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In Situ Remediation Activities of Rock Phosphate In Heavy-Metal Contaminated Cocoa Plantation Soil In Owena, South Western, Nigeria

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Abstract: The continuous use of copper-based fungicide over the years in the control of black pod disease has led to heavy-metal accumulation in cocoa soils due to its non biodegradable nature. Considering the limited, available forest, it is therefore, necessary for the existing heavy-metal contaminated cocoa soils to undergo soil remediation in order to reduce the bioavailability of metals for plant uptake and ultimately minimize the risk of heavy metal toxicological effects in consumers of cocoa products. Polypropylene pots were filled with 2.5kg heavy-metal contaminated soil collected from a cocoa plantation in Idanre, Ondo State, Nigeria. The soil samples were thoroughly mixed with Sokoto rock phosphate at the rate of 20, 40 and 60g phosphate per kg soil before sowing cocoa beans in the soils. The experiment was in a completely randomized block design (RCBD) replicated thrice. At six months after planting, the seedlings were removed and processed according to standard procedure. Seedlings' leaves were analyzed for Cu and Pb using Atomic absorption spectrophotometer. Results showed that, bioavailable Cu in soil was reduced by 19, 35 and 42% due to application of 20, 40 and 60g phosphate per kg soil respectively while, Pb was reduced by 12, 23 and 25% respectively. The application of 20g, 40g and 60g rock phosphate reduced foliar Cu by 80, 69 and 85% while foliar Pb was reduced by 88, 89 and 77% respectively. The findings showed that, Sokoto rock phosphate which is readily available is a potential candidate for the remediation of heavy metal contaminated cocoa soils.

Key words: Cocoa · Remediation · Sokoto · Rock Phosphate · Heavy Metal

INTRODUCTION

As human activity impacts the environment, metal contamination issues are becoming increasingly common [1]. Metals are a natural part of terrestrial system and occur in soil, rock, air, water and organisms. A few metals including copper (Cu), manganese (Mn) and zinc (Zn) are required by plants in trace amount. It is only when metals are present in bioavailable forms at excessive levels that they have the potential to become toxic to plants. Part of the sources of metals in agricultural soils is use of fertilizers, sewage sludge and animal wastes used as fertilizers, pesticides and irrigation water [2]. Many cocoa soils in Nigeria have accumulated Cu in them due to long-term application of Cu-based fungicide in the control of black pod disease of cocoa [3, 4]. Copper being a transition metal is not biodegradable by soil microorganisms. Hence, it accumulates in soils. The build-up of Cu in soil can lead to its undue absorption and translocation to various vegetative parts of the tree including the beans, which is the economic part of the crop. In order to keep the level of Cu residue in cocoa beans within the acceptable limit set by the European Union, it became necessary to remediate the existing heavy-metal contaminated plantations. Soil remediation technologies based on the excavation, transport and land filling of metal contaminated soil and wastes are highly effective at lowering risk; however, their use in cocoa plantations where trees are grown may not be practically feasible because the soil act as support for the crop. In this circumstance, in-situ immobilization technique becomes a more appropriate means of remediating the heavy-metals contaminated soil. In-situ chemical immobilization is a remediation technique that decreases the concentration of dissolved contaminants by sorption or precipitation. Increased sorption and decreased solubility can reduce pollutant transport and redistribution from contaminated soils. Compared with

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other remediation techniques, in-situ chemical immobilization is less expensive and may provide a long-term remediation solution through the formation of low solubility minerals and/or precipitates. Research on chemical immobilization of heavy metals has included alkaline-and phosphate-based materials that adsorb, chelate, or complex heavy metals in soil. Alkaline materials used as chemical immobilization treatments include calcium oxides and calcium carbonate [5, 6]. Alkaline amendments reduce heavy metal solubility in soil by increasing soil pH and concomitantly increasing metal sorption to soil particles [7, 8]. Increased soil pH and carbonate buffering can lead to the formation of metal-carbonate precipitates, complexes and secondary minerals [9] that decrease metal solubility and reduce metal transport. Addition of phosphate materials have proven effective as a chemical immobilization treatment for Pb. Experiments involving treatment of metal contaminated soils with rock phosphates (apatite and hydroxyapaptite) have shown that formation of metal-phosphate precipitates and minerals reduced heavy metal solubility. Insoluble and geochemically stable lead pyromorphites such as hydroxypyromorphite $[Pb_5(PO_4)_3OH]$ and chloropyromorphite $[Pb_5(PO_4)_3Cl]$ have been found to control Pb solubility in apatite amended contaminated soils [10-12]. In addition to reducing metal solubility, rock phosphate amendments are also effective at reducing metal bioavailability associated with accidental ingestion of soil by humans [13, 14]. This research work was carried out to evaluate the potential of Sokoto rock phosphate in in-situ immobilization of Cu, Pb and Zn in heavy metal contaminated cocoa plantation soil obtained from Owena, in Ondo State, Nigeria.

MATERIALS AND METHODS

Materials: Surface soil (0-30cm in depth) contaminated with Cu was collected with soil auger in a cocoa plantation in Owena, South-Western part of Nigerian. The farm has the history of twenty-five years of continuous application of copper fungicide. The soil samples were air-dried and then ground and passed through 2mm sieve prior the introduction of Sokoto rock phosphate. Chemical analysis of the soil sample used for the study was also carried out.

Treatments: Sokoto rock phosphate (36% P) was purchased from Glamour Nigeria Limited, Ibadan, Nigeria. The pulverized rock phosphate was sieved through 266µm before application. The rock phosphate material contained 2.5mg/kg cadmium. For pot experiment, 20, 40 and 60g rock phosphate were mixed with the soil sample. There were four treatments including the control pot in which no rock phosphate was added. Rock phosphate application rate was based on the specific P/total metal molar ratio. Total metals, for the purpose of the immobilization treatments in this study, were defined as the sum total of Cu, Pb, Zn and Cd which was determined by Atomic Absorption Spectrophotometer. This application rate was chosen with the intent to immobilize the total concentrations of the four main metal specie of interest in the studied soil. In related work, authors have used the ratio of 3/5 P/M total as the basis of hydroxyapatite and apatite treatments to Pb-contaminated soils [15-17]. This ratio corresponds to the P/Pb ratio for chloropyromorphite $[Pb_5(PO_4)_3Cl]$. However, due to the solubility of rock phosphate in soils, since the total P may not react with insoluble Pb, higher P/Pb molar ratios (up to 11.2) have been suggested by Basta and Gradwohl [18, 19].

Pot Experiments: Two and half kilogram of the treated and untreated soil samples were packed into each polypropylene pot. Four treatments were: a control with no rock phosphate amendment, amendment with 20, 40 and 60g RP. The treatments were kept moist and incubated for one month before the sowing of cocoa beans. This was done to allow the solubilization of rock phosphate in soil solution in order to make the phosphate active in the stabilization of the heavy metals in soil. The seedlings were allowed to grow for six months after which the experiment was terminated. At termination the plant was removed from the pot and washed with distilled water, sun-dried and kept in oven for 4 hours at 60°C. The leaves, stem and roots were pulverized and digested with $HCl/HNO_3/HClO_4$(3:2:1, v/v/v) and the concentration of Cu, Pb, Zn and Cd was determined. But for this report Cd is not included.

Metal Speciation: After the removal of cocoa seedlings from the rock phosphate treated soils, some portion of the remaining soil samples in the various pots were air-dried and sieved with 2mm sieve prior to sequential extraction of Cu, Pb, Zn and Cd. One gram of each of the samples including the control was weighed into 30ml sample bottles and the procedure of Tessler [20] was used to separate the heavy metals into various fractions. Mobility factor was calculated by the formula MF=F1+F2+F3/F1+F2+F3+F4+F5+F6 X100.

Statistical Analysis: The data generated from the various chemical analyses were subjected to Analysis of variance (ANOVA) analysis using SPSS Version 15 and differences ($P < 0.05$) between means were determined using Duncan test.

RESULTS

Result showed that, foliar bioaccumulation factor of Cu was 2.48 in the control cocoa seedlings while bioaccumulation factor of Cu was 0.5, 0.76 and 0.38 in seedlings from soils treated with 20, 40 and 60g kg^{-1} phosphate respectively (Table1). Bioaccumulation factor is the ratio of heavy metal in plant to the heavy metal in soil. This result is an indication of significant reduction in the absorption and accumulation of Cu in plant tissue as a result of immobilization of soil Cu by the applied rock phosphate. The bioaccumulation factor of Pb in the foliage of control cocoa seedlings was 24.94 while bioaccumulation factor of Pb was 3.12, 2.73 and 5.84 in seedlings from soils treated with treated with 20, 40 and 60g kg^{-1} phosphate respectively. Bioaccumulation factor of Zn in foliage of control seedlings without rock phosphate was 21.66 while bioaccumulation factor of Zn was 13.13, 5.20 and 5.00 in seedlings planted in 20, 40 and 60g kg^{-1} rock phosphate treated soils respectively. Result show that, Zn bioaccumulation factor in all the treated seedlings were significantly lower than that of the control. Result also show that, bioaccumulated Cu, Pb and Zn in foliage of cocoa seedlings planted in soils treated with 20, 40 and 60g were significantly ($P<0.05$) lower than the control (Table 1). Translocation factor of Cu in foliage of the control seedlings was 0.49 while translocation factor of Cu was 0.10, 0.15 and 0.08 in seedlings planted in soil treated with 20, 40 and 60g kg^{-1} rock phosphate respectively. Translocation factor of metal in foliage of cocoa seedlings is the ratio of heavy metal in leaves and metal in the roots. There was significant reduction in translocated Cu in foliage of seedlings planted in soils treated with rock phosphate than the control, which had no rock phosphate treatment. Translocation factor of Pb in foliage of the control experiment was 6.86 while factor of 0.86, 0.79 and 1.64 was obtained in foliage of seedlings planted in soil treated with 20, 40 and 60g kg^{-1} phosphate respectively. Translocation factor of Zn in the control seedlings (0.39) was significantly higher than the translocation factors (0.24, 0.09 and 0.09) of Zn in foliage of seedlings planted in soil treated with 20, 40 and 60g kg^{-1} rock phosphate respectively. Result also showed that, the application of rock phosphate significantly ($P<0.05$) reduced the uptake of zinc from the soil treated of seedlings treated with rock phosphate. Figure 1 show the reductive effect of rock phosphate on Cu, Pb and Zn mobility in Owena cocoa soil. Result showed that, the treatment of the contaminated soil with 20, 40 and 60g kg^{-1} rock phosphate reduced Cu mobility by 19, 35 and 42% respectively while Pb mobility was reduced by 12, 23 and 25% respectively zinc mobility was reduced by 38, 54 and 54% by the application of 20, 40 and 60g kg^{-1} phosphate respectively to the contaminated soil.

Table 1: Effect of rock phosphate on soil pH.

	Month1	% Inc	Month 2	% Inc	Month 4	% Inc	Month 6	% Inc
Control	5.72^{b}	-	5.54^{c}	-	5.59^{b}	-	6.55^{b}	-
20g/kg	6.57^{a}	14.86	6.60^{b}	19.13	6.68^{a}	19.50	7.34^{a}	12.06
40g/kg	6.79^{a}	18.70	6.73^{ab}	21.48	6.67^{a}	19.32	7.35^{a}	12.21
60g/kg	6.63^{a}	15.91	6.79^{a}	22.56	6.66^{a}	19.14	7.34^{a}	12.06

Key: % Inc = Percent Increment in soil pH

Table 2: Effects of rock phosphate on heavy metal translocation and mobility factors.

	Translocation factor			Bioaccumulation factor		
Treatments	Cu	Pb	Zn	Cu	Pb	Zn
Control	$0.49^{a}±0.03$	$6.86^{a}±0.05$	$0.39^{a}±0.03$	$2.48^{a}±0.06$	$24.94^{a}±0.3$	$21.66^{a}±1.53$
20g P/kg soil	$0.10^{c}±0.02$	$0.86^{c}±0.02$	$0.24^{b}±0.02$	$0.50^{b}±0.02$	$3.12^{c}±0.14$	$13.13^{b}±1.62$
40g P/kg soil	$0.15^{b}±0.02$	$0.79^{c}±0.02$	$0.09^{c}±0.02$	$0.76^{b}±0.04$	$2.73^{c}±0.17$	$5.20^{c}±0.45$
60g P/kg soil	$0.08^{c}±0.01$	$1.64^{b}±0.01$	$0.09^{c}±0.03$	$0.38^{c}±0.03$	$5.84^{b}±0.21$	$5.00^{c}±0.52$
	% Reduction in Translocation factor			% Reduction in Bioaccumulation factor		
	Cu	Pb	Zn	Cu	Pb	Zn
20g P/kg soil	69.39	87.46	38.46	80.00	87.50	39.42
40g P/kg soil	68.39	88.48	76.92	69.23	89.06	75.96
60g P/kg soil	83.67	76.09	77.00	81.03	76.56	76.92

Key: Different alphabets on same column are significantly different ($P<0.05$)

Table 3: Linear regression of metals in leaves and various metal fractions

	Lead (Pb)	
Fractions	y	R^2
Water soluble	0.0037x + 0.886	0.371
Exchangeable	0.0157x + 13.747	0.850
Carbonate	-0.0014x + 3.84	0.150
Fe-Mn oxide	0.0124x + 2.169	0.266
Copper (Cu)		
Water soluble	0003x + 0.42	0.839
Exchangeable	-0.0017x + 1.924	0.079
Carbonate	0.0021x + 1065	0.432
Fe-Mn oxide	0.0063x + 6.415	0.027
Zinc (Zn)		
Water soluble	0.006x + 0.239	0.614
Exchangeable	0.0219x + 2.66	0.820
Carbonate	0.0157x + 18.42	0.717
Fe-Mn oxide	-0.0076x + 7.94	0.269

The relationship between metals in cocoa foliage and heavy metal fractions in Owena cocoa soil is presented in Table 3. Linear regression of data generated from the determination of metals in cocoa seedlings' foliage and heavy metal fractions of rock phosphate-treated soil showed that, Pb in exchangeable fraction had the highest R^2 value with foliar Pb (0.85).

Relationship between water soluble Pb and foliar Pb had R^2 value of 0.37 while carbonate bound Pb had R^2 value of 0.15 with foliar Pb. Fe-Mn oxide bound Pb had R^2 value of 0.26 with foliar Pb. R^2 values between water soluble Cu, exchangeable Cu, carbonate bound Cu, Fe-Mn oxide Cu and foliar Cu was 0.83, 0.07, 0.43 and 0.02 respectively. Regression analysis between zinc in the various metal fractions in soils and foliar Zn had R^2 values of 0.61, 0.82, 0.71 and 0.26 for water soluble, exchangeable, carbonate bound and Fe-Mn oxide respectively.

DISCUSSION

Metals Remobilization: The effect of rock phosphate on heavy metals transformation from non residual to residual fraction was shown by the reduction in mobility factor of the various metals in the treated soil samples (Figure 1). The reduction in mobility factor was an indication that, the metals were stabilized in soil by undergoing remobilization from bioavailable to non bioavailable state. The Fs are the various fractions of heavy metals in soil. When a particular heavy metal is in its labile state (soluble) it can easily be bioavailable. But when the metal is transformed or remobilized from available (non residual) state to unavailable state (residual). Such metal becomes inactive, stable and unavailable for plant uptake. By this, success would have been made in reducing the toxicity

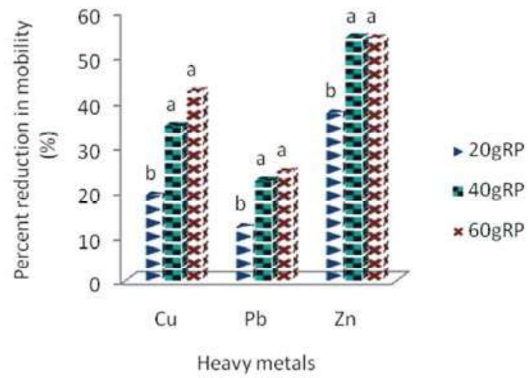

Fig.1: Reductive effects of rock phosphate on heavy metal mobility factor

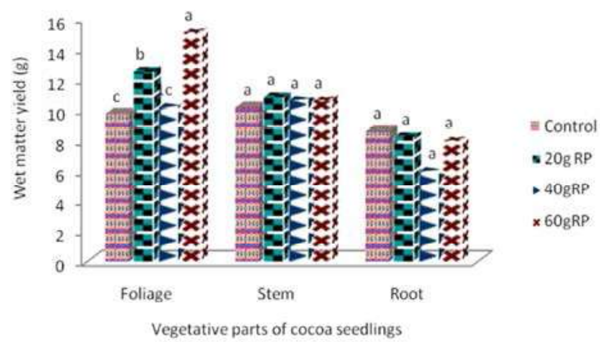

Fig. 2: Effect of rock phosphate on biomass yield of cocoa seedlings.

and contamination of such metal in soil environment. The non-residual fractions include; water soluble fraction, exchangeable, carbonate, Fe-Mn oxide and organic fractions. Heavy metals bound or associated with the water soluble, exchangeable and carbonates are bioavailable to plants and the environment. The remaining two fractions in the non-residual fraction (Fe-Mn oxide and organic) are not readily available except there are favorable chemical reactions like oxidation, reduction or dissolution of organic matter which could enhance the release of bound metals into soil solution.

Effect of Rock Phosphate on Heavy Metal Uptake in Cocoa Seedlings: The concentrations of metals in foliage of cocoa seedlings sown in rock phosphate - treated soil were significantly ($P< 0.05$) lower than the concentration of metals in foliage of control cocoa seedlings which had no rock phosphate treatment (Table 1). It was an indication that the applied rock phosphate significantly immobilized Cu, Pb and Zn in the contaminated soil thereby, reducing the amount of metals taken by cocoa seedlings. Translocation and bioaccumulation factors were used in this work to evaluate the ability of Sokoto rock phosphate to reduce the uptake of heavy metals from the treated soils. Translocation factor was estimated by dividing the concentration of metal in foliage by the concentration of metal in root while bioaccumulation factor was estimated by dividing the concentration of metal in foliage by concentration of metal in soil. [21] reported significant decrease in shoot Pb, Cu and Zn in wheat planted in soils treated with phosphate compound. Application of P have been reported to cause a decrease in bioavailability of Zn and Cu [22, 23]. There are so many mechanisms responsible for the immobilization of heavy metals ions by rock phosphate in soil solution. But the sorption process, which generally involves species attachment from soil solution to its co-existing solid surface by three types namely surface adsorption, absorption or diffusion into the solid and precipitation or co-precipitation appears to be the governing mechanism for the retention of metal ions by rock phosphate. It has been shown that the primary mechanism of metal ion removal by rock phosphate is governed by its dissolution in acidic environment followed by subsequent precipitation.

Apart from the mechanism where P in rock phosphate helps in precipitating metal ions in soil, the possibility of isomorphic substitution of calcium with heavy metals divalent ions which is correlated to their ionic radius and electronegativity exists [24]. The hydrated radius of an ion is a function of charge and ionic radius which dictates the removal phenomenon. The higher reduction in translocation and bioaccumulation factor of lead (Pb) compared to copper and zinc was due to the fact that Pb has electronegativity and ionic radius (1.20Å) very close to the ionic radius of Ca^{2+} (0.99 Å). Cu^{2+} (ionic radius 0.70 Å) having smaller ionic radius than Ca^{2+} and high electronegativity show intermediate behavior. On the other hand, zinc with ionic radius 0.75 Å smaller than Ca^{2+} and low electronegativity was exchanged to lesser extent than copper. [25] suggested that, Pb has a higher probability of being incorporated into the apatite structure by isomorphic substitution because of Pb^{2+} ionic radius (0.133nm) being greater than Ca^{2+} radius (0.094nm). The isomorphic substitution for those metals with lower radius, Cu^{2+} (0.069nm) or Zn^{2+} (0.074nm) would be less favorable. [26] reported that the mechanism involved in the immobilization of lead as pyromorphite identified by XRD, was ion exchange between Ca^{2+} ions in hydroxyapatite lattice and metal ions in solution. The work of [27] also demonstrated that reaction of Pb with hydroxyapatite was controlled by hydroxyapatite dissolution and the formation of a new lead and calcium solid solution $Pb_{(10-x)}Ca_x(PO_4)_6(OH)_2$(PbCaHA) which transforms in hydroxypyromorphite with time, with Pb^{2+} ions occupying Ca^{2+} sites. The existence of PbCaHA as an intermediate phase was confirmed by XRD and the use of electron microscopy analysis [28]. In foliage of cocoa seedlings planted in soil treated with 60g phosphate, it was observed that, translocated and bioaccumulated Pb was higher than those treated with 20 and 40g rock phosphate which appeared to be a deviation from the trend of Cu and Zn. This suggests the likelihood of the release of Pb from the rock phosphate which is a confirmation of the report of [29] and [30] who stated that rock phosphate compounds contain a range of metals. According to McLaughlin *et al.* addition of phosphate compounds to soils does not only help to overcome the deficiency of some of the essential trace elements, but could also introduce toxic metals. However due to the fact that, rock phosphate slowly dissolves, its recommendation has been made for use by many authors without fear of soil contamination. Result (Table 3) further show that, among the various fractions of heavy metals studied, the water soluble and extractable fractions are well correlated with heavy metals in the foliage which suggest that, bio availability of Cu, Pb and Zn for cocoa seedlings uptake in rock phosphate amended soil depend on the water soluble and exchangeable fractions. This implies that, the bulk of these metals found in cocoa tissue are mainly mined from these two fractions. According to some authors, metals in the water soluble and exchangeable fractions are the most available for plant uptake. Metals within the soil solution are the only soil fraction directly available for plant uptake [31-33]. The increase in biomass with increase in concentration of rock phosphate can be explained on the basis of ionic population of phosphate and calcium. When the concentration of phosphate and calcium increased in soil, pH and cation exchange capacity of the soil is also increased thereby increasing the fertility of the soil. According to [34], rock phosphate fertilizers have higher content of calcium ranging from 24-33%, which makes rock phosphate beneficial in increasing soil pH and cation exchange capacity resulting in yield increase of oil palm. [35] noted incorporation of rock phosphate into the soil ensures a sustainable supply of P over a long period and also created a high rooting density for better nutrient exploration. This is the reason why soil treated with the highest concentration of rock phosphate (60g phosphate) produced the highest biomass of cocoa seedlings compared with other treatments.

CONCLUSIONS

This study has shown that, Sokoto rock phosphate is a potential remediation material for copper, Pb and Zn contaminated soil by significantly reducing bioaccumulated and translocated metals in cocoa seedlings tissue. In this regard, Sokoto rock phosphate has demonstrated multipurpose potentials in agricultural soils by its ability to increase biomass yield as well as reducing contamination and toxicity in plant and soil. However, due to the presence of trace metals in rock phosphate, its application should be based on calculated total heavy metals to be immobilized in soils so as to avoid undue introduction of trace metals into the environment.

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