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EFFECTS OF CALCIUM HYDROGEN PHOSPHATE ON LEAD UPTAKE BY COWPEA (*VIGNA UNGUICULATA*)

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ABSTRACT
The effects of calcium hydrogen phosphate on lead (Pb) uptake by cowpea (*Vigna unguiculata*) were investigated in a growth chamber experiment coupled with atomic absorption spectrophotometry (AAS) and colorimetry analyses. Cowpea seeds were planted and harvested in soil samples treated with Pb^{+2} added as lead nitrate at the rates of 0, 1000 and 3000 mg/kg followed by calcium hydrogen phosphate ($CaHPO_4$) at 0, 10, 50 and 250 mg/kg. Increases in shoot yield brought about by phosphate fertilization were observed to oppress the shoot lead concentration as a result of growth dilution, where the actual amount of lead in shoots was unaffected by phosphate treatment. This work revealed that there is no substantial effect on lead uptake by cowpea, as a result of phosphate fertilization. Therefore, phosphate fertilization is a good means of cowpea production with minimal lead uptake and hence safe for human consumption.

Keywords: Cowpea, growth chamber, calcium hydrogen phosphate, fertilization, lead.

INTRODUCTION

Soil factors controlling lead availability to plants have been well documented. The elevation of pH promotes the formation of Pb precipitates as hydroxides, phosphates and carbonates, as well as insoluble complexes with organic matter. Organic material associated with the elevation of pH slows the absorption of Pb by plants, as the Pb^{2+} ion forms complexes with humic compounds of the soil and becomes less available (Kabata-Pendias and Pendias, 2001). Lead still has low mobility in soil due to its different interactions with the environment (Wuana and Okieimen, 2011).

Though, the widespread use of Pb has discontinued in many countries of the world, it is still used in many industries like car repair, battery manufacturing and recycling, refining, smelting, etc. The visual general symptoms of lead toxicity are fast inhibition of root growth, underdeveloped growth of the plant, blackening of root system and chlorosis. Lead inhibits photosynthesis, let downs mineral nutrition, water balance and enzyme activities (Sharma and Dubey, 2005). These disorders upset normal physiological activities of the plant. At high concentrations, lead may finally result to cell death (Seregin and Ivanov, 2001).

Similarly, lead inhibits germination of seeds and retards growth of seedlings, decreases germination percent, germination index, root/shoot length, tolerance index and dry mass of roots and shoots (Mishra *et al.,* 2006). This work is precisely aimed at investigating the influence of phosphate addition to plant, with respect to lead uptake and disorders in physiological activities of the plant caused by the presence of lead.

MATERIALS AND METHODS

All reagents, lead nitrate and calcium hydrogen phosphate supplied by Merck (Darmstadt, Germany), nitric acid obtained from Thornton and Ross (Huddersfield, England) used in this study were of analytical grade purity and used without further purification. Deionized water was prepared using a Milli-DI water purification system (Molsheim, France). Glassware and plastic containers were washed with detergent, rinsed with distilled water, soaked in 10% HNO_3 for 24 hr and finally dried in an oven at 80°C for 24 hr.

Sampling

Both soil and cowpea seed (V*igna unguiculata*) samples used for this study were collected from International Institute of Tropical Agriculture (IITA) farm in Wasai village, Minjibir Local Government Area of Kano State (Figures 1).

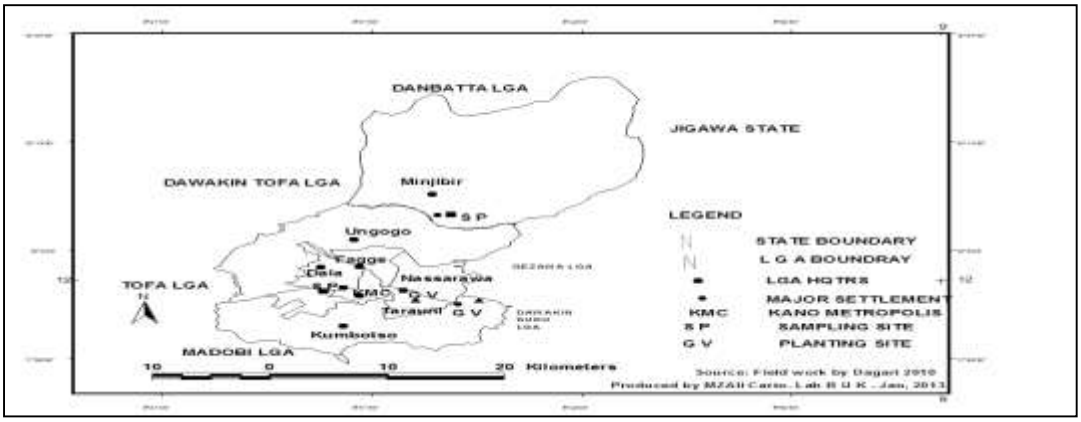

Figure 1: Map showing sampling and planting sites

Plant Preparation

A growth chamber which regulates the temperature at 26 ± 2°C during a daily 14-hr photoperiod and at 18 ± 2°C during darkness was used to provide uniform atmospheric and climatic conditions for the plant growth. In the factorial experiment with 3 rates of soil Pb^{+2} treatment (0, 1000, 3000 mg/kg applied as lead nitrate, $Pb(NO_3)_2$ and 4 rates of calcium hydrogen phosphate (0, 10, 50, 250 mg/kg) arranged in randomized blocks with 4 replications, plants were grown in 4-litre plastic pots each containing 3 kg oven dried soil.

The plants were allowed to grow for twelve weeks under glasshouse conditions before taking the first of two shoot harvests, shoots being cut about 3 cm above the soil surface and dried in paper bags at 80°C for 48 hr. The second shoot harvest was taken seven weeks later.

Dry Weights of Shoots and Roots

The dry weights of both shoots and roots were carried out by collecting the harvested portions into dried polyethylene bags and stored in a dark cupboard until constant weights were obtained.

Digestion of samples

The samples were washed with tap water to remove soil particles, then with 1% nitric acid for 30 sec to remove lead and phosphate adhered to the outside surfaces. Finally, samples were rinsed with deionised water and allowed to dry in an oven at 80°C for 48 hr (Wong and Lau, 1985).

Statistical Tool

Collection, processing and statistical analysis of the data followed the computer methods described previously by SAS (2002). All data were statistically treated and significance test was performed at 95% confidence level.

Instrumentation

All determinations were carried out in three (3) replicates using Agilent Atomic Absorption Spectrophotometer (AAS) for lead and an auto-analyzer (colorimeter) for phosphate. The phosphate was analysed according to the stannous chloride method (Allen, 1989).

RESULTS AND DISCUSSION

As previously determined, the soil had 1.16% oxidizable organic matter, 3.44 millieqv/100g of cation exchange capacity, 4.37 exchangeable sodium percentage, 0.02 mS/cm electrical conductivity, 2.60 µg/g water soluble phosphate and pH 7.08 (Dagari *et al.,* 2020).

Dry Weights of Shoots and Roots

The variations of dry weights of shoots and roots with added weights of calcium hydrogen phosphate are shown in Figures 2 to 4

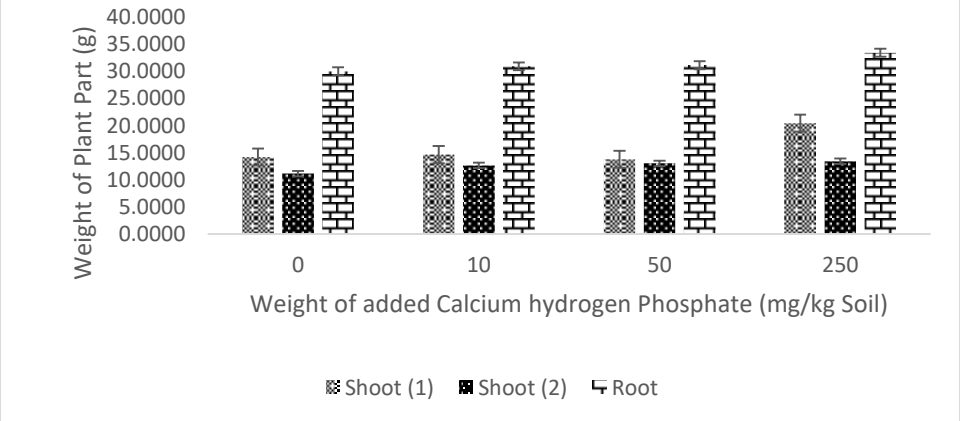

Figure 2: Variations in weights of plant parts against weight of added calcium hydrogen phosphate at 0.0 mg Pb^{+2}

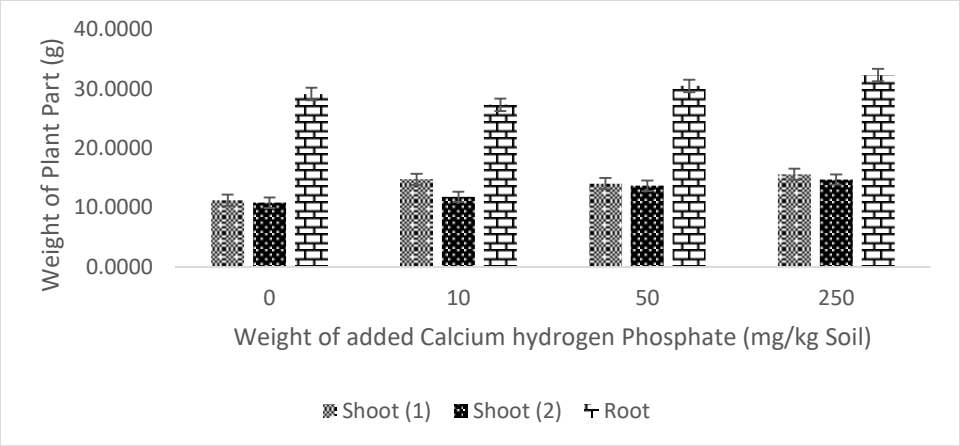

Figure 3: Variations in weights of plant parts against weight of added calcium hydrogen phosphate at 1000 mg Pb^{+2}

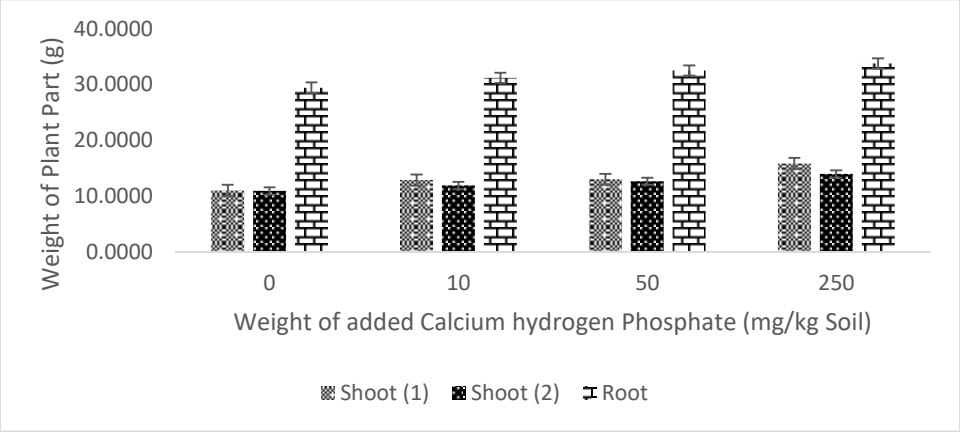

Figure 4: Variations in weights of plant parts against weight of added calcium hydrogen phosphate at 3000 mg Pb^{+2}

Figures 2, 3 and 4 show changes in dry weights of shoots and roots with added weights of calcium hydrogen phosphate were insignificant ($P>0.05$). The dry weights ranged from 11.0353±0.3405 to 16.1208±2.9486 mg/kg for first shoot harvest, 10.8052±0.6701 to 14.6984±0.5751 mg/kg for second shoot harvest and 27.3279±1.5730 to 33.7873±0.0832 for roots. In all instances, 10 mg/kg of calcium hydrogen phosphate was sufficient to bring about the increase in yield.

The first and second shoot yields increased from 11.2146±0.4778 to 14.7366±4.1556 and 10.8052±0.6701 to 11.7856±0.7554 mg/kg for soil amended with 1000 mg/kg Pb^{+2} and 10 mg/kg calcium hydrogen phosphate (Figure 3).

Lead Concentration in Shoots and Roots

Addition of lead to soil significantly increased shoot lead concentrations in both harvests and root lead concentrations ($P<0.05$) as shown in Figures 5 to 7.

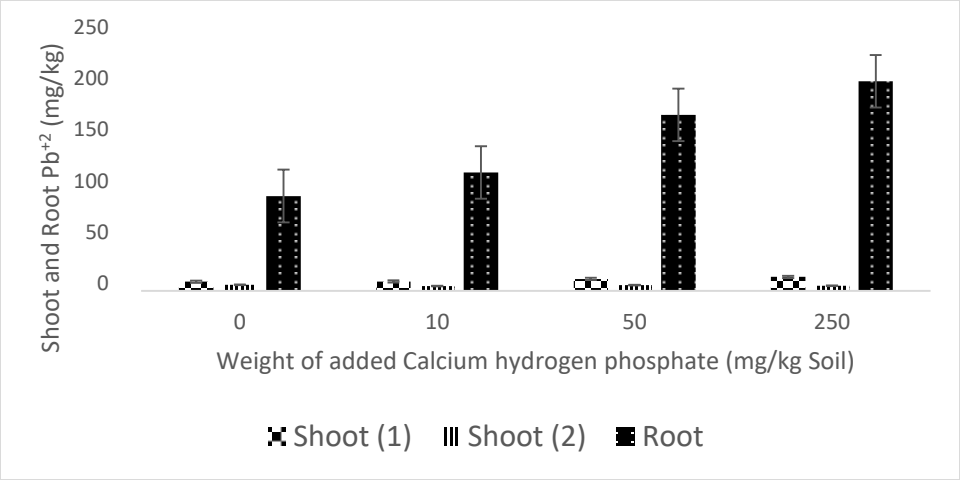

Figure 5: Changes in shoot and root lead concentrations against weight of added calcium hydrogen phosphate at 0 mg Pb^{+2}

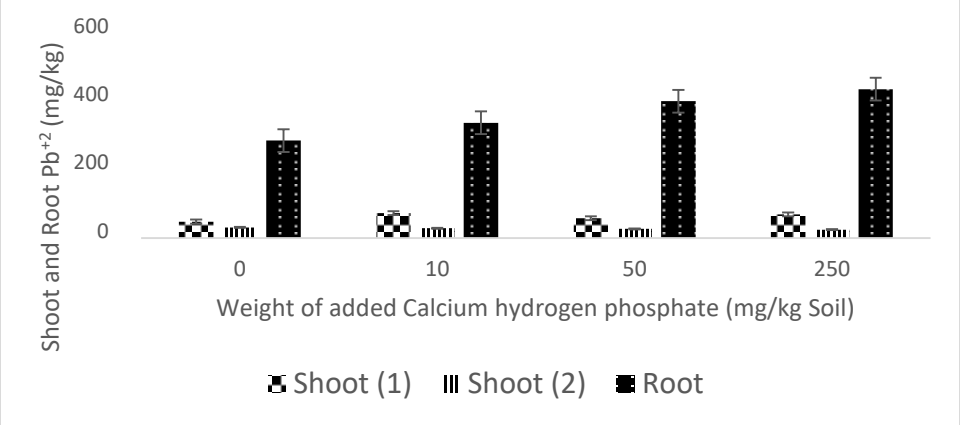

Figure 6: Changes in shoot and root lead concentrations against weight of added calcium hydrogen phosphate at 1000 mg Pb^{+2}

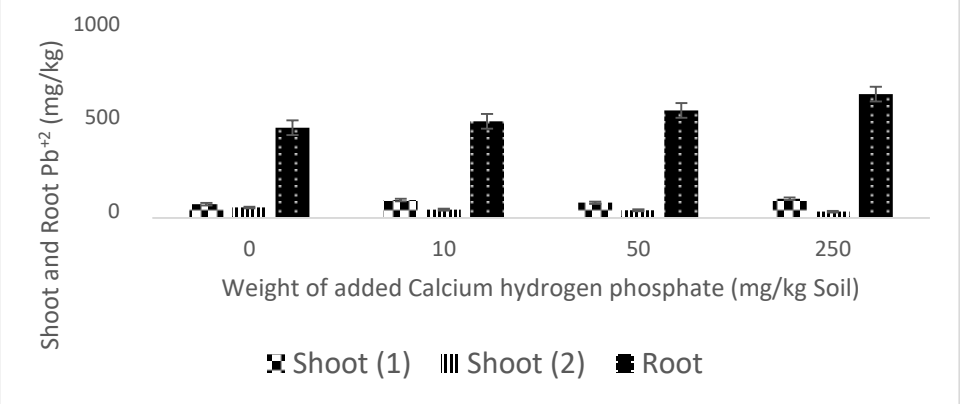

Figure 7: Changes in shoot and root lead concentrations against weight of added calcium hydrogen phosphate at 3000 mg Pb^{+2}

From the first harvest as shown in Figures 5, 6 and 70, the shoot lead concentration in lead control soil was 8.9760±1.7190 mg/kg compared with 48.4595±5.1124 and 74.6107±4.5802 mg/kg in soils amended with lead at 1000 and 3000 mg/kg respectively. Root lead concentration increased from 92.5183±4.3547 mg/kg in lead control soil to 437.0264±15.8298 and 665.2758±26.3897 mg/kg in soils amended with 1000 and 3000 mg/kg Pb^{+2} and 250 mg/kg phosphate respectively. Root lead concentrations therefore were clearly much greater than shoot concentrations.

Phosphate addition to soil had no influence over shoot lead concentrations in the first and second harvests in lead control soil. In soil amended with 1000 mg/kg Pb^{+2} (Figure 6), addition of 50 mg/kg phosphate significantly lowered shoot lead

concentration in the second harvest from 31.3257±2.5056 to 26.9898±4.9509 mg/kg (P<0.05). With 250 mg/kg phosphate addition, the shoot lead concentration was further reduced to 24.9530±6.4124 mg/kg. Although root lead concentrations demonstrated an increasing trend with increased phosphate addition, no statistically significant effect of phosphate was observed.

Total Phosphate in Shoots and Roots

Figures 8 to 10 show the changes in shoot and root total phosphate against weight of added calcium hydrogen phosphate.

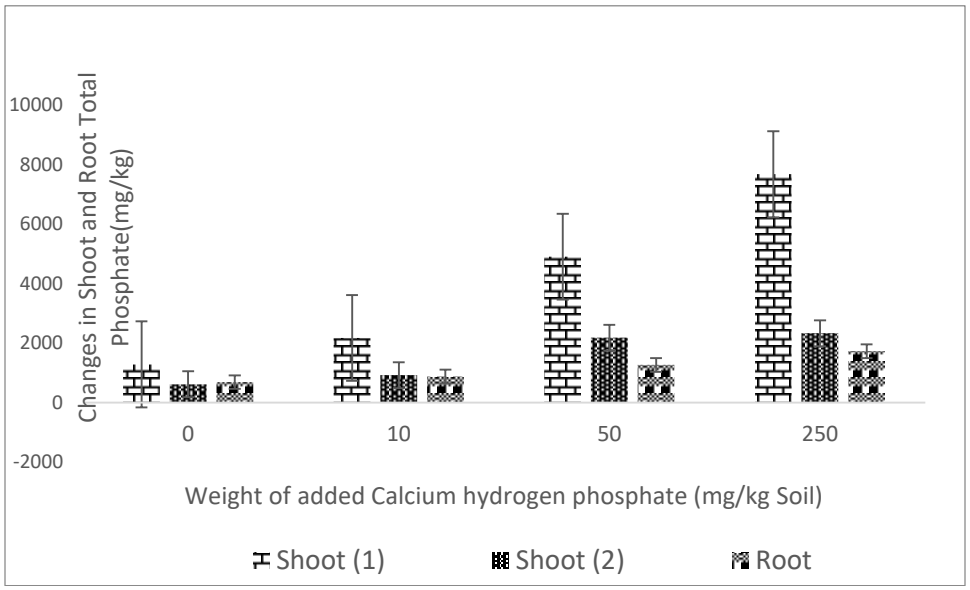

Figure 8: Changes in shoot and root total phosphate against weight of added calcium hydrogen phosphate at 0 mg Pb^{+2}

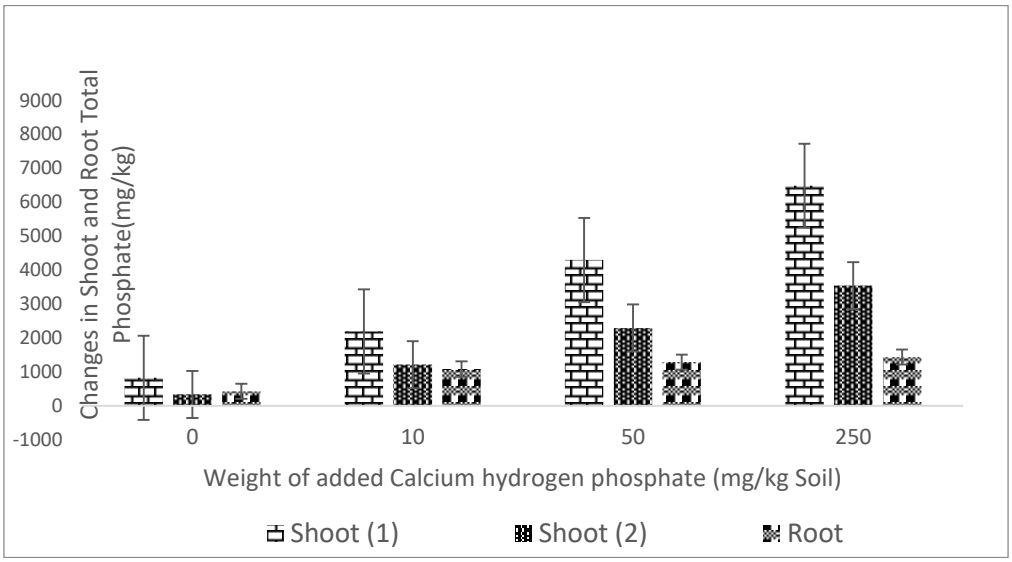

Figure 9: Changes in shoot and root total phosphate against weight of added calcium hydrogen phosphate at 1000 mg Pb^{+2}

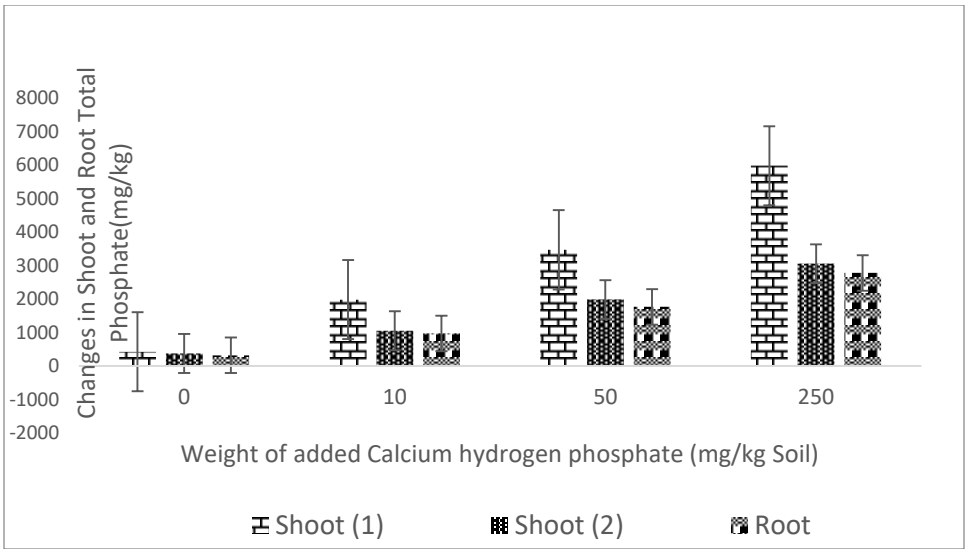

Figure 10: Changes in shoot and root total phosphate against weight of added calcium hydrogen phosphate at 3000 mg Pb^{+2}

Addition of lead to soil did not significantly affect shoot phosphate concentrations in the first or second harvest, nor did it affect root phosphate concentrations. Phosphate additions however raised shoot phosphate concentrations (both harvests) and root phosphate concentrations ($P<0.001$). In each instance addition of 250 mg/kg phosphate resulted in the maximum increase in phosphate concentration (Figures 8, 9 and 10). Considering the second harvest, shoot phosphate concentrations in lead control soil increased from 618.2317±28.4316 mg/kg to a maximum of 2335.4851±59.6481 mg/kg when 250 mg/kg phosphate was added to the soil (Figure 8). In soil amended with 1000 mg/kg Pb^{+2} however, shoot phosphate concentrations increased from 338.6138±23.6812 mg/kg in unfertilized soil to 3543.2273±47.2614 mg/kg where 250 mg/kg phosphate was added to soil (Figure 9).

Many authors including Seregin *et al. (*2004), Sharma & Dubey, (2005), Kopittke *et al*. (2007), Meyers *et al.* (2008) have demonstrated decreased yield in metal contaminated soil similar to results observed in this study. Heavy metals have been shown to be toxic to plant biochemical pathways and hence to growth if present in sufficient concentration. Liu *et al.* (2008), Qufei and Fashui (2009) demonstrated inhibition of photosynthesis by elevated lead concentrations. Steps in the formation of reactive oxygen Romanowska *et al.* (2008), Sengar *et al.* (2009) and Bhattacharjee (2005) will interfere with plant function, thus decreasing either production of carbohydrate, or the rate at which this is metabolised, so resulting in decreased growth. Addition of 3000 mg/kg Pb^{+2} to soil is a relatively high level of contamination and could bring about such effects.

Shoot yield in the first harvest was increased by the addition of 250 mg/kg phosphate to soil which suggests that the available phosphate concentration in control soils was less than optimal for growth. It is likely that added lead will complex with available phosphate in soil to produce an insoluble compound which is not taken up by plant roots and this could reduce phosphate availability. This may further decrease yield that is already low due to the naturally sub-optimal conditions. Where soils were fertilized with phosphate, any effect of lead addition was largely removed. This suggests that addition of phosphate reduced lead uptake to a level where physiological functions were not impaired to an extent which decreased yield. It also indicated *amelioration in* the physiological dysfunction caused by lead as demonstrated by Macpherson and Martin (1994).

Although there was a strong increasing trend with increasing phosphate addition, no significant difference in root lead concentration or total root lead levels was found between soil phosphate treatments. Despite root yield increasing with addition of phosphate to lead amended soil, no greater uptake of lead was observed to coincide with this greater utilization of soil. The root mass was so great in most of the soil treatments that the greater portion of soil was probably being utilized by the plant anyway. With comparable soil lead concentrations between phosphate treatments, any differences in shoot lead concentration may arise either through growth dilution or through precipitation of lead on roots due to the presence of increased concentrations of phosphate.

Results obtained in this study are in consonance with the report of Macpherson and Martin (1994). They observed that large lead and phosphate deposits in dictyosomes of plant roots were transported through the cytoplasm where they became fused with the cell wall thus removing soluble lead from sites of biochemical importance to relatively stable or inactive stores. This suggests that root phosphate status had no influence on lead translocation to shoots.

CONCLUSION

Increases in shoot yield brought about by phosphate fertilization decreased the shoot lead concentration. The increase in dry weights ranged from 11.0353±0.3405 to 16.1208±2.9486 mg/kg for first shoot harvest, 10.8052±0.6701 to 14.6984±0.5751 mg/kg for second shoot harvest and 27.3279±1.5730 to 33.7873±0.0832 for roots. In all instances, 10 mg/kg of calcium hydrogen phosphate was sufficient to bring about the increase in yields. The total amount of lead in shoots was unaffected by phosphate treatment. No evidence was found to suggest a phosphate mediated reduction in lead availability in soil, or decreased translocation in plants.

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