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LEAD ACCUMULATION IN MAIZE GRAINS, LEAVES AND OTHER PARTS OF THE PLANT: A POSSIBLE ROUTE FOR LEAD POISONING

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ABSTRACT

Lead (Pb) accumulation in parts of maize plant as a possible route for Pb poisoning was investigated. Experimental design adopted was a 3 factorial combination (4 x 1 x 1). Prior to planting of seeds, soil sample was analyzed for its physicochemical properties. At maturity, maize crop and parts of the plants were harvested and analyzed for their Pb contents. TF, BCF and BAF were as well determined. Physicochemical properties' results revealed that pH was 6.75, Ec = 1.62 dSm-, K = 1.66 %, Mg =12.87 cmol/kg, Ca =1.9 cmol/kg, N = 0.6%, P = 0.98%, BD = 1.25 g/cm^3 and MC = 40.84%. TF, BCF and BAF value obtained were < 1. Similarly, Pb found in the grains, leaves, shots, roots and soil were within 0.3133 - 1.0533 ppm; 4.7133 - 7.100 ppm; 8.070 - 14.09 ppm; 14.02 - 26.00 ppm and 172 - 551.34 ppm for grains, leaves, shoots, roots and soil Pb concentration respectively. However, consumption of vegetables and crops cultivated in Pb contaminated soil will pose serious health risk to the consumers as Pb found in the grain and leaves of maize were above the maximum permissible limit for health safety (0.2 ppm) recommended by joint FAO/WHO.

Keywords: Accumulation, Grain, Lead (Pb), Leaves, Maize, Poisoning

INTRODUCTION

Food security is a prerequisite for human survival and fundamental guarantee for human health, (Hu *et al.*, 2019). However, a variety of food contamination issues have led to food scarcity and public attention to food safety in recent years (Sriprachote *et al*., 2012; Akhtar, 2013; He *et al*., 2013). Several human activities have adverse effect on the environment by polluting or contaminating the water we drink, the air we breathe, and the soil in which plants grow (Manisalidis *et al*., 2020). Research has shown that heavy metals have adverse effects on soil microbial community structure (Aponte *et al*., 2021). Metals are all over the earth including the atmosphere, earth crust, water bodies and can also accumulate in biological organisms such as plants and animals (Engwa *et al.*, 2019). There are 35 natural existing metals in the globe of which 23 of them possess high specific density above 5 g/cm^3 and atomic number greater than 40.04 and are known to be heavy metals (Duffus, 2002; Li, 2017). This category of metals termed heavy metals has not only been known for their high density but most importantly for their adverse effects to the ecosystem and living organisms (Bradl, 2002). It has been observed that long term accumulation of heavy metals in the body may result in slowing the progression of physical, muscular and neurological degenerative processes that mimic certain diseases such as Parkinson's disease and Alzheimer's disease (Monisha, 2014). More so, repeated long-term contact with some heavy metals or their compounds may even damage nucleic acids, cause mutation, mimic hormones thereby disrupting the endocrine and reproductive system and eventually lead to cancer (Jarup, 2003). Soil heavy-metal pollution causes severe damage to microorganisms, reducing microbial activity, altering microbial community structure and leading to changes in soil enzyme activity (Li *et al*., 2021). Heavy metals constitute an ill-defined group of inorganic chemical hazards, and those most commonly found at contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni) (Lu *et al*., 2011).

Pb is one out of four metals that have the most damaging effects on human health which is a potent poison and is harmful in even small amount, and it is one of a limited class of elements that can be described as purely toxic. It is widely distributed in the environment (Seema and Tripathi, 2012). The toxic action of lead in the body is traced in part to the enzyme (ALAD) inhibition by the Pb++ ion. It has been reported that 24 hours' exposure to 10 and 150Mg/kg BW of Pb acetate induced increase in the renal thiobarbituric acid reactive substances (TBARS) content that acts as an indicator of lipid peroxidation as well as SOD and CAT activities in kidney of Balb-c mice (Sharma and Singh, 2014). Pb can affect the central nervous function leading to mental disorder, damage the blood constituents and may damage the lungs, liver, kidneys and other vital organs promoting several disease conditions (Monisha, 2014). Pb and Cd are considered potential carcinogens and are associated with etiology of a number of diseases, especially cardiovascular, kidney, nervous system, blood as well as bone diseases (Jarup, 2003). Soil and vegetables polluted with Pb and Cd can lead to decrease of human life expectancy within the affected areas. Pb poisoning is mostly related to the gastrointestinal tract and central nervous system in children and adults (Markowitz, 2000). Acute exposure of lead can cause headache, loss of appetite, abdominal pain, fatigue, sleeplessness, hallucinations, vertigo, renal dysfunction, hypertension and arthritis while chronic exposure can result in birth defects, mental retardation, autism, psychosis, allergies, paralysis, weight loss, dyslexia, hyperactivity, muscular weakness, kidney damage, brain damage, coma and may even cause death (Martin, 2009). Thus, Pb can accumulate through the food chain and affect humans as well, causing irreversible damage, particularly in children (Liu et al., 2018; Kelepertzis *et al.*, 2021).

In 2019, pollution was responsible for approximately 9.0 million premature deaths (Fuller *et al*., 2022). Air pollution (both house hold and ambient air pollution) remains responsible for the greatest number of deaths, causing 6.7 million deaths in 2019. Water pollution was responsible for 1.4 million premature deaths. Pb pollution was responsible for 900,000 premature deaths (Fuller *et al*., 2022). To clean and create a safer and healthier environment, several remediation techniques have been adopted. Chiwetalu *et al*. (2021) used organic materials such as poultry droppings, mashed cassava peels and slightly mashed grasses to remediate soil alkalinity for soil sustainability and productivity. Similarly, Chemical precipitation, ion-exchange, evaporation, electrochemical treatment, and filtration and even phytoremediation have been applied to clean up Pb and other heavy metal polluted soil in order to reduce their negative effects on the ecosystems and human health (Malik 2004; Yang *et al*., 2021).

Phytoremediation techniques may also be more publicly acceptable, aesthetically pleasing, and less disruptive than the widely used techniques of physical and chemical process (Salido *et al*., 2003). It encompasses a number of different methods that can lead to contaminant degradation (Rodriguez, 2005). Advantages of this technology are its effectiveness in contaminant reduction, low-cost, being applicable for wide range of contaminants (Liu *et al.*, 2000), and in overall it is an environmental friendly method. Regardless of the remediation methods used, investigation of Pb levels in polluted soils and a thorough understanding of their physical, chemical, and biological characteristics remains a pending subject. Therefore, the aim of the study is to evaluate lead (Pb) accumulation in maize grain, leaves and other parts of the plant as a possible route for Pb poisoning while the objective of the study is to determine the accumulation level of Pb in the different parts of maize cultivated in Pb contaminated soil.

MATERIALS AND METHODS

The Study Area

The study was carried out in the Faculty of Agriculture practicing farm, Enugu State University of Science and Technology (ESUT), Agbani is in Nkanu West L.G.A. of Enugu State, Nigeria. ESUT is situated in Agbani, the headquarters of Nkanu West L.G.A. of Enugu State. ESUT lies approximately between latitude 6° 51' 24" N and longitude 7° 23' 45" E. The annual rainfall in the area is between 1500 mm to 2000 mm, the mean monthly rainfall is about 140 mm (NIMET, 2015), and the surface pressure is about 985.5 hpa the relative humidity ranges between 40% and 89%. The temperature ranges from 26. 6°C to 32°C and the mean annual temperature is 27°C, (NIMET, 2015) though, in recent years, the temperature range, especially in the dry season period (November to March), approaches 40 - 42°C. Agbani like the rest of other places in Enugu State has the tropical savanna type of climate, according to Koppen's (1987) climate classification. Agbani has some of the few characteristics of the rainforest zone, but due to human activities in the area, the natural rainforest has been destroyed (Okwu-Delunzu, 2018). The people are predominantly farmers (Adekola and Nwoye, 2016). Figure 1 is a map of Enugu State indicating the study location. The people are predominantly farmers (Adekola and Nwoye, 2016).

Materials and Equipment Used for the Study

The materials and equipment used for the study were as follows:

Maize: This was the crop that was used for the study.

Soil auger: This was used for collection of soil samples

Disposable hand gloves: They were worn for safety measures

Permanent ink marker: It was used for identification of samples

Distilled water: It was used for rinsing the conical flask before digestion and analysis

Oven: It was used for drying of samples

Lead (II) nitrate ($Pb(NO_3)_2$): This was used for contamination of the soil artificially

Atomic absorption Spectrophotometer (AAS): It was used to analyze the Pb content of the soil and also Pb found in the different parts of the plant.

Aqua regia: This was used for digestion

Digital weighing balance: It was used for measurement of soil sample, dried grounded maize stover and grain.

Filter paper: This was used for filtering of the sample before AAS analysis.

Experimental Design

The experimental design adopted for the study was a 3-factorial combination (4 x 1 x 1), four different concentrations of Pb adopted for the study, a study condition (field experimental study) and a plant species. Ten different containers were filled with 15 kg of soil contaminated at the required concentration of Pb and used for the study. The containers were well labeled for identification and three maize seeds were sown per pot. The seedlings were thinned to one seedling per bucket after eight days of germination. They were allowed to grow up to eight days after germination in order to identify the most viable seedling that will be used for the study. Plastic buckets of about 120 cm^3 were perforated by the side. The perforation was for improved aeration and the choice of the points of perforation was to minimize the rate at which liquid will flow out of the container. Figs. 1, 2 and 3 are the experimental site, potted maize plant and sample of maize seeds used for the study respectively.

Figure 1: Experimental site

Figure 2: Potted maize experiment

Figure 3: Maize seeds used for the study

The experimental set-up was designed as follows: sample 1, ordinary soil without contamination (0 Mg/kg, control); sample 2, Soil contaminated soil at 200 Mg/kg of Pb concentration; sample 3, Pb contaminated soil at 400 Mg/kg; sample 4, Pb contaminated soil at 600 Mg/kg. Samples 2, 3 and four were replicated in triplicate.

Acquisition and Preparation of Maize Seed Used for the Study

Maize seeds used for the study were procured from ENADEP (Enugu State Agricultural Development Program). The choice of procuring maize seeds from ENADEP was to obtain an improved and viable variety of maize that will be capable of withstanding stress of different forms and as well give a substantial result.

Contamination of the Soil

Different concentrations of Pb used for the study were obtained by using lead (11) nitrate salt as source of contamination. 0 mg/L (without Pb contamination), 200 mg/kg, 400 mg/kg and 600 mg/kg of Pb contaminated soil were obtained by adding equivalent quantity of Pb in 1000 mL cylindrical flask containing distilled water. The concentrations obtained were used to contaminate the soil at the required concentrations for the study. The concentrations obtained were mixed thoroughly with the measured quantity of soil in order to spike the soil (Kabata and Pandias, 1984). Those varying levels of contamination obtained as described in the experimental set up were planted with maize and monitored till maturity.

Sample Preparation

Soil samples were dried at ambient temperature for about 2 weeks. The dried soil samples were homogenized using a well washed and dried wooden mortar. The soil samples were further sieved with 2 mm sieve to remove heterogeneous materials and stored in cellophane bags. Similarly, the plant materials were as well dried at ambient temperature for 24 hours and then dried further in a hot air oven at approximately 105ºC for 48 hours. Dried plant materials were homogenized using a manual hand grinder and sieved and stored in cellophane bags (Kumhomkul and Panich-pat, 2013; Daniela et al., 2010; Turkekul *et al*., 2004.)

Determination of Some Physical and Chemical Properties of the Soil

Samples of soil were collected randomly at the site for analysis in Soil and Water laboratory unit of Agricultural and Bio-resource Engineering Department, Enugu State University of Science and Technology. Those samples were analyzed for their soil class, electrical conductivity, organic matter content, pH and bulk density. Particle size analysis of the soil was carried out using different types of sieves to obtain the class of the soil. The soil sample was air-dried, pulverized, and sieved using a set of sieves ranging from 9.5mm to 75µm in size. The physical property of the soil was determined and the soil was classified using AASHTO M 145. Potential hydrogen test was determined using a digital pH meter. The bulk density of the soil sample used was determined following the Core Cylindrical Method described in the Soil Quality Test Kit Guide by (Arshad *et al.*, 1996). More so, electrical conductivity was determined using a digital conductivity meter (model DDS-307). Organic matter content of the soil sample was determined following the methods described by **ASTM D 2974** – Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Organic Soils.

Analysis of Pb in the Soil and Some Parts of the Crop

1.5 g of soil, well ground root, stem, leaves and harvested maize crop (oven-dried at 60ºC) were measured into different conical flasks that were previously washed with HNO_3 and distilled water. Thereafter, 4 ml of perchloric acid, 25 ml concentrated HNO_3 and 2 ml of concentrated H_2S0_4 were added. Furthermore, the contents were mixed and heat gently at low to medium heat on a hot plate under perchloric acid fume hood. Heating continued until dense white fumes appeared, then each mixture was allowed to cool and then 40 cm^3 distilled water was added and boiled for a minute or half under the same hot plate. The solutions were allowed to cool and then filtered for atomic absorption spectrometry readings in accordance with Perkin-Elmer (1968).

Determination of Bioaccumulation Factor for Pb in the Maize Grain and Some Other Parts

Bioaccumulation factor (BAF) is a unit less value that describes the degree to which substances are taken up or accumulated into tissues of aquatic organisms from water directly and from food or other ingested materials containing the accumulated substances, and is usually measured as a ratio of a substance's concentration in tissue versus its concentration in water/soil in situations where exposure to the substance is occurring from both water, soil and the food chain. Bioaccumulation factor was determined as stated in equation 1. In this study, bioaccumulation factors were determined for the followings; transfer of Pb from soil to root; soil to maize stem; soil to maize leaves and soil to maize grain according to the formula proposed by (Malik 2010).

$$BAF = M_L/M_S \qquad 1$$

Where:

BAF is the bioavailability factor; M_L stands for mg of Pb per kg leaves of maize while M_S is the total concentration of Pb per kg of soil.

Determination of Biological Concentration Factor and Translocation Factor

The biological concentration factor (BCF) was calculated as the concentration of metal in the plant roots to that of the soil. The equation for determination of BCF is given in equation 2. The transfer factor is described as the ration of heavy metals in plants' shoots to the plants' roots. However, equation 3 was used to determine TF.

$$BCF = M_{root}/M_{soil} \qquad 2$$

$$TF = M_{shoot}/M_{root} \qquad 3$$

Where:

M_{root}, Pb concentration in the root, M_{soil} is the concentration of Pb in soil and M_{shoot} is the concentration of Pb in maize shoot.

RESULT AND DISCUSSION

Results obtained for some of the analyzed physicochemical properties of the soil and the organic matter content of the soil are given in Table 1.

Table 1: Soil physical, chemical properties and macronutrient elements

pH	EC (dSm⁻)	K (%)	Mg (cmol/kg)	Ca (cmol/kg)	N (%)	P (%)	BD (g/cm^3)	MC (%)
6.75	1.62	1.66	12.87	1.9	0.6	0.98	1.25	40.84

pH result of the soil samples used for study showed that the soil is slightly acidic and almost neutral. The pH of the soil was found to be within a range that is good for cultivation of maize (Sirisuntornlak *et al*., 2021). Organic matter content (OM) of the soil was within the acceptable range of 2% to 3% of the soil (Loveland and Webb, 2003). The Electrical conductivity (EC) result obtained indicated that the soil's EC value fell within the acceptable range (0 – 8 dS/m) which is an indication that the soil is not saline. The Ec value of the soil showed that the soil is also fertile (1.1 dS/m - 5.7 dS/m) i.e. optimal range in the soil. More so, the macro-nutrient elements results were as well adequate for production of maize. Table 2 is the analyzed results of concentrations of Pb found in the maize grain for soil contaminated at different levels.

Table 2: Pb content of the grain

Conc. Mg/kg	AAS value (ppm)	Conc. (ppm)
00	00	00
200	0.00047	0.3133
400	0.0013	0.8667
600	0.00158	1.0533

Results of Table 2, Pb content of the maize grain indicated that maize grown in the soil contaminated at 600 Mg/kg of soil had the highest concentration of Pb in their grains and was followed by the one cultivated in soil contaminated at 400 Mg/kg of soil. The least concentration of Pb in maize grain was obtained from soil contaminated at 200 Mg/kg of soil. This result agreed with the study conducted by Mwilola *et al*., (2020) that reported high concentration of Pb in the grains of un-amended soil and lower concentration of Pb from the grains of maize cultivated with chicken manure (droppings). Though the macro-nutrient elements' compositions of the soil were within the acceptable limits, chicken manure was added to all the experimental crops so as to maintain adequate nutrient for crop growth, development and yield. Thus, the ascertained low concentration of Pb in maize grains of different concentration may be as a result of amendment (chick manure). However, these concentrations obtained from the grains cultivated in three level of Pb contamination are higher than the joint FAO/WHO maximum permissible limit of 0.2 Mg/kg for health safety (FAO/WHO, 2018). Table 3 is the analyzed Pb content of the leaves.

Table 3: Pb content of the leaves

Conc. Mg/Kg	AAS value (ppm)	Conc. (ppm)
00	00	00
200	0.0707	4.7133
400	0.0753	5.02
600	0.1065	7.10

Pb content of the leaves shown in Table 3 were all high and above the 0.2 Mg/kg maximum permissible limit stipulated by the joint FAO/WHO. The implication is that the leaves of these crops are not good for consumption as high dosage of Pb in the body system is linked to several ill health. Moreover, it was observed that the concentration of Pb in leaves increased with increase in the concentration in soil. The result of this study aligned with the study conducted by (Sridhar, 2001) who asserted that Pb in the different parts or organs of the plants used for their study were positively correlated to the Pb content of the soil. Table 4, presents the results obtained for analyzed Pb content of maize shoots.

Table 4: Pb content of the Shoot

Conc. Mg/Kg	AAS value (ppm)	Conc. (ppm)
00	00	00
200	0.1210	8.07
400	0.1711	11.41
600	0.2143	14.29

The results found in Table 4 indicated that concentration of Pb in the shoot were greater than that which was obtained in the leaves and the grains of the crop. It was also observed that the level Pb found in the shoot increased with increase in available Pb in the soil. Again, some studies have shown that uptake of metals from the soil depends on different factors such as the soluble content in the soil, pH of the soil, plant growth stages, types of species, fertilizers and soil (Ismail *et al*., 2005; Sharma *et al* 2006). Increased quantity of Pb found in the stem of maize can be aligned with the higher biomass obtained from the stem. Table 5 presents the concentration of Pb found in the roots of maize crops used for the study.

Table 5: Pb in the roots

Conc. Mg/Kg	AAS value (Mg/L)	Conc. (ppm)
00	00	00
200	0.2103	14.02
400	0.267	17.80
600	0.39	26.00

The results obtained showed that the Pb content of the root was higher than the concentration found in the soil. The reason can be attributed to the proximity between the root of maize and soil which is housing the contaminant. And can be supported by a study conducted by Markert (1993) who asserted that uptake of Pb is probably passive and translocation from roots to other parts of the plant is low. More so, the concentration in the roots increased with increase in the concentration in the soil. Maize crop(s) cultivated or grown in the pots contaminated at 200 Mg/kg showed the lowest concentration of Pb in their root followed by the pot(s) contaminated at 400 Mg/kg and lastly the pot(s) contaminated at 600 Mg/kg of Pb yielded the highest Pb in the root. This is in agreement with the study conducted by Kaewsringgam *et al*. (2014) who asserted that increase of metal content in plants was consistent with the concentration in the soil. In other words, high dose of Pb or other metals in the soil will influence their uptake by plants. Apart from concentration, root depth, number of roots developed and soil chemistry can as well influence adsorption of Pb from the soil. Nonetheless, Table 6 contains the result of the analyzed Pb content of the soil at the end of the crop growth period.

Table 6: Pb in the soil at the end of the experiment

Conc. Mg/Kg	AAS value (Mg/L)	Conc. (ppm)
00	00	00
200	2.580	172.00
400	5.4705	364.70
600	8.2701	551.34

Results presented in Table 6 is the Pb concentrations in the soil at the end of the study. The result showed that maize is capable of removing Pb from the soil since meaningful concentration was conducted into the plant tissues. This is in line with the study conducted by Chiwetalu *et al*. (2020). This result also agreed with the work of Mojiri (2014) that studied, "the potentials of maize in phytoremediation of soil contaminated with Cd and Pb". His study showed that increasing the concentration of Pb in the soil increases the concentration of Pb found in the plant's parts. Mojiri (2014) noted also that Pb could hardly move from soil into plant thus, the quantity accumulated in the soil were far higher than what was obtained in the roots, shoots, leaves and grains. However, Table 7 shows the result of translocation factor, bio-accumulation factor and bio-centration of the contaminant.

Table 7: Translocation Factor, Bioconcentration Factor and Bioaccumulation factor of Pb for all the concentration used for study

Conc.	TF	BCF	BAF
00	00	00	00
200	0.5756	0.0815	0.0274
400	0.6410	0.0488	0.01371
600	0.5496	0.0472	0.0129

Table 7 showed that the translocation factor obtained for the study were 0.576, 0.641 and 0.5496 for soils contaminated at 200 Mg/Kg, 400 Mg/kg and 600 Mg/Kg respectively. Translocation factor is a parameter that quantifies the mobility of heavy metals in plants (Ondo *et al*., 2012). TF obtained for this study were all less one. This means that lead is not easily or quickly transferred from roots to shoots in maize plant. On the other hand, the bio-concentration factor obtained were 0.815, 0.049 and 0.047 for 200 Mg/Kg, 400 Mg/kg and 600 Mg/Kg Pb contaminated soils respectively. Bioconcentration factor increases with increase in concentration of Pb in the soil. Studies have affirmed that Pb concentration in exposed plants increased with increase in the contaminant's concentration in the soil. Similarly, (Omolara *et al*., 2019) asserted that *Zea may* is a significant accumulator of Pb and Cd. Bio-accumulation factor obtained for the study were 0.0274, 0.01371 and 0.0129 for 200 Mg/kg, 400 Mg/kg and 600 Mg/kg of lead contaminated soils respectively. The BAF results obtained for this study were in line with the findings of research conducted by Omalara *et al.* (2019), a review on the bioaccumulation factor of some selected heavy metals in *Zea mays.* Though BAF of Pb obtained in this study was less than 1, Cd and Pb have been reportedly accumulated in maize above the level used to classify metal hyperaccumulators (Omolara *et al*., 2019). Furthermore, the transfer and accumulation of heavy metals from soil to plants is an extreme complex process affected by several factors. Some factors like pH of the soil, organic matter content of the soil, plant species, use of polluted irrigation water and even climatic conditions affect transfer and bioaccumulation of Pb and some other heavy metals from the soil (Ondo *et al*., 2012). Figs. 4 and 5 are graphical representation of the Pb content of the leaves and grain against the initial concentration of Pb in the soil and Pb obtained in the shoot of the crop against the initial concentration of Pb in the soil respectively.

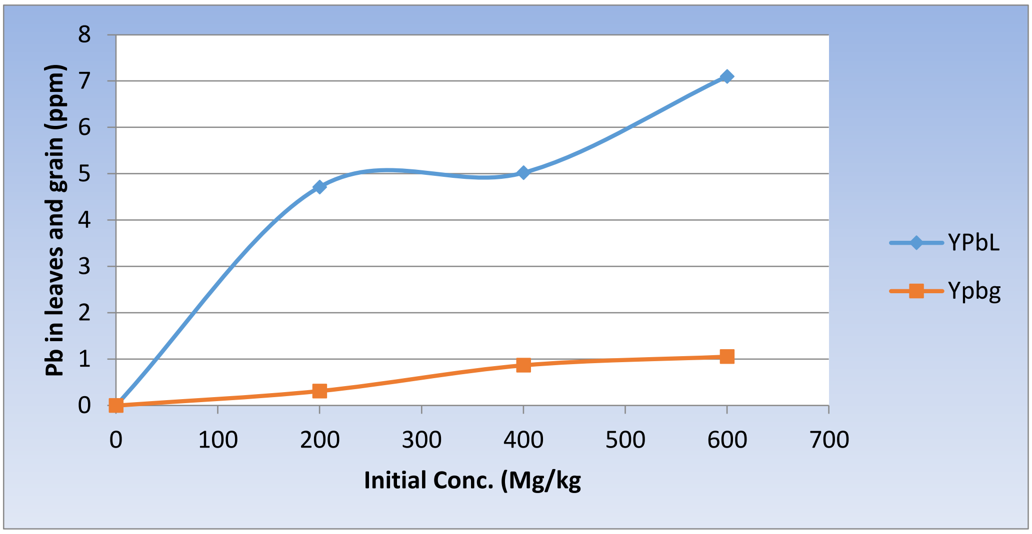

Figure **4:** A plot of Pb content of the maize leave and grain against the initial concentration of Pb in the soil.
Note: Pb_L is lead in the leaves, Pbg is Pb content of the grain
X axis represents the initial concentration of Pb in the soil before planting

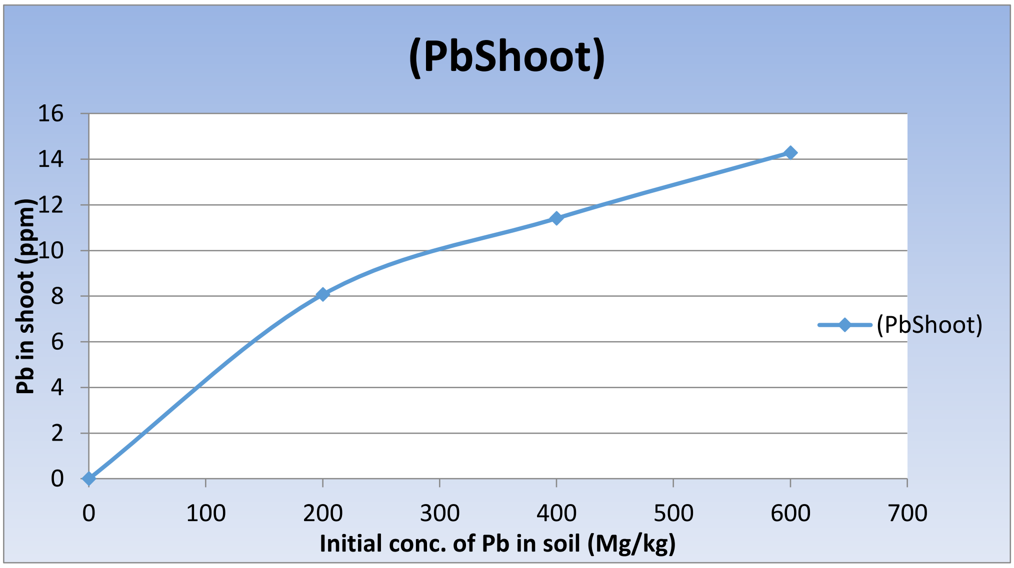

Figure 5**:** A plot of Pb content in the shoot against initial concentration in the soil
Note: Pb_{sh} is lead in the root
X axis represents the initial concentration of Pb in the soil before planting

Figures 4 and 5 showed the trend in the concentration of Pb obtained from the leaves and grain of maize plotted against the initial concentration of Pb in the soil and the concentration of Pb found in the shoot of maize plotted against the initial concentration in the soil. Though the transfer factor was small but it is evident that Pb can move from the soil system to the different parts of the crop when crops are cultivated in Pb contaminated soils.

CONCLUSION

The results of the study revealed that the use of edible plants in phytoremediaton of Pb contaminated soil will pose severe risk to human and even animals that consume such plant or crop used for phytoremediation of contaminated soil or water. Though, the TF, BCF and BAF obtained were small, the values obtain in different parts of the plants can pose serious risk and even poison the consumers when it constantly and gradually accumulates in the body system. Accumulation of the contaminant (Pb) in the parts of the plants tested were in the order of Pb concentration in roots were higher than the concentration obtained in the shoots. Similarly, the concentration found in the shoot weres greater than the concentration in the leaves while the least concentration of Pb was obtained from the grains. Bioavailability of Pb and other toxic metals in the plants used to remediate heavy metals stands to become a limiting factor in using edible plants for remediation purposes for avoidance of transfer of contaminants into the food chain and human body in particular.

DECLARATION OF INTEREST

Authors declare no conflict of interest in publishing this work

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