SOIL CONTAMINATION BY LEAD ACID BATTERY WASTES INHIBITS SEED GERMINATION AND INDUCES MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES IN SEEDS OF MAIZE (*ZEA MAYS* L.) AND *JATROPHA CURCAS.*

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ABSTRACT

High concentration of heavy metals in agricultural soils has been reported to affect plan physiological processes and seed germination. There is, therefore need to ameliorate the effect of metal toxicity on germinating seeds. Greenhouse and laboratory studies were carried out to determine the effect of mixing lead (Pb) contaminated soil with uncontaminated soil on seed germination as well as on the physiological process and morphological features of germinating seeds. Greenhouse experiment involved the mixing of lead contaminated soil with uncontaminated soil in ratios; 10:90, 25:75, 50:50, 75:25, 100:0 and 0:100 to give six soil types of 10, 25, 50, 75, 100 and 0% contamination respectively with four replications. Maize and Jathropha crops were used as test crops for the experiments. The leachates collected from the six soil types were used to carry out germination test in the laboratory. They were labelled as 0, 10, 25, 50, 75 and 100% contamination. Distilled water alone and 0% contamination were used as checks. The experiments were arranged using completely randomized design replicated three times. The results showed that the Pb concentration in this contaminated soil was very high (16,130.00 mg/kg). Though, soil mixing with contaminated soil reduced the soil Pb concentration compared to control (100% contamination), none of the mixtures could support seed germination. The laboratory experiment revealed that leachates from different soil mixtures also inhibit seed germination. There was 100% reduction in germination percentage. Water uptake by the seeds was reduced compared to control. There was shrinkage in the seeds sown in all the contaminated leachates. The nitrogen and protein contents of Jatropha seeds raised with the 100% contaminated soil were reduced compared to control (leachates from uncontaminated soil). However, there was an increase in nitrogen and protein contents of maize seeds raised with 100% lead-contaminated leachate as compared to control. High lead concentration in soils contaminated by lead acid battery wastes inhibits seed germination and induced plasmolysis.

Keywords: Lead, soil contamination Remediation, Maize, Jatropha, Protein,

Introduction

Lead (Pb) is one of the major heavy metals of antiquity and has gained considerable importance as a potent environmental pollutant. Lead has a long history of toxicity. Its exposure is becoming a great concern not only because of its toxic nature, but its wide spread occurrence and long life in biological system. Excess lead accumulation in soils is toxic to humans and other animals due to food chain transfer. The major sources of lead in soil include weathered bedrock, parent material from lead mine, smelting operations, use of lead arsenate, use of tetra methyl lead as anti knocking additive, exhaust fumes of automobiles, chimneys of factories using lead, effluents from battery industries, metal plating and finishing operations, fertilizers, pesticides and additives in pigments and gasoline (Pallavi and Dubey 2005).

Lead acid battery contains 80% of Lead and 40% of Sulphuric acid. The consequences of the indiscriminate dumping of the slag wastes are the most frightening as it releases lead and sulfuric acid into the environment (Oldema, 1994; Onianwa and Fakayode, 2000). The dumping of lead-acid battery wastes is said to be the main culprit in the introduction of lead to agricultural soils and the environment in general. Small amount of lead in the cell is capable of causing a wide range of adverse effects on physiological processes. Lead phyto-toxicity leads to inhibition of enzyme activities, disturbed mineral nutrition, water imbalance, change in hormonal status and an alteration in membrane permeability (Seregin and Ivanov, 2001). Heavy metal toxicity, according to Hossain *et al.* (2012), is responsible for the excessive accumulation of Reactive Oxygen Species (ROS) and Methyl-Glyoxal (MG), both of which can cause peroxidation of lipids, oxidation of protein, inactivation of enzymes, DNA damage and interaction with other vital constituents of plant cells.

Seed germination is the most important phase in the life cycle of a plant and it is highly responsive to the existing environment (Saritha *et al.,* 2007). Seeds generally contain the embryo, endosperm tissue, and seed coat. One of the main purposes of endosperm is to serve as a nutrient source for the germinating embryo. Germination is assumed to be completed when the radicle emerges from endosperm and seed coat. Carbohydrates, triacyl glycerols, and proteins are the major constituents of the endosperm. These macromolecules are mobilized during germination and seedling growth with the action of different enzymes. Storage proteins are said to be the major sources of amino acids for the growing embryo, and the released amino acids are used to make the necessary enzymes and components for seed germination and seedling growth. A germinating seed relies almost exclusively on it's food reserves and water to supply metabolites needed for respiration. The success of these however, depends on the prevailing environmental conditions. For example, contamination of the soil has been reported to adversely affect germination and growth of the plants (Muhamad *et al.,* 2008). Before seeds can germinate, they need to encounter a favourable set of environmental conditions. A pollution free soil is, therefore important for the successful germination of seeds.

Heavy metal toxicity is one of the major chemical parameters of an environment which has serious consequences on seed germination and determines the success or failure of plant establishment (Meagher, 2000; Raskin and Ensley, 2000). Inhibition to germination and retardation of plant growth has been reported due to lead toxicity (Jaffer *et al.*, 1999; Wierzbicka and Obidzinska, 1998). A lot of work has been carried out on the effects of heavy metal toxicity on seed germination (Mishra and Choudhuri, 1997; Bansal, 2001; Deep *et al.*, 2002). However, this trial focused on the effect of a remediation technique (mixing of Pb contaminated soil with normal uncontaminated soil) on lead concentrations as they affect germination, imbibitions, protein content and morphology of maize and jatropha seeds both in the greenhouse and laboratory.

MATERIALS AND METHODS

Experimental site

The experiment consisted of greenhouse and laboratory studies which were carried out at the Department of Crop Protection and Environmental Biology, University of Ibadan. Greenhouse experiment was done at the Rooftop Greenhouse while, laboratory experiments were carried out in the Crop Physiology Laboratory.

Sources of experimental materials

Soil collection

The soil for the experiment was collected from the lead – acid battery wastes contaminated site at Lalupon in Lagelu Local Government area of Oyo State of Nigeria. The soils were randomly collected from different points on the site, bulked together and brought to the Department. About 250 kilograms of the soil was evacuated from the site. The soil was air-dried, sieved (2mm) and composite samples taken for physico-chemical analysis before packing them into the pots. Uncontaminated soil was collected from the fallow ground beside the Department of Crop Protection and Environmental Biology.

Seeds

The seeds used for the experiment; jatropha, and maize were collected from the Forest Research Institute of Nigeria (FRIN), and the International Institute of Tropical Agriculture (IITA), respectively.

Experimental procedure for the greenhouse studies

Contaminated soil was mixed with normal (uncontaminated soil) in ratio10:90, 25:75, 50:50, 75:25, 100:0 and 0:100 to give six soil types of 10, 25, 50, 75, 100 and 0% contamination, respectively with four replications, Hundred percent contaminated soil and uncontaminated soil served as checks. This was done to achieve varying levels of contamination to give a total of six soil types. Twenty-four experimental pots of 250 gram capacity were used for the experiment. Each pot was filled with two hundred (200) grams of soil, in 6 (six) different ratios of uncontaminated to contaminated soil. The different soil mixtures are as follows;

75% contamination:150g contaminated +50g uncontaminated soil

25%contamination: 50g contaminated +150g uncontaminated soil

50% contamination:100g contaminated +100g uncontaminated soil

0% contamination:0g contaminated +200g uncontaminated soil

100% contamination:200g contaminated +0g uncontaminated soil

10% contamination:20g contaminated +180g uncontaminated soil.

Each soil ratio was mixed thoroughly and each soil mixture was represented as S1, S2, S3, S4, S5 and S6, respectively. These were analyzed to know the initial soil Pb concentration. The experiment was replicated four times. One week after mixing, maize and Jatropha seeds were sown. The experiment was terminated one month after sowing. Data collection was only on seed germination and soil lead (Pb) concentration in the various soil mixtures.

Soil Analysis

Analysis for lead concentration of the various soil combinations were carried out before and after planting. This was estimated using Atomic Absorption Spectrophotometer under different wavelengths after wet digestion. The wet digestion was done by adding 10mls of 2M Nitric acid (HNO_3) to 1g of soil in a centrifuge tube (50ml), which was then heated for two hours in a water bath at 100^0C. It was then cooled and the soil filtered through Whatman Filter paper. The resulting solution was then made up to 50mls in a standard flask and analyzed with an Atomic Absorption Spectrophotometer (AAS).

LABORATORY EXPERIMENT: *In-vitro* study of the effects of leachates from lead contaminated soil on seed germination.

The laboratory study was carried out to investigate the effects of Pb toxicity on seed germination. Effects of Pb contamination on percentage germination and imbibitions of water by the germinating seeds were studied. The leachates collected from the six soil types were used to carry out germination test in the laboratory. They were labelled as 0, 10, 25, 50, 75 and 100% contamination. Distilled water alone was used as control. Fifty millimetres of leachates were obtained from each of the various soil mixtures. The leachates were then dispensed on cotton wool placed in petri-dishes. Ten clean seeds of maize and jatropha were first weighed, washed with 10% NaOCl (to remove microbial growth) and rinsed with distilled water before being placed inside different petri-dishes already laden with cotton wool and soaked with 50mls of respective leachates. The treatments were replicated three times in a Completely Randomized Design (CRD). Data collection was on Imbibitions 48 hours after sowing, Percentage Seed Germination after 72 hours and biochemical analysis of the seeds in the various leachate concentrations.

Imbibitions after 48 hours

Six seeds of maize and jatropha of known weight were sown in the Petri-dishes containing cotton wool soaked in the various leachate concentrations and distilled water (control). Forty-eighthours after sowing, the seeds were re-weighed. The Imbibition was then calculated as:

$$\text{Weight after 48 hours - Initial Dry weight}$$

The mean for each of the treatments was calculated and used as the Imbibition rates of the seeds.

Percentage germination after 72 hours

After 72 hours, the number of seeds in each petri-dish containing the various treatments, already showing radicle and plumule elongation was recorded. Percentage seed germination was then calculated as:

$$\frac{\text{Number of germinated seeds}}{\text{Total number of Seeds in petri – dish}} \times 100$$

The means for each treatment was then taken and recorded as the percentage germination in the treatment.

Protein determination

The seeds in the leachates of 100% Pb-contaminated soil and those of uncontaminated soil (0% contamination) were removed, oven-dried, ground and 0.5g of each used for analysis. The amount of nitrogen in each sample was determined using the Kjeldahl method and the protein content (%) was calculated by multiplying the N amount of each sample by 6.25.

RESULTS

Physico-chemical characteristics of the contaminated soil used for the experiment

Results of soil analysis showed that lead concentration in the soil was high (16,130 mg/kg), compared to the concentration found in the uncontaminated soil (63 mg/kg) (Table 1). Nitrogen and organic matter contents in the contaminated soil were also abnormally low (0.3 g/kg and 3.34 g/kg, respectively) compared to that in the uncontaminated soil with 27.6g/kg and 36.4 g/kg, respectively. Contaminated soil was slightly acidic (pH 5.2) and was sandy-loam in nature (Table 1). On the effect of different soil ratios on lead concentration in the soils, the concentration in the various soil mixtures showed that mixing of contaminated soil with uncontaminated soil reduced lead concentration progressively in 75, 50, 25, 10% contamination, respectively compared to the concentration in the 100% contamination which had the highest concentration of 16,130 mg Pb/kg. Lead concentration in 0% contamination (control) was within the acceptable limit of 300 mg/kg (Table 2).

Effect of different soil ratios of soil mixtures on seed germination

No germination was recorded in all the soil mixtures, i.e., 100%, 75%, 50%, 25%, and in the lowest, 10% contamination. Only the soil with 0% contamination (control) supported maize seed germination (Table 3). The seeds in the contaminated soil mixtures were still intact even at one month after sowing.

Table 1. Physico-chemical analysis of contaminated soil and the effects of compost on lead-contaminated soil

Soil characteristics	Uncontaminated soil	Contaminated soil
pH (H_2O)	6.50	5.20
Total N (g/kg)	27.6	0.30
Org. matter (g/kg)	36.40	3.34
Heavy metal		
Pb (mg/kg)	63.00	16,130.00
Ni (mg/kg)	0.03	20.00
Cd (mg/kg)	0.00	3.20
Particle Size Distribution		
Sand (g/kg)	818.00	538.00
Silt (g/kg)	80.00	300.00
Clay (g/kg)	102.00	162.00
Textural Class (USDA)	Loamy sand	Sandy loam

Table 2. Lead concentration in different soil ratios at mixing point and one month after mixing

Treatment	At mixing point (mg/kg)	One month after mixing (mg/kg)
S_1	2835.00	2870.00
S_2	1786.75	4900.00
S_3	2697.50	492.50
S_4	63.00	179.00
S_5	16130.60	2020.50
S_6	1688.20	1152.00

S_1=75%, S_2=25%, S_3=50%, S_4=0%, S_5=100%, S_6=10%

Table 3. Percentage seed germination of maize in the varying lead concentrations at one month after planting

Treatment	% seed germination
0% lead contamination	90.6
10% lead contamination	0
25% lead contamination	0
50% lead contamination	0
75% lead contamination	0
100% lead contamination	0

In-vitro **study of the effects of leachates from lead contaminated soil, uncontaminated soil and distilled water on seed germination.**

Effect of Lead-Contaminated Leachates on Water Imbibitions by Maize and Jatropha Seeds

With increasing concentration of lead in the leachates, the amount of water imbibed by both maize and jatropha seeds sown in the leachates after 48 hours was reduced compared to the control. Distilled water showed the highest rate of Imbibitions followed by leachates with 0% contamination. As the concentration of Pb increased from 10% to 100% contamination, Imbibitions was drastically reduced (Fig.1).

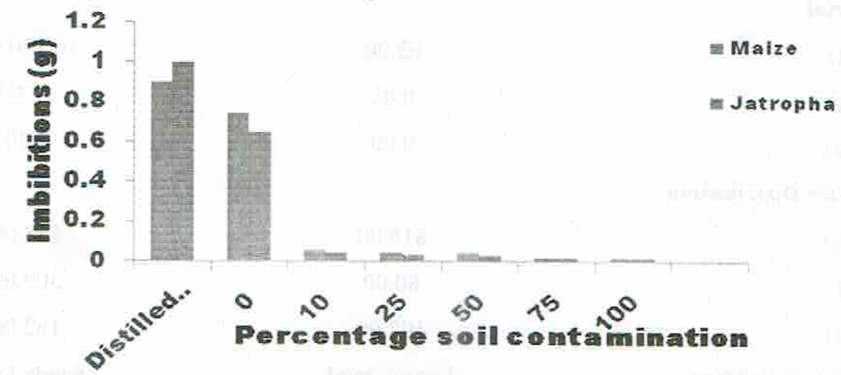

Fig 1: Effect of varying concentrations of lead contaminated leachates on water Imbibitions of maize and jatropha seeds

Effect of Lead Contaminated Leachates on Seed Germination of Jatropha and Maize

Results of the germination test showed that there was no germination in all the contaminated leachates. However, in uncontaminated leachate and distilled water (control), maize and jatropha seeds germinated. At 7 days after planting, plumule lengths of germinating maize seeds attained mean lengths of 7cm and 5.5cm in uncontaminated leachate and distilled water, respectively. The plumule length of Jatropha seeds were 3cm and 2.8cm for uncontaminated leachate and distilled water, respectively at 7 days after soaking (DAS)(Table 4). It was observed that the extract from uncontaminated soil enhanced plumule elongation more than distilled water in both maize and Jatropha .

Morphological effects of treatments on germinating seeds

When dissected, it was observed that the cotyledons in the seeds from uncontaminated leachate were still green and swollen up while those of the 100% Pb-contaminated leachates were shrunk and blackish (Plate 1). It was also observed that during the germination test, there was a formation of crystals on seeds sown in contaminated leachate, (Plate 2). These crystals were not readily soluble in water and more crystallization occurred with the addition of water to the petri-dish. A test carried out to determine the composition of the crystals using $BaCl_2$ showed a whitish solution with $BaCl_2$ indicating the presence of sulphate (most probably $PbSO_4$) It shows that the crystal was made up of $PbSO_4$. Jatropha seeds from the contaminated leachates were plasmolysed and the seed coats were separated from the embryo when split into two halves, confirming osmotic stress induced by high salt concentration. On the other hand, the seeds from uncontaminated leachate were swollen with plumule emergence (Plate 3).

Table 4: Percentage seed germination and mean plumule length of maize and jatropha in contaminated leachates.

Crop	Treatment (% contamination)	% seed germination after 48 hrs	Mean plumule length (cm) 7 DAS
Maize	0	60a	7 a
	10	0b	0 b
	25	0b	0 b
	50	0b	0 b
	75	0bb	0 b
	100	0b	0 b
	Distilled water	50a	5.5 a
Jatropha	0	55a	3.0a
	10	0b	0 b
	25	0bb	0 b
	50	0b	0 b
	75	0b	0 b
	100	0b	0 b
	Distilled water	40a	2.8a

Means in the same column with the same letters are not significantly different by DMRT(p=0.05). DAS = Days after Soaking

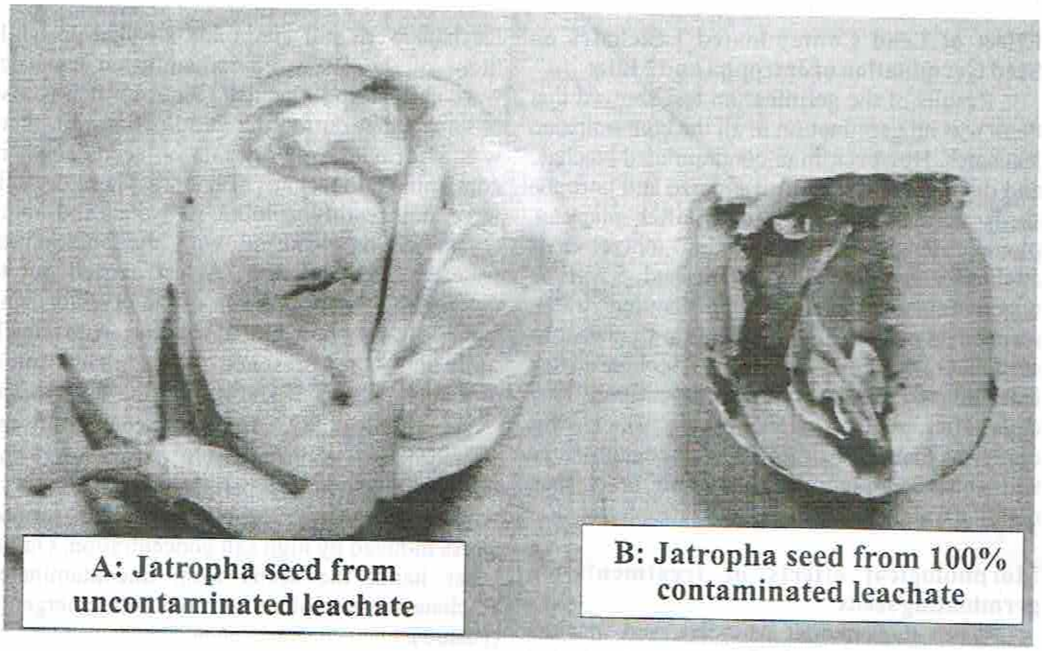

Plate 1. Effects of uncontaminated (A) and Pb-contaminated leachates (B) on germinating jatropha seeds

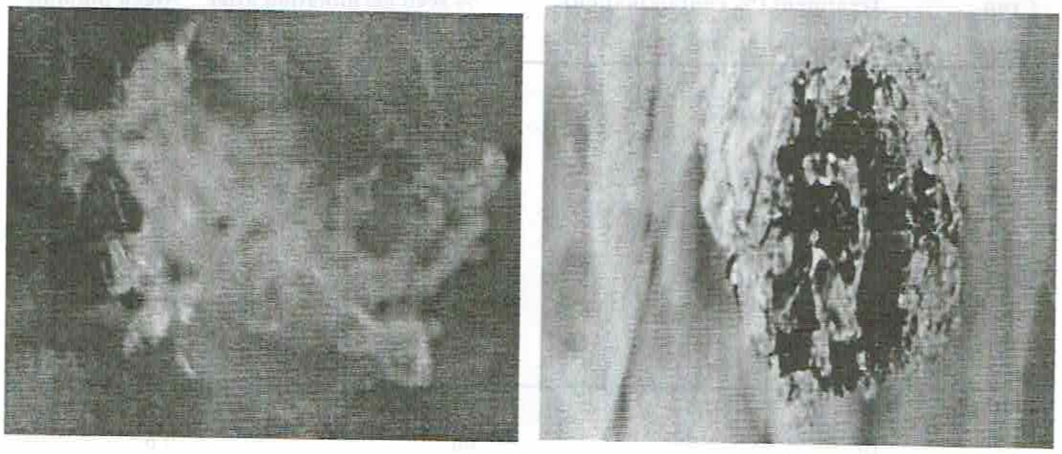

Plate 2: Maize and jatropha in lead-contaminated leachate showing the crystal

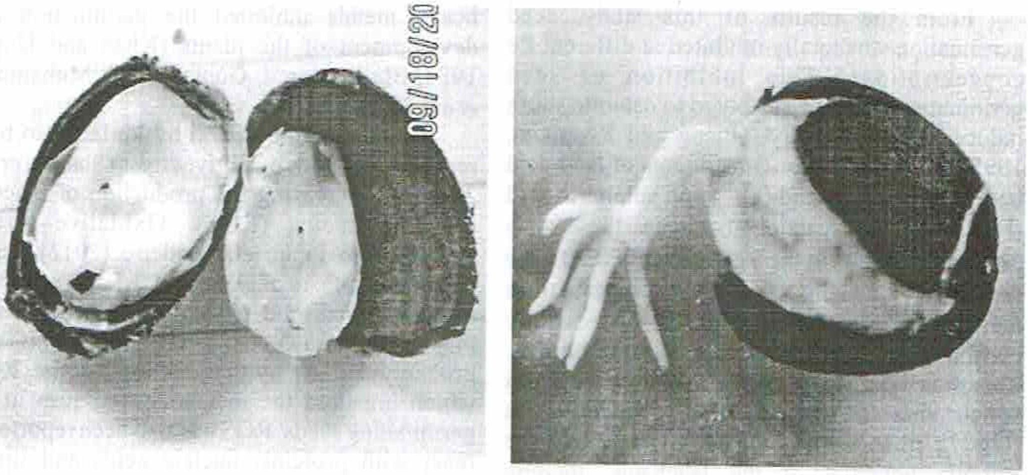

Plate 3. Effect of 100% Pb-contaminated leachate (A), distilled water (B) and uncontaminated leachate (C) on the plumule of germinating jatropha and maize seeds

Nitrogen and Protein in the germinating seeds sown with leachates from normal and 100% Pb-contaminated soils

There was an increase in the protein content of maize seeds from Pb contaminated leachates (7.44%) compared to the protein content of the maize seeds from uncontaminated soil leachates (1.31%). However, protein content of jatropha in the contaminated leachates was reduced (0.69%) compared to that of uncontaminated soil leachates (7.92%). Comparatively, the protein content of jatropha , though lower than that of maize in the contaminated leachates, was more than that of maize in uncontaminated leachates. Jatropha had 7.92% protein in uncontaminated leachates whereas maize had 1.31% protein (Table 5).

Table 5. Effect of soil contamination on percentage nitrogen and protein in the germinating seeds sown with leachates from normal and 100% Pb contaminated soils

Treatments (%) contamination	Crop	Nitrogen (%)	Protein (%)
0	Maize	0.21	1.31
	Jatropha	1.26	7.92
100	Maize	1.19	7.44
	Jatropha	0.11	0.69

0 = Leachate from Normal soil and 100 = Leachate from 100% Pb contaminated soil

DISCUSSION

Lead concentration (16,130.0mg/kg), in the soil used for the experiment was too high compared to that of the uncontaminated soil. The value was beyond the European Union permissible level, which is in the range of 2-300 mg/kg (Ogundiran and Osibanjo, 2008). The concentration also exceeds the Federal Environmental Protection Agency (FEPA) of Nigeria limit of 0.05mg/kg for agricultural soil (FEPA, 1991). The pH of the contaminated soil was slightly acidic (5.2), which suggests that the toxicity effect was not due to acidity, but rather due to the formation of lead sulphate ($PbSO_4^{2-}$) in the soil. This is a deviation from what would have been expected from a heavily lead (Pb) acid battery waste contaminated sites which usually have pH range between 3-4.

From the results of this study, seed germination was totally inhibited at different Pb concentrations. This inhibition of seed germination could be attributed to osmotic stress induced by Pb toxicity (Huang and Redmann, 1995). Since the major constituents of lead-acid battery wastes are lead (Pb^{2+}) and sulphuric acid ($H_2SO_4^{2-}$). The reaction between these two substances results in the formation of $PbSO_4$.The formation of the $PbSO_4$ crystals is suspected to be the reason why all attempts at successfully germinating seeds in the soil proved abortive. This was confirmed by the imbibitions result which showed that high salt concentration ($PbSO_4$) in this soil could have increased the osmotic potential of the leachates, thereby reducing the water intake by the seeds (Gill *et al.*, 2002). Germination of the seeds was, therefore inhibited as a result of decrease in water movement into the seeds during imbibitions (Hadas, 1977). This is because seed germination is initiated by water uptake. Water imbibitions by seeds induces the physiological processes, which in turn would result in the breakdown of reserves, the mobilization and utilization of the broken-down products, and the growth and expansion of the embryo. According to Younis *et al.* (1991) low moisture intake under salt stress could lead to cessation of metabolism or inhibition of certain steps in metabolic sequences during germination. Intake of toxic ions during seed germination on contaminated soil may also alter certain enzymatic or hormonal activities of the seeds (Smith and Comb, 1991). Moreover, several reports suggest that hyper-saline environments cause delayed germination (Prado *et al.*, 1995) by reducing hydrolytic enzyme activities and retarding the mobilization rate of metabolites (Ashraf *et al.*, 2002; Bhushan and Gupta, 2008). It is most probable that salt stress limited hydrolysis of food reserves from storage tissues and impaired their translocation from storage tissue to developing embryo axes (Dubey, 1985; Dkhil and Denden 2010). The results further confirmed the previous findings that high concentrations of Pb caused 14 to 30 % decrease in germination and reduced the growth of seedlings by more than 13 to 45 % (Verma and Dubey, 2003). Many studies have also been carried out on the effects of heavy metals on plants and it was shown that certain amounts of heavy metals inhibited the germination and development of the plants (Khan and Ungar, 1984; Bhushan amd Gupta, 2008; Muhammad *et al.*, 2008).

Contamination of soil by Pb has also been reported to induce oxidative stress (Sharma *et al.*, 2012) by increasing the production of reactive oxygen species (ROS). Oxidative stress, according to Pinho and Ladeiro (2012), could affect the molecular, biochemical, morphological, and physiological processes in plant. High salt concentrations in this soil probably led to overproduction of the ROS, which impaired the metabolic activities in the germinating seeds. ROS has also been reported to react with proteins, nucleic acids and lipids, thereby causing deleterious effect on various cellular processes (Møller *et al.*, 2007). It is said to have high affinity for sulfhydryl and oxygen containing molecules, which could result in the blockage of the essential functional groups (Noctor *et al.*, 2006). This probably could have been responsible for the changes in the structural components of the cotyledon and the necrotic lesion in the seeds from Pb contaminated leachates. More importantly, oxidative stress induced by Pb toxicity can cause peroxidation injury of the cell membrane,which is said to lead to leakage of cellular contents due to a breakdown in cell structural integrity (Scandalios, 2005,). Hence, the shrinkage or plasmolysis of the cell as was observed in the seeds sown in Pb contaminated leachates could be based on this.

Toxicity of Pb, apart from inhibiting germination of seeds, also affects the protein content of the germinating seeds. Though at the cellular level, Pb has been reported to inhibit the activities of the enzymes containing sulphydryl (-SH) groups, it also influences the activities of a wide range of enzymes of different metabolic pathways (van Assche and Clijsters, 1990; Smiri, 2011). Activities of several enzymes are reported to be enhanced by Pb treatment. Such apparent enhancement results from changes in enzyme synthesis, or as a result of effector molecules which are synthesized under Pb phytotoxicity (Bhattacharjee, 2011). Increase in the protein content of maize seeds treated with Pb-contaminated leachates could be as a result of increased production of enzymes. This was confirmed by previous reports that tissues injured by oxidative stress generally contain increased concentrations of carbonylated proteins which are widely used as marker of protein oxidation (Imlay and Linn, 1988; Møller and Kristensen, 2004). It has also been reported that plants react to abiotic stresses by the alterations in metabolic rates, protein turnover, osmolytes, membrane function, and gene expression (Møller, 2001).

High protein content of jatropha in the uncontaminated soil more than that of maize could be due to the fact that it is oily and more proteinous than maize (Raja *et al.,* 2011) but the oily nature and high protein content on the other hand could make jatropha seeds to be more vulnerable to lipid peroxidation and protein degradation under stress conditions in contaminated leachate. This could have induced the degradation of the protein molecules in Jathropha and reduced the protein content (Møller and Kristensen2004).

Conclusion

There was high concentration of Pb in the experimental soil and attempt made to remediate it by mixing with uncontaminated soil proved abortive. The method was unable to reduce the toxicity effects of Pb on seeds sown in this soil. Germination of maize and jathropha seeds were reduced by 100% in all the mixtures. It could, therefore be concluded that high Pb concentration in lead-acid battery wastes contaminated soil is injurious to seed germination and crop growth. It increases the osmotic potential of the soil solution which dries up the germinating seeds. Mixing of contaminated soil with normal (uncontaminated soil) could not ameliorate the effect on the seeds.

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