2004), plants that can accumulate heavy metals belong to the *Brassicaceae* and *Fabaceae* families. Heavy metals cannot be biodegraded in soils, but tend to bioaccumulate and persist in soils for very long time (Euras, 2009). Heavy metals can be translocated into plants primarily through root uptake from contaminated soils, whereas atmospheric particulates may penetrate foliar tissues via deposition and absorption through the leaf surface (Rana et al., 2000). Although heavy metal bioaccumulation has been reported in the medicinal plant *Centella asiatica* in Peninsular Malaysia (Ong et al., 2011), corresponding data for *Arachis hypogaea* are still lacking.
(Pb) is considered to be one of the most dangerous environmental contaminants which commonly enters the aquatic environment from various types of industrial wastewaters. It is a basic ingredient in the production of storage batteries, steel products, leaded glass, gasoline, pigments and photographic materials (Nadeem et al., 2006). Likewise, copper (Cu) contamination caused by smelting processes, iron foundries, and combustion-related activities causes high Cu accumulation in industrial areas (Department of Environment, 2009). Cu may also be contributed by excess use of fertilizers, bactericides, fungicides and algicides from agricultured sites (WHO, 1998). Cu can enters human bodies through the drinking of water and eating of food. Severe Cu poisoning can cause Alzheimer's disease (Bewer, 2010, Siddiq *et al*., 2024). Therefore, the determination of Pb and the level of Cu in plants is essential in biomonitoring studies.
Groundnut (*Arachis hypogaea*), which belongs to the family Fabaceae, comprises many nutrients that are beneficial to our health (Kaprovickas and Gregory, 1994). Groundnut (*Arachis hypogaea L*) is a major oilseed crop cultivated across nearly 20 million hectares of agricultural land spanning the tropical and subtropical regions globally (Wynne *et al*., 1991). Plant species that accumulate minimal concentrations of heavy metals in their edible tissues, remaining within permissible limits, are suitable candidates for cultivation in metal-contaminated soils (Barman *et al.,* 2000). Accordingly, this study aimed to evaluate the accumulation patterns of copper (Cu) and lead (Pb) in various plant parts (roots, stems, leaves, and seeds) of *Arachis hypogaea* grown under both contaminated and uncontaminated soil conditions.

2.0 Methodology

2.1 Experimental design

The experiment was carried out at Universiti Putra Malaysia's University Agricultural Garden (Taman Pertanian Universiti). About 3.5 kg of air dried soil was put in a poly bag 17 x 18 cm with the soil mixture (loam soil: organic fertilizer: sand) in the ratio of 3:2:1 for the different treatments (Harini, 2002). An extra set of poly bags without heavy metals were also used as the control (Rana and Masood, 2001) and one seedling was thinned per poly bag.
All treatments were also fertilized with nitrogen, phosphorus and potassium (NPK) in the ratio of 34: 56: 56 kg / ha. The type of irrigation used was overhead irrigation also known as Sprinkler in the evening every day. Groundnut seeds were obtained from the Division of Seed Management, Malaysian Agricultural Research and Development Institute (MARDI).
Three replications and a randomized complete block design (RCBD) was employed to minimize the influence of positional effects in the experiments (Khan et al., 2006). For plants treated with Cu and Pb about 100ml of solution were applied twice during the flowering period on weeks 4 and

5 as shown in Table 1. The test plant for the accumulation of Cu and Pb was harvested after 90 days germination. The selected concentrations of 1 ppm for Pb and Cu were based on reported levels of these metals in moderately contaminated agricultural soils (0.5–5 ppm) near industrial, mining, or wastewater-irrigated areas (Department of Environment, 2009; WHO, 1998). These concentrations reflect realistic contamination conditions, ensuring that the experimental setup closely represents potential field exposure scenarios.

Table 1: Experiment Methodology

Sample	Treatment
Control plant	natural soil (Control)
Cu treatment plant	natural soil with 1 ppm Cu
Pb treatment plant	natural soil with 1 ppm Pb

2.2. Heavy metal determination

For determining metal accumulation in plants, samples were divided to four fractions: shoots, stems, roots, and seeds (with hulls). Plant tissue and soil samples were dried in an oven at 60 °C until a constant weight was achieved (approximately 72 h) before analysis. An accurately weighted quantity (500 mg) of each dried plant tissue was transferred into a digestion tube and added with concentrated nitric acid (10 mL) (AnalaR grade, 69% BDH) for acid digestion.

For the soil samples, particles smaller than 63 µm were used and directly digested using aqua regia. Each of the treatments was replicated three times. All digestion tubes were placed in a hot block digester after being maintained for one hour at 40 °C and for at least three hours at 140 °C. (Ong et al., 2011; Yap et al., 2003). The digested samples, after cooling, were diluted with doubly de-ionized water to a total volume of 40 ml. The solutions, after passing through Standard cellulose (Whatman No. 1) filter paper, were transferred to acid-washed containers and stored properly until metal analysis.

The air-acetylene flame and the Perkin-ElmerTM AAnalyst 800 Flame Atomic Absorption Spectrophotometer were used for the heavy metals analysis of digested plant and soil samples. Standard calibration solutions of Cu and Pb were prepared from stock solutions of 1000 ppm MERCK Titrisol, and the concentration of metals was measured by using dry weight (µg/g).

2.3 Quality control

All glassware and equipment were pre-cleaned by rinsing and soaking in a 10% nitric acid solution for 2–3 days to minimize contamination during metal analysis, and subsequently rinsed thoroughly with double-distilled water. After digestion, the samples were transferred into acid-washed containers and securely stored until metal concentration analysis.

2.4 Statistical Treatment and Data Interpretation

Statistical treatment and data interpretation were conducted by using SPSS software. One-way ANOVA was performed to assess variations in heavy metal concentrations among plant parts, sampling sites, and treatment groups, and the significance among groups was evaluated using the Student–Newman–Keuls (SNK) post hoc test.

3.0 Results

As shown in Table 2, under Cu treatment, Cu concentration (in µg/g dw) was highest in the roots (110.9), followed by leaves (14.10), stems (12.70), and seeds (10.11). In the control, the highest Cu concentration was also observed in the roots (4.27), followed by leaves (3.76), stems (2.37), and seeds (2.27). Therefore, the sequence of accumulation of Cu in the treatment and control was roots > leaves > stems > seeds.

Table 2: The mean concentrations (±SE, µg/g dry weight) of Cu in the different parts of *Arachis hypogae* in control and Cu treatment plants.

Parts	Cu	mean	±	SE	t-test
leaves	control	3.76	±	0.139	P<0.05
	treatment	14.10	±	0.046	
stems	control	2.37	±	0.071	P<0.05
	treatment	12.70	±	0.046	
roots	control	4.27	±	0.071	P<0.05
	treatment	110.90	±	29.24	
seeds	control	2.27	±	0.071	P<0.05
	treatment	10.11	±	0.096	
soils	control	23.55	±	0.352	P<0.05
	treatment	236.60	±	0.833	

For the Pb treatment, the Pb concentration (in µg/g dw) in roots was the highest (19.92), followed by seeds (4.96), stems (3.87), leaves (3.17) (Table 3). In the control, the roots (3.31) also had the highest Pb level value followed by seeds (2.11), stems (1.33) and leaves (0.67) as shown in Table 3. Therefore, the sequence of accumulation Pb in the treatment and control was roots > seeds > stems > leaves.

Table 3: The mean concentrations (±SE, µg/g dry weight) of Pb in the different parts of *Arachis hypogae* in control and Cu treatment plants.

Parts	Pb	mean	±	SE	t-test
leaves	control	0.67	±	0.190	P<0.05
	treatment	3.17	±	0.027	
stems	control	1.33	±	0.027	P<0.05
	treatment	3.87	±	0.201	
roots	control	3.31	±	0.027	P<0.05
	treatment	19.92	±	0.046	
seeds	control	2.11	±	0.187	P<0.05
	treatment	4.96	±	0.027	
soils	control	17.86	±	14.06	P<0.05
	treatment	67.71	±	13.93	

4.0 Discussion

For Cu levels, both control and Cu treated plants exhibited the highest accumulation in roots, while the lowest levels were observed in seeds. It was supported by Johnny *et al*. (2008) who found that accumulation of Cu was higher in the roots of peanuts (*Arachis hypogaea*). The hull functions as a selective barrier regulating the translocation of heavy metals to the seeds (Angelova *et al*., 2004). A limited amount of Cu may traverse the hull, as this element serves both structural and catalytic roles in numerous metalloproteins and enzymes that participate in essential metabolic processes (Pilon *et al*., 2006). Consequently, Cu accumulation in seeds was the lowest among the examined plant parts, despite their proximity to the soil via the root system.

As mentioned by Singh and Sinha (2005), the pronounced retention of heavy metals in root tissues can be attributed to the formation of complexes with sulfhydryl groups, thereby reducing their mobility and subsequent translocation to the shoots. The higher Cu concentration in roots could also result from metal penetration into the plasma membrane, leading to inactivation and precipitation processes that form slowly mobile complexes with organic substances. Similar findings were reported by Rout et al. (2001) and Street et al. (2009), who reported higher metal accumulation in the roots compared to the shoots of *Merwilla plumbea* and *Oryza sativa*. Consistently, Hülya et al. (2010) found that root tissues contained higher concentrations of Pb^{2+}, Cd^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Ni^{2+} and Zn^{2+} compared to leaves and stems in Verbascum bombyciferum.
The lowest level of Cu in seeds in control was supported by Khan *et al*. (2008) in *Euphorbia helioscopia.* In the unpolluted area, the Cu level (in µg/g dw) was highest in leaves (1.05) followed by the stem (0.83), roots (0.66) and seeds 0.50). In the polluted area, the higher level of Cu was in seeds 2.25 mg/kg followed by the stem (1.46), leaves (1.12) and roots (0.94). The finding of the highest Cu accumulation in seeds in the polluted area was different compared to our results that seeds accumulated the least amount of Cu in our Cu treatment. This was because the hull of *A. hypogaea* acted selectively to Cu uptake resulting in reduced the Cu uptake into roots and translocation to other plant parts such as leaves, stems and seeds. A high level of Cu is highly toxic even in small quantities. Påhlsson (1989) reported that copper (Cu) concentrations as low as 0.1–0.2 ppm can interfere with plant metabolic activities and hinder growth. When present in excessive concentrations, it can interfere metabolic processes and suppress plant growth, even at concentrations only marginally higher than expected reading (Fernandes and Henriques, 1991).
For Pb concentrations, both the control and Pb-treated plants exhibited a similar pattern, where roots showed the highest accumulation and leaves the lowest. Lead (Pb), with its high atomic number (82) and low mobility, is not readily translocated from roots to aerial parts such as leaves (Kabata, 2000). Due to this limited mobility, Pb levels were significantly accumulated higher in the roots than in the leaves. Similar observations were reported by Zheljazkov et al. (2006) in Anethum graveolens (dill), Mentha piperita (peppermint), and Ocimum basilicum (basil). The high level of Pb content in the roots under the Pb treatment was further supported by Singh and Sinha 2005; and Tang et al., 2009, explained that the roots are always in contact with with soil, are the first organs exposed to metal contaminants. Consequently, this Continuous exposure enhances Pb accumulation in root tissues. Moreover, the root system of groundnut is shallow, normally reaching a depth of depth of 20–30 cm, which allows for efficient absorption of metals present in that soil layer. This closeness to the soil surface increases the likelihood of root exposure to metal contaminants increases with enhanced metal accumulation.
Furthermore, the expanded surface area provided by root hairs enhances the capacity of roots to adsorb and absorb metals, and the symbiotic associations within the rhizosphere contribute to improved nutrient acquisition. (Yap et al., 2010). Since roots serve as the primary site because all metal uptake must occur for water and nutrient absorption through osmosis, through them before translocation to other plant parts (Clemens et al., 2002; Onget al., 2011).
Pb is highly toxic to plants; thus, plants have developed different mechanisms that limit its entry and mobility in tissues. Because Pb is a nonessential the uptake of this element is generally limited or actively avoided by plants. Mehra and Tripathi, 2000. Contrary to the findings presented by Khan et al. (2008), who reported higher Pb accumulation in leaves from both polluted and unpolluted areas, the current findings indicated low Pb accumulation in leaves. under Pb treatment. This may be due to the negative effects of high Pb concentrations that disturb regular physiological and metabolic functions in plants. When plants are exposed to heavy metals, they produce reactive oxygen species. ROS as metabolic by-products (Marschner, 1995; Singh, 2007). These ROS are highly reactive and can damage cellular components such as membranes, lipids, proteins, and DNA, ultimately leading to cell injury or death in leaf tissues.
Based on the present findings, seeds were highly accumulative of Pb after roots. This could be due to the hulls and seeds being digested together for determining the Pb accumulation level. This study showed that powdered Peanut hulls can be effectively utilised as biosorbents for the removal of anionic dyes from aqueous solutions. With their easy availability and low cost, the use Peanut hulls are an inexpensive, available means of wastewater treatment. The seeds even had a higher level of

Pb though it was not significantly ($P<0.05$) higher compared to leaves and stems. Cu treatment seeds did not demonstrate higher Cu accumulation compared to leaves and stems unlike in Pb treatment. This was because Pb is toxic and non-essential Metal which is injurious to plants whereas Cu is one of the essential metals for normal metabolism in plants (Sawidis et al., 1995).
The accumulation of Cu and Pb in the edible parts of groundnut has serious ecological and public health ramifications. Groundnut is a popularly consumed oilseed crop, and chronic exposure to Pb through soils may result in serious health issues in humans, including neurological and developmental effects. While Cu is required in trace amounts, it might prove toxic at higher concentrations that can induce oxidative stress in plants and possibly bioaccumulate in the food chain. These findings emphasize the continuous monitoring of agricultural soils and soil management practices like phytoremediation and crop rotation to minimize heavy metal uptake in food crops.

5.0 Conclusion

The highest accumulation of metals in both experiments was observed in roots, while seeds accumulated the lowest amount of Cu and leaves the lowest amount of Pb after separate exposure to Cu and Pb, which indicates different mechanisms of metal uptake and storage in different parts of A. hypogaea. All these results suggest that the groundnut plant could be used as a potential biomonitoring plant in agricultural soils, especially those susceptible to contamination from fertilizers, industrial effluents, or mining activities. The metal concentrations in groundnut roots and hulls may provide an early warning indication of soil pollution under field conditions and thus help farmers and environmental managers to monitor and manage polluted agricultural lands with far greater efficiency before the metals enter the food chain.
Such findings need to be complemented by studies on the performance of A. hypogaea as a biomonitor in real field conditions with varying types of soils and contamination levels. These comparative studies with other leguminous and non-leguminous crops would help in drawing more insights on the species-specific pattern of metal uptake. Further, the study with different concentrations of metals and long-term exposure trials will establish threshold levels for safe cultivation of crops and inform agricultural guidelines on the management of heavy metal contamination.

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Conflict of Interest
The authors declare no conflict of interest.

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