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EFFECT OF ROCK PHOSPHATE AND COW DUNG ON THE HEAVY METALS CONCENTRATION IN *AMARANTHUSVIRIDIS* LINN GROWN IN A FIELD

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ABSTRACT: *This research work investigated the effect of the application of rock phosphate (RP)and different rates of cow dung fertilizer on the heavy metals uptake in Amaranthus viridis collected from experimental and control sites in Ile -Ife, Nigeria. The residual concentrations of the elements lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu) and arsenic (As) in the crop following the fertilizer treatments were calculated as the transfer factor (TF). A total of 18 subplots containing the crops were treated with the fertilizers and the treatments consisted of RP alone; RP + 1.0 tha-^{1}; ; RP + 2.0 tha-^{1}; RP + 3.0 tha-^{1}RP + 4.0 tha-^{1} and minus RP and cow dung as the control. The experimental design used was the Randomized Complete Block Design (RCBD) which consisted of a control and the ttreatments had PR added singly and in a combination with different levels of cow dung. All the treatments were replicated thrice. Composite topsoil samples were collected using the Dutch soil auger while the plant leaves were collected pre- flowering; both were subjected to routine analyses for heavymetals using atomic absorption spectrometer (AAS). The results showed that PR application alone and in combination with various levels of cow dung increased the soil solution concentrations of Pb, , Zn, Cu, As and Pin the soil but were below the recommended standard by WHO and USEPA. Except for Cd the enrichment of the topsoil with heavy metals were not above the normal and critical value. Cadmium had a high value of 5.30µgg^{-1} which was above the critical value of 3.0 µgg^{-1}. The heavy metals content of the plant under different treatments showed that Pb content did not increase with treatments. The study further revealed a high transfer ratio (TR) of heavy metals between soil and Amaranthussp and ranged from Pb (1.72 -1.86), Zn (0.22 -0.67), Cu (14.38 -29.8), Cd (0.16 -0.32)and As (0.9 -2.1). The Cd concentration in the crop was negatively correlated ($r = -0.51, p< 0.05$). It can be concluded that both RP and cow dung contributed significantly to soil and crop contamination with heavy metals.*

KEYWORDS: rock phosphate, cow dung, heavy metals concentration, amaranthusviridis, linn grown

INTRODUCTION

Nutritious food provision and production is increasingly gaining global attention in many research studies because it is the inalienable right of the consumer to have access to quality and nutritious food products. Bationo *et al* (2006) observed that inappropriate land use, poor management and lack of inputs to soils have led to the decline in productivity, soil erosion, salinization and loss of vegetation. They further said that most African soils have low organic carbon due to low root growth of crops and natural vegetation, as well as the rapid turnover rates of organic materials with high soil temperature and micro fauna. The reasons and effects above makes the application of chemical and biological soil amendments to improve soil fertility becomes inevitable.

Sanchez (2002) noted that the prices of inorganic fertilizers in Africa are higher than in other regions of the world due to subsidy removal, transportation and transaction cost among others. Organic manures are produced from naturally available animal and vegetable materials.

Roadside agricultural soil contamination with heavy metals may result from vehicular emissions and elevated heavy metals uptake by crops affects food quality and safety (Ho and Tai, 1988). Vegetables are basic components of human diet and sources of important nutrients like protein, minerals, anti-oxidants, fibers etc.Leafy vegetables grown in heavy metals contaminated soils bio accumulate higher amounts of metals compared to those grown in uncontaminated soils (Oyedele*et al*., 1995; Al Jasser*et al*.,2005). Heavy metals accumulation in plants depend upon plant species, soil properties and the efficiency of different plants in absorbing metals is evaluated by either plant uptake or soil – plant transfer factor of the metals (Rattan *et al*., 2005).

Heavy metals transfer from soils to vegetables is one of the key ways of metal contamination through the food chain. Transfer factor (TF) or plant Concentration Factor (PCF) is a parameter used to describe the transfer of trace metals from soil to the plant body. Heavy metals can accumulate to toxic levels from phytoextraction and bio accumulation causing phytotoxic effects in plants which are not hyper accumulators. Excessive content of metals beyond the Maximum Permissible Level (MPL) leads to a number of nervous, cardiovascular, renal, neurological impairment as well as bone diseases and several other health disorders (WHO, 1992; Stealand and Boffetta, 2000).

Rock Phosphate fertilizer in the native state are used by many peasant farmers in the South West part of Nigeria in the cultivation of vegetables and other arable crops probably because of the ease of acquisition by way of its easiness to procure due to low cost. The processed or acidulated has a higher agronomic potential than the former but it is expensive to procure. Hammond et *al* (1986) observed that the soft rock phosphate has a higher agronomic value with respect to the high water solubility of its phosphorous content making it readily available to the crops but it is more expensive when compared to the hard rock phosphate. In Nigeria, rock phosphate deposits have been reported in Sokoto, Anambra, Ogun, Benue and Imo States respectively (Aduayi *et al.*2002). In spite of its advantage in increasing crop production, it is known to contain some metallic contaminants such as cadmium, zinc (Hammond *et al.,*1986). Taylor(1997) noted that the increase in soil cadmium in agricultural soils when compared to non-agricultural soils is associated with phosphate fertilizer application.

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Appropriate and adequate fertilizer application and proper management of soil is very relevant for soil fertility and productivity in most regions of the world. This can be considered for improved and increased crop production which is necessary for food security. According to Wu *et al* (2003) extremes of pH in soils will lead to rapid increase in negative surface charge and thus increases soil affinity for metal ions. It was observed by Vegelar *et al* (2009) that soil available nutrients such as N, P and K coming from mineralization and available components of fertilization can be directly absorbed by plants contributing greatly to soil fertility.The objective of this research is to investigate the effects of the combined application of rock phosphate and cow dung fertilizers on the uptake of phosphorous and some heavy metals in the vegetable *Amaranthus viridis* a staple arable crop widely consumed as a vegetable in Nigeria.

MATERIALS AND METHODS

Study Area

The study was done in Ile –Ife which is the headquarters of Ife Central Local Government Area of Osun State in South West Nigeria. It lies roughly between latitude 7°69´N to 70°50´N and longitude 3° 55´ E to 4° 69¨ E. The climate is equatorial with wet and dry seasons and relatively high relative humidity. The mean annual temperature is about 27° C. Rainfall figures over the state varies from an average of 1200mm at the onset of heavy rains in June/ July to 1800mm at the peak of the wet season in August/ September. The vegetation is the evergreen low forest type and the soil is the loamy soil derived from the pre – Cambrian gneiss. The soil is waterlogged in many parts during the wet season and becomes a mixture of fine grained loamy/ humus soil that supports the cultivation of cereals, vegetables and root crops.The experiment was conducted an designated sites widely used for arable crops production.. The experiment was laid out using the Randomized Complete Block Design having six treatments including the control with three replications. A total of 18 subplots each of dimension 3.0m by 2.5m containing the crop understudy The treatments were done using PR and different levels of cow dung fertilizers and are itemized below;.

Table 1: Treatments and application rates of PR and Cow dung Fertilizers

Treatments	Application Rates
(Control)	No RP and no cow dung applied
RP	RP Only
RPCD 1	RP + 1tha $^{-1}$ of cow dung
RPCD 2	RP + 2 tha^{-1} of cow dung
RPCD 3	RP+ 3tha^{-1} of cow dung
RPCD 4	RP + 4tha^{-1} of cow dung

Soil sampling

Soil samples were collected from experimental site surface (0 -15 cm) soil depth using a Dutch soil auger and placed in clean plastic bucket from where composite ,days and sieved through a 2 mm sieve.

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Plant sampling

Seeds of *Amaranthus* obtained from the National Horticultural Institute, Ibadan were sown in the subplots and harvested for their leaves pre flowering.

Samples analyses

Heavy metals contents of cow dung, RP and crop samples were analyzed using routine methods. Soil pH was determined in 1 :1 soil water suspension using a glass electrode pH meter. Available phosphorous in all samples were extracted using Bray 1 method and Pin the extract ant was measured by colorimeter. Cadmium, Pb^{2+}, Zn $^{2+}$, $Cu^{2+}$and As^{2+} were extracted from the soil samples and digested using *aqua regia* while the plant samples were digested with H_2O_2 .The concentrations of heavy metals in the extract ants were measured using the Buck Scientific Model 2000 (East Norwalk Connecticut, USA) Atomic Absorption Spectrophotometer.Transfer factor was computed as the ratio of the concentration of the metals in the plant tissue relative to its corresponding soil concentrations according to the method of Oyedele*et al* (1995).

Data Analysis

Data collected from the analyses of soil and crop samples were subjected to appropriate statistical analysis. The Duncan Multiple Range Test (DMRT)was used to test the significant difference among the treatments. The relationship between the heavy metals contents of soil and crop were determined using correlation analysis and the ANOVA Test. All the statistical analyses were done using the SAS package for Windows (2000).

RESULTS

Heavy metals contents of PR and Cow dung fertilizers are shown in Table 2. All the heavy metals composition of PR ranged between as follows, Pb(0.004µgg^{-1}), Cd (0.08µgg^{-1}) while cow dung metals composition ranged between ; Cd (0.00 µgg^{-1}) to 0.28 µgg^{-1} for Cu.

Table2: Some heavy metals contents of RP and Cow dung

Sample	Pb	Zn	Cu	Cd	As
			µgg^{-1}		
RP	0.004	0.02	0.01	0.08	0.01
Cow dung	0.10	0.26	0.28	0.01	0.01

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Heavy metals level in soil

Table 3 shows that the combined application of PR and Cow dung fertilizers affected the top soil chemical properties. Treatment with PR alone had the highest concentration of lead (7.30µgg^{-1}) when compared to other treatments and the control but showed no significant differences. Combination of PR and cow dung treatments did not significantly affect soil concentrations of Zn, Cu and As. The situation for cadmium was different showing an increase in content with increase in the rate of organic fertilizer application. Phosphorous content of the surface soil in the treatment having 4.0 tha^{-1}of cow dung was significantlydifferent from the control and RP treatment alone. The pH of the soil solution showed significant difference between RPCD1/ RPCD2 and the control only

Table 3: Effect of RP and different levels of cow dung and its combination on some heavy metals and available P.

Treatment	pH	P	Pb	Zn	Cu	Cd	As
			µgg^{-1}				
-RPCD	5.6b	9.4c	6.0ab	7.6a	6.7a	3.5bc	0.9a
RP	5.8ab	10.3bc	7.2a	8.7a	7.5a	3.3c	0.9a
RPCD1	6.1a	11.5abc	6.8ab	8.7a	7.1a	3.9bc	0.9a
RPCD2	6.0a	12.9ab	6.6ab	8.4a	8.4a	4.3b	0.7a
RPCD3	5.9ab	12.9ab	6.5ab	8.2a	7.9a	4.4ab	0.9a
RPCD4	5.8ab	14.4a	6.5ab	8.7a	7.8a	5.3a	1.0a

Means within the same column followed by the same letters are not significantly different from each other at $P < 0.05$ according to the Duncan Multiple Range Test (DMRT)

RP = Rock Phosphate

CD = Cow dung

RPCD 1 - 4 = Represents RP and tons of cow dung

-RPCD represents no rock phosphate and cow dung application

Metal content of plant

Combined application of RP and various levels of organic manure affected the Pb, Cd and Cu contents of the plant while the concentrations of P, Zn and As were unaffected. The Pb level in the control differed significantly from that of RPCD3 and RPCD4 respectively while Cd concentration showed that only the treatment RPCD2 was significantly different from the rest treatments including the control.

Table 4 :Effect of the combined application of RP and various levels of cow dung on then utrient contents of *Amaranthusviridis*

Treatment	P	Pb	Zn	Cu	Cd	As
			$\mu g g^{-1}$			
-RPCD	0.35a	13.33a	3.50a	12.33b	1.49a	1.10a
RP	0.36a	10.56b	3.40a	20.67a	1.49a	1.43a
RPCD1	0.32a	12.67ab	3.16a	17.00ab	0.99ab	1.23a
RPCD2	0.35a	12.33ab	3.23b	12.67b	0.73b	1.33a
RPCD3	0.36a	10.80b	3.60a	15.67ab	1.16ab	1.23a
RPCD4	0.34a	10.55b	3.30a	15.07b	1.09ab	1.20a

Means within the same column followed by the same letters are not significantly different from each other at $P < 0.05$ according to the Duncan Multiple Range Test (DMRT)RP = Rock Phosphate

CD = Cow dung

RPCD 1 - 4 = Represents RP and tons of cow dung

RPCD represents no rock phosphate and cow dung application

Correlation coefficient

The correlation coefficient between the topsoil chemical properties, crop heavy metals and available phosphorous are presented in Table 5. The result showed that soil pH was significantly negatively correlated (r= -0.51, p < 0.05) with plant Cd.

Table 5: Pearson correlation coefficient between surface soil elements and elements concentration in *Abelmuschus* leaves.

	Plant property					
Soil	P	Pb	Zn	Cu	Cd	As
Property						
pH	ns	ns	ns	ns	-0.51*	ns
Zn	ns	ns	ns	ns	ns	ns
Cu	ns	ns	ns	ns	ns	ns
Pb	ns	ns	ns	ns	ns	ns
As	ns	ns	ns	ns	ns	ns
Cd	ns	ns	ns	ns	ns	ns
P	ns	ns	ns	ns	ns	ns

*= significant at p< 0.05

Ns = not significant

N = 18 correlation prob> (r)) under Ho: Rho =0

Correlation matrix

The correlation marix among the soil chemical properties are presented in Table

5b. The result showed a significant positive correlation (r= 0.67, p< 0.01) between Cu and soil Cd only while the rest associations were insignificant.

Table 5b: Pearson correlation matrix between top (0 – 15 cm) soil heavy metals, P and pH

	pH	P	Pb	Zn	Cu	Cd	As
pH	ns	ns	ns	ns	ns	ns	ns
P		ns	ns	ns	ns	ns	ns
Pb			ns	ns	ns	ns	ns
Zn				ns	ns	ns	ns
Cu					ns	0.67**	ns
Cd						ns	ns
As							ns

*= significant at p< 0.05

** = significant at p < 0.01

Ns = not significant

N = 18 correlation prob> (r)) under Ho: Rho =0

Transfer factor

The results in Table 6 indicates the levels of heavy metals and phosphorous taken up by *Amaranthus*sp under different fertilizer treatments and it showed variable results depending on the treatment. The transfer ratio for P was lowest for the treatment RPCD1 relative to the other treatments. The metal lead TF was lowest in RP treatment alone (11.4) while RPCD2 had the highest value of 32.1. The TF values for Zn indicated that that the control showed the least value of32.7 relative to the rest with RPCD3 and RPCD4 having the highest amounts of43.2respectively. The TF for Cu indicated that the value for the control recorded the largest relative to the rest treatments while for Cd the trend showed the opposite where the control had the least value when compared to the rest treatments. Arsenic TF was highest in the control relative to the other treatments.

Table 6: Influence of RP and Cow dung on the transfer ratio of P and some heavymetals in *Amaranthus viridis*

Treatment	P	Pb	Zn	Cu	Cd	As
-RPCD	0.04	2.22	0.46	1.83	0.41	1.22
RP	0.03	1.47	0.41	2.75	0.45	1.78
RPCD1	0.03	1.86	0.36	2.46	0.25	1.75
RPCD2	0.03	1.87	0.38	1.50	0.16	1.90
RPCD3	0.04	1.66	0.43	1.98	0.26	1.36
RPCD4	0.03	1.63	0.38	1.90	0.20	1.09

Means within the same column followed by the same letters are not significantly different from each other at $P < 0.05$ according to the Duncan Multiple Range Test (DMRT) RP = Rock Phosphate

CD = Cow dung

RPCD 1 - 4 = Represents RP and tons of cow dung

RPCD represents no rock phosphate and cow dung application

DISCUSSION

The results from the study revealed that the concentrations of the elements varied in accordance with the different treatments because RPis reported to contain some trace amounts of heavy metals and is rich in phosphorous (Taylor, 1997). According to the United States Environmental Protection Agency (USEPA) the normal background levels of heavy metals in uncontaminated soil range from Cd, 0.1 to 1.0µgg^{-1},Pb 4 to 61 0µgg^{-1}, Cu 1 to 50µgg^{-1}, As 3 to 12µgg^{-1}, and Zn 9 to50 µgg^{-1}.In this study the concentration of the metals in the top soil range arePb 6.0 to 7.20µgg, Zn 7.60 to 8.80µgg^{-1}, Cu 6.70 to 8.40µgg^{-1}, Cd 3.33 to 5.20µgg^{-1}and AS 0.70 to 1.0µgg^{-1}and Available P 9.4 to 14.4µgg^{-1} , These values are all within the normal range for uncontaminated soil except for Cd and P which were higher than the normal ranges. According to

KabataPendiasndPendias (1984) the critical or threshold values for the metals Zn is 300µgg^{-1} , Cu is 100µgg^{-1} , Cd is 3µgg^{-1} and As is 50µgg^{-1} while Stewart *et al.(* 1974) said that the normal range for Available P is 0.01 to 0.2µgg^{-1} . The topsoil composition of Cd increased with rock phosphate fertilizer application in combination with various levels of cow dung. This result is in conformity with the findings of Williams (1994); ; Taylor(1997).

The high level of Available P may not be unconnected with the applications of the fertilizers treatments.

Heavy metal content of crop

The results from the study showed that the responses to heavy metal accumulation from RP treatments affected the concentrations of the elements. According to Stewart *et al*. (1974) the naturally occurring normal concentrations of plant tissue heavy metals are as follows; 0.01 to 0.03µgg^{-1} for Cd; 0.5 to 3µgg^{-1} for Pb; 2.5 to 25µgg^{-1}for Cu; 15 to 100µgg^{-1} for Zn; 0.01 to 1.0µgg^{-1} for As and the element P is 0.05 to 0.3µgg^{-1} . The results revealed that Pb , Zn, Cu and P were within the normal recommended range with the exception of Cd and As which were above the recommended range showing heavy metals contamination with both elements from the treatments.

The transfer ratios (TR) for the elements Pb, Zn, Cu and P were within the normal recommended range while the elements Cd and As were above the normal recommended range. The high transfer ratios of the heavy metals into the crop samples and eventual consumption by man and domestic animals poses a high health risk to both organisms as the heavy metal Cd is recalcitrant and non biodegradable when it accumulates above the maximum permissible level.

In conclusion rock phosphate fertilizer application in combination with cow dung the levels of the heavy metals and P in the soil solution but only Cd was above the normal recommended range and was enough to cause soil pollution with the metals. The fertilizer treatments increased the tissue concentrations of Cd and Pb above the normal recommended range making them hyper accumulators of the metals and could be useful in phytoremediation.

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