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DISTRIBUTION OF HEAVY METALS IN COCOA BEAN AND SOILS ALONG TOPOSEQUENCE OF COCOA PLANTATIONS

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Abstract
This study was conducted in April, 2018 to evaluate the relationships between Cu, Fe, Mn and Zn in cocoa bean and in soils along a toposequence of cocoa-growing areas of Abia State, Nigeria. Twelve composite soil samples and 10 plant tissues (leaves and cocoa pods) were randomly collected from twelve (12) different cocoa plantations along a toposequence (crests, upper, middle, lower and bottom) for laboratory analyses. The concentrations of Cu, Fe, Mn and Zn in both plant tissues and soils were determined. Results showed that the soils were sandy clay loam, strongly acidic, low in organic matter and sufficient in the four heavy metals contents. The content of coca-cola extractable Cu, Fe, Mn and Zn in soils, cocoa bean and leaf varied significantly along the soil toposequence. The bioavailability of the metals in soils and in plant tissues were in the order: Cu>Zn>Fe>Mn, while, the comparative abundance of the four metals followed the order: cocoa bean > leaves > soil. Significant positive relationships were also established between the metals in cacao tissues and corresponding levels of bioavailability in soils. This suggests that, the primary source of the heavy metals in cocoa beans could be linked to its primary uptake from the soils and secondary from the leaf sprayed with metals containing pesticides. This result can possibly be used as predictive parameter for evaluating Cu, Fe, Mn and Zn levels in cacao bean and cocoa products.

Keywords: Cocoa bean, coca-cola extractant, heavy metals, bioavailability, toposequence, soil, and leaf

Introduction

In Nigeria, agriculture is the mainstream industrial activity and cocoa is one of the major cash crops grown. Nigeria is the fourth largest producer of cocoa in the world (Aikpokpodion *et al.,* 2012). Cocoa is a crop of economic importance with more than 650,000ha of land cultivated in Nigeria (Sanusi and Oluyole, 2005; Aikpokpodion *et al,* 2012). To boost cocoa production, cocoa estates have over the past decades received heavy doses of agrochemical application (Idris *et al.,* 2013). These agrochemicals however, may contain heavy metals and it is therefore likely that the metals may have accumulated in the soils and plant residues. Aikpokpodion *et al*., (2013a), observed that when crops are treated with pesticides to destroy pests and pathogens, only 15% of the applied pesticide is taken by the target while the remaining 85% is distributed within the air and the soil. Soils that received repeated applications of pesticides, exhibited high concentrations of bioavailable metals and subsequently resulted in increased heavy metal concentrations in runoff and leaching of minerals. It has been reported that 11% of the total copper residue in cocoa beans was absorbed from the applied copper fungicide via the pod (Aikpokpodion *et al*.,2013b).

Previous studies by Aikpokpodion *et al*. (2010), IOC (2012) and WCF (2012), indicated that high accumulation of heavy metals in cocoa plantations ecosystems have been associated with the use of pesticides. For instance, soil samples collected from cocoa plantations which were treated with pesticides over the years in Abia, Ondo and Cross River States of Nigeria, were confirmed contaminated with heavy metals (Aikpokpodion, 2010; Aikpokpodion, *et al.*, 2012; Ajiboye *et al.*, 2015; Illori and Shittu, 2015). In plants, metals such as Cu, Fe, Mn, and Zn, are considered essential micronutrients, which are absorbed from soils and subsequently distributed among its various tissues, including leaves, pods and beans. Moreover, when accumulated in excess, these essential metals can accumulate to toxic levels in the tissues of plants used for human nutrition (Reilly, 2002; Valerie and Feller, 2005). Generally, the intake of these metals in very small amounts by humans is unavoidable, with agricultural produce being a major source (Beccaloni *et al.*, 2013).

Due to its health benefits, cocoa consumption continues to increase globally (Aikpokpodion, *et al.* 2013a). In spite of the health benefits of cocoa (EFSA, 2012), there has been a high food safety concern regarding the concentrations of heavy metals, such as Cu, Fe, Mn and Zn, both in cocoa beans and cocoa products (cocoa butter, cocoa liquor, cocoa powder and chocolates). Ramtahal *et al.* (2016), reported that content of heavy metals in cocoa bean has been implicated in chocolates. Therefore, heavy metals contamination has impacted the production of cacao and subsequently chocolate worldwide. It is therefore believed that levels of heavy metals in cocoa beans are because of their uptake by cacao plants from heavy metal contaminated soils. Previous studies by Rankin *et al.,* (2005), Aikpokpodion, (2010), Aikpokpodion *et al.*, (2013b) and Ajiboye *et al.*, (2015), have indicated that cocoa beans, pods, leaves and soils in cocoa growing areas have been susceptible to heavy metal contamination, unfortunately, no information is available regarding Cu, Fe, Mn and Zn concentration in soil, cacao plants, and cacao beans along the toposequence of cocoa plantations in Abia State. This information is important for developing management solutions to the contamination of cacao bean by these metals. The potential to relate copper, iron, manganese and zinc levels in soils via correlations with cacao beans and cacao leaves were investigated to provide a basis for subsequent studies in their absorption and distribution in cacao trees (Dube *et al.,* 2001; Valerie and Feller, 2005). Such relationships can be used to assess the effectiveness of soil treatments to reduce the metals uptake and redistribution in cacao tissues, especially the bean seeds.

Materials and Methods

Description of Sample Location

The study was carried out on cocoa plantation soils of Abia State (latitude 5°32'51"N and longitude 7°33'34"E). The selected cocoa plantations have received Cu and Pb-based pesticides for more than twenty-five years (Corporate Nigeria, 2010; CRIN, 2018). The climate of the area had a fairly uniform mean daily temperature from 27°C all through the year which rarely exceeds 35°C. It is located within the tropical rainfall zone with an annual rainfall total ranging between 1512 and 2731mm, relative humidity ranges from 51 to 87% and 3-7 hours of sunshine. The vegetation of the area is tropical rain forest consisting mainly trees, shrubs, grasses and other herbaceous plant (CRIN, 2018). The experimental soils are derived from shale parent material, an Ultisol and are classified as Typic Paleudults (SSS, 2010). The soil is able to hold enough water necessary for the cultivation of cocoa, rice, maize, cassava, etc., which are grown on it. Sampling and data collection under this study were subdivided into soil and plant sampling.

Soil Sampling

A random sampling method was employed to generate composite soil samples from twelve (12) different cocoa plantations. The soil samples were collected from two depths 0-30 and 30-60cm considered as topsoil and subsoil with soil auger at different slope gradients (crests, upper, middle, lower and bottom). A control (background) soil sample was also collected from uncultivated adjacent forests to the cocoa farms at the same depths. For each slope and the control, two (2) composites, made up of topsoil and subsoil were taken for further analysis. A total of 12 composite soil samples were obtained (2 depths x 5 slope gradients and 1 control). The sampling was limited to this area because the most feeding roots of cocoa are concentrated within that depth (Aikpokpodion 2010).

Plant Sampling

In each of the cocoa plantations, three cocoa trees were randomly selected where two ripe cocoa pods and index leaves along the toposequence were collected for analysis. In line with Aikpokpodion (2010), the pods were broken with wooden stick and later fermented for six days. The fermented beans were sun-dried for six days. The beans and the leaves samples were later oven-dried at a temperature of 65°C for 48 hours until constant weight was attained. The dry beans and leaves samples were ground and sieved to pass through 0.05mm sieve.

Analysis of some soil properties

The selected physico-chemical parameters are the basis for the growth and development of cocoa and determinants of the metal's bioavailability in cocoa ecosystem. The soil samples were analyzed for particle size composition using the hydrometer method (Gee and Or, 2002). Soil pH was determined in a 1:2.5, using soil: $CaCl_2$ suspension method (Thomas, 1996). Soil organic carbon was measured using the wet oxidation colorimetric method (Nelson and Sommers, 1996). Organic carbon was converted to OM by multiplication using a factor of 1.724 (Van Bemmelen factor). Exchangeable bases (Ca, Mg, K, and Na) were extracted with neutral NH_4OAc. Ca and Mg were determined in the extract by EDTA titration, while K and Na were determined using the flame photometer. Exchangeable acidity was determined by leaching with KCl and the leacheate titrated with 0.05N NaOH. The effective cation exchange capacity (ECEC) of the soils were determined by summation of the total exchangeable bases (TEB => Ca^{+2}, Mg^{+2}, K^{+1} and Na^{+1}) and the exchangeable acidity (EA => H^{+1} + Al^{+3}), using the standard method proposed by Sumner and Miller, (1996).

Analysis of soil samples for bioavailability of Cu, Fe, Mn and Zn

The bioavailable Cu, Fe, Mn and Zn were extracted from the soil samples using Coca-Cola solution methods, as described by Schnug *et al*. (1996). After shaking for 120mins, the Cu, Fe, Mn and Zn concentrations in the supernatant were determined using an atomic absorption spectrophotometer (AAS), employing atomization in an air/acetylene flame using

PG-Model AA-500. Coca-cola solution was chosen for its excellent extractant of Cu, Fe, Mn and Zn, for many soils. Further advantages of Coca-Cola as an acid extractant are its ubiquitous availability, readiness for use, easy and safe handling and, the fact that the procedure has no harmful impact compared to the other extracting solutions (Schnug *et al*., 1996).

Analysis of the plant materials for Cu, Fe, Mn and Zn
In order to determine the concentration of Cu, Fe, Mn and Zn in cocoa bean and leaf, the plant samples were dried in a forced air oven at 70°C for 48hrs. The plant samples were digested and analyzed for total elements using a tri-acid mixture of H_2SO_4:HNO_3: $HClO_4$ (1:2:1) in Teflon crucible, heated on a hot plate, a method described by Shuman, (1985), Whitney, (1988) and Sims and Johnson (1991). The Cu, Fe, Mn and Zn concentrations in the filtrate were determined by atomic absorption spectrophotometer (UNICAM model SOLAAR 32: Astm D1691).

Data analysis
Data collected from the soils and plant materials were subjected to analysis of variance (ANOVA) procedure, using generalized linear model. Significant means were separated using Fisher's Least Significant Different were appropriate (two-tailed). Also, Pearson correlation was used to evaluate the relationship between the bioavailable Cu, Fe, Mn and Zn in soils and in cocoa bean. The statistical analysis was performed using Genstat 4 edition and PASW Statistics software, version 18 for Window 7.0 was used for the correlation analysis, while WPS spreadsheet Office 16 was used for the graphics presentation.

Results and Discussion
Soil properties
The analyzed soil properties showed that the mean soils texture is sandy clay loam with pH which are strongly acidic (4.44), high in organic matter (44.82gkg^{-1}) and low in ECEC (4.81 Cmolkg^{-1}). The soil properties reported in this study are similar to other soils of cocoa production area reported by Ololade, (2010) and Ajiboye *et al.,* (2015) in Ondo State.

Effects of toposequence on concentrations of Cu, Fe, Mn and Zn in cocoa bean, leaf and soil sampled
Figure 1 showed that the concentrations of Cu, Fe, Mn and Zn in the cocoa beans, leaf and soil of cocoa plantation farms along the toposequence, increased down the slopes, suggesting that the metals might be from non-lithogenic sources.

Copper (Cu): Copper ranked first in abundance as element detected in the cocoa beans which varied significantly (P<0.05) from 30.24 - 53.36mgkg^{-1}, and these were obtained from the crest and lower slope respectively with a mean of 41.02 mgkg^{-1}. Moreover, concentrations of Cu in the cocoa leaf were found to be highest (48.48 mgkg^{-1}) at the lower slope, and lower (25.32 mgkg^{-1}) at the crest with a mean of 36.69 mgkg^{-1}. While, the extractable Cu in soil varied significantly from 23.94 – 41.07mgkg^{-1} at the crest and lower slope respectively with a mean of 32.61mgkg^{-1}. The mean values of Cu obtained in cocoa beans of all the 12 cocoa farms were below the maximum critical levels of 50mgkg^{-1} regulated for extractable Cu by the EFSA (2012). Amankwaah *et al*., (2015), Aikpokpodion *et al*., (2012) and Rehman (2012) reported a range of Cu concentration in cocoa bean from 10.47 to 55.17mgkg^{-1}, 5.250- 41.950mgkg^{-1}, 5-43.65mgkg^{-1} and 15.22-24.50mgkg^{-1} respectively. Lowor and Shiloh (2013), reported 26.76mgkg^{-1}, while Aikpokpodion, *et al*., (2013a) reported levels of 25mgkg^{-1} and 26.1mgkg^{-1} for Ogun and Ondo States respectively, and 10–24mgkg^{-1} with an average value of 18 mgkg^{-1} was reported for Cross River State. Similar results were reported by Ajiboye *et al*., (2015) on the cocoa growing areas of Etung, Cross River State. The authors noted that the mean values of Cu concentration at 30-60cm depth were higher than those of 0-30cm depth, when the soils of Ajassor and Agbokim of Etung by depth were compared. This agrees with the result by Illori and Shittu (2015), who observed that the Cu content was generally higher in the subsurface horizons than in the surface horizons. The results suggest the possibility of recording varying levels for Cu for different locations within the same country. However, the results from the literatures may be because the use of copper-based fungicide to prevent black pod disease over time may have accumulated levels of Cu in plant tissues especially the beans. The presence of higher accumulation of Cu in both the cocoa beans and the leaf tissue might have come from Cu contaminated soil due to the 85% of the pesticide containing Cu that drops on the soil. This indicates that some of the Cu intrusions were retained in the leaf tissue, while some others passed into the bean (Koka *et al.*, 2011). Aikpokpodion *et al*., (2010), noted that about 15% of applied fungicides get to the target, while the remaining 85% end up in the soil and therefore could be suggested that, the continual application of copper-based fungicide will result in the elevated levels of copper in the soil.

Zinc (Zn): The content of Zinc ranked second highest metal detected in the cocoa beans after copper (Figure 1). This is because Zn is widely abundant in nature and the source of zinc in most cocoa beans is mainly from the parent material from which the soils are formed (Lowor and Shiloh 2013). The concentration of Zn in cocoa beans and leaf, varied significantly (P<0.05) from 12.14 - 22.81mgkg^{-1} and 10.82 – 20.21mgkg^{-1} at the crest and bottom slope respectively. Cocoa beans and leaf had average concentrations of 30.73mgkg^{-1} and 27.97mgkg^{-1} respectively. The extractable Zn content in soil were significantly different from Zn in cocoa beans and leaf, and ranged from 4.93 – 14.74mgkg^{-1}at the crest and lower slope respectively with a mean of 21.56mgkg^{-1}. The Zn concentration obtained in this study was lower than that of Aikpokpodion *et al.,* (2013a) who reported 79–180mgkg^{-1} with a mean value of 108mgkg^{-1} and suggested that the high concentrations may be due to the inherent ability of the *Theobroma cacao* to absorb zinc from the soil. Other results by Amankwaah *et al*., (2015), reported ranges of Zn concentration from 24.05-68.25mgkg^{-1} and 24.05-50.79mgkg^{-1} in beans respectively. Nartey e*t al*., (2012), reported a mean value of 47.17mgkg^{-1} in cocoa bean, and these were also higher that the values obtained in this study. Zinc is widely known to interfere with Cu metabolism but there is dearth of knowledge about its toxicity in human (Kabata-Pendias, 2011).

Iron (Fe): Iron is the third most abundant element detected in the cocoa beans with a range of 12.52 – 35.36mgkg^{-1} obtained at the crest and lower slopes respectively, and a mean concentration of 20.09 mgkg^{-1}. The concentration of Fe in leaf and the extractable value varied from 19.14 – 41.79mgkg^{-1}and 22.77 - 48.43mgkg^{-1} in the crest and bottom slopes respectively. Cocoa leaf and extractable Fe had average concentrations of 23.80mgkg^{-1} and 27.87mgkg^{-1}, respectively. Unlike Cu, where higher accumulation was domiciled in plant tissues, Fe concentration was more in soil than in leaf and bean samples (Figure 1). Amankwaah *et al*., (2015), reported a range of Fe concentration from 0.50 - 72.36mgkg^{-1} and 0.5-160.60mgkg^{-1} with a mean of 27.420mgkg^{-1} in beans. The lower concentration of Fe in the plant tissues may be due to an excess of manganese and copper interaction which attributed to their oxidizing effects in the conversion of soluble Fe^{2+} to the more insoluble Fe^{3+}(USEPA, 2003).

Manganese (Mn): The concentration and mobility of Mn from soil to cocoa residues followed a similar trend with that of Fe (Figure 1). Manganese was the third most abundant element detected in cocoa beans with a range of 18.73–18.73mgkg^{-1} and a mean concentration of 13.35mgkg^{-1} obtained at the bottom slope and the crest respectively. Similarly, the concentrations of Mn in leaf and the extractable soil, varied significantly ($P<0.05$) from 8.23-21.15mgkg^{-1} and 10.02-24.07mgkg^{-1} at the crest and lower slopes with means of 15.31 and 17.69 mgkg^{-1}, respectively. Amankwaah *et al*., (2015) and Aikpokpodion (2010), reported a range of Mn concentration from 4.45 to 72.64mgkg^{-1} and 0.12 - 77.04mgkg^{-1} with an average value of 61.18 mgkg^{-1} respectively. Nartey *et al.,* (2012) reported a mean value of 33.60mgkg^{-1} in cocoa beans. The results obtained is supported by Illori and Shittu (2015,) who indicated that the distribution of Mn gradually increases down the slope in most of the profiles though it did not follow a particular pattern.

In summary, the result obtained from the matrix (soil, leaves and bean seeds) measured, shows that, the distribution of the four metals in the cocoa plantations are in the order of Soil: Cu (32.61mgkg^{-1}) >Fe (27.86mgkg^{-1}) > Zn (21.56mgkg^{-1}) > Mn (17.69mgkg^{-1}); Cocoa leaf: Cu (36.69mgkg^{-1}) > Zn (27.97mgkg^{-1}) >Fe (23.80mgkg^{-1}) > Mn (15.31mgkg^{-1}) and Cocoa bean: Cu (41.02mgkg^{-1}) > Zn (30.73mgkg^{-1}) >Fe (20.09mgkg^{-1}) > Mn (13.35mgkg^{-1}) (Figure 1). The concentration of Cu was higher than that of Zn in the cocoa beans and their mean difference was significant ($P< 0.05$). This could be because of the regular application of copper-based fungicides to the cocoa tress and pods as a means of controlling black pod disease and Mirids on the cocoa farms (Aikpokpodion, 2010; Aikpokpodion *et al*., 2012). Koka *et al*., (2011), observed that cocoa pods are also a route through which copper gets into the cocoa beans. Aikpokpodion *et al*. (2013) and Carrillo-Gonzalez *et al.,* (2006), reported the two possible pathways through which copper gets into cocoa beans in the field; uptake and translocation of Cu from soil and permeation of cocoa pod cuticle by copper after fungicide application.

Correlation matrix for predicting Cu, Fe, Mn and Zn in Cacao Bean

Correlation analyses were used to evaluate and show relationships between Cu, Fe, Mn and Zn concentrations in the various cacao tissues and soils (Table 1). The results in Table 1 showed a significant ($P< 0.05$) positive correlation between concentration of copper in soil and cocoa beans. The correlation analysis between Cu in soil and in plant tissues indicates that Cu in soil was positively corrected with Cu in bean ($r = 0.726^{**}$) and Cu in leaf ($r = 0.489^{ns}$), while the Cu in bean was significantly correlated with Cu in leaf ($r = 0.963^{**}$). This implied that, the concentrations of Cu in the cocoa beans could be because of the concentrations of Cu from the soil and leaf tissue. Moreover, the highly positive correlation of the concentration of Cu in cocoa beans indicates that increase in the extractable Cu in soil increases the concentrations in cocoa beans. Whereas, Cu in soils showed no significant correlation with Cu in cocoa leaves, indicating that Cu in leaf is independent of the concentration in soil (i.e., concentration of Cu in leaves may not necessary come from the soil), hence, it can be attributed to anthropogenic source such as the foliar application of Cu-based fungicides in the study area (Kabata-Pendias, 2011).

The correlation analysis between Fe in soil and Cu in plant tissues indicates that Fe in soil was positively corrected with Fe in bean ($r = 0.832^{**}$) and in leaf ($r = 907^{**}$), while the Fe in bean was significantly correlated with Fe in leaf ($r = 0. 0.977^{**}$). The concentration of Mn in cocoa bean also had positive and significant correlation with Mn in leaf ($r = 0.813^{**}$), and soil Mn ($r = 0.758^{**}$). Mn in cocoa leaf was also significant and positively correlated with soil extractable Mn ($r = 0.898^{**}$). Similarly, the concentration of Zn in cocoa bean was significant and positively correlated with Zn in leaf ($r = 0.955^{**}$), and soil Zn ($r = 0.819^{**}$). Concentrations of Zn in cocoa leaf was also significant and positively correlated with extractable Zn ($r = 0.898^{**}$). These results are similar to the trend of results reported by Wang *et al.,* (2003), Aikpokpodion *et al.*, (2013a; 2013b), and Lowor and Shiloh (2013).

This result generally indicates that the concentrations of Cu, Fe, Mn and Zn in cacao beans and leaves vary proportionately with each other. It also suggests that the mineral distribution and concentration ratios within tissues are related. Such strong correlations suggest that Cu, Fe, Mn and Zn levels in cacao beans can be estimated through its other tissues, when pods are not extractable. This may offer a means of early screening of cacao cultivars for these metals' accumulation, to allow cacao growers to meet the standard of EFSA, (2012) for Cu, Fe, Mn and Zn in cocoa beans.

The positive and non-significant correlation between the metals in cocoa bean, leaf and soils may be because of their partial distribution in the cacao tree as a result of the mobilization of protective mechanisms in plants, which inhibit the transport of metals to other tissues and organs (Carrillo-Gonzalez *et al.,* 2006; Kabata-Pendias, 2011). It could also suggest that the measurement of the extractable Cu, Fe, Mn and Zn may not be appropriate for estimating their availability to plants. This is because, the bioavailability of metals in soils for uptake by plants is often not the same as their total metal concentrations, since only a fraction may be extractable for absorption (Carrillo-Gonzalez *et al.,* 2006; Aikpokpodion *et al.*, 2013a). It is notable that such correlations were obtained for a wide variability of cacao growing trees along the toposequence of the cocoa plantations. The study on the relationship between cacao tissues and soils demonstrated that selective extraction methods for soil Cu, Fe, Mn and Zn will provide stronger correlations between cacao beans and soils. Further study on the uptake and distribution of Cu, Fe, Mn and Zn from soils to cacao tissues should therefore use combination of extractable and total to predict the concentrations of these metals in cocoa bean seeds.

Conclusion

This study showed that strong and significant correlations exist between Cu, Fe, Mn and Zn levels in cacao tissues and those extracted over a wide range of soil along the toposequence of cocoa plantations. These findings suggest that it may be possible to predict Cu, Fe, Mn and Zn levels in cocoa beans from those in mature leaves and pods. Similarly, the effectiveness of soil treatments to minimize the heavy metals uptake from soils by cacao plants can be monitored through tissue. The study further shows that, the bioavailability of the metals in soils and in plant tissues were in the order: Cu>Zn>Fe>Mn while, the comparative abundance of the four metals followed the order: cocoa bean > leaves > soil.

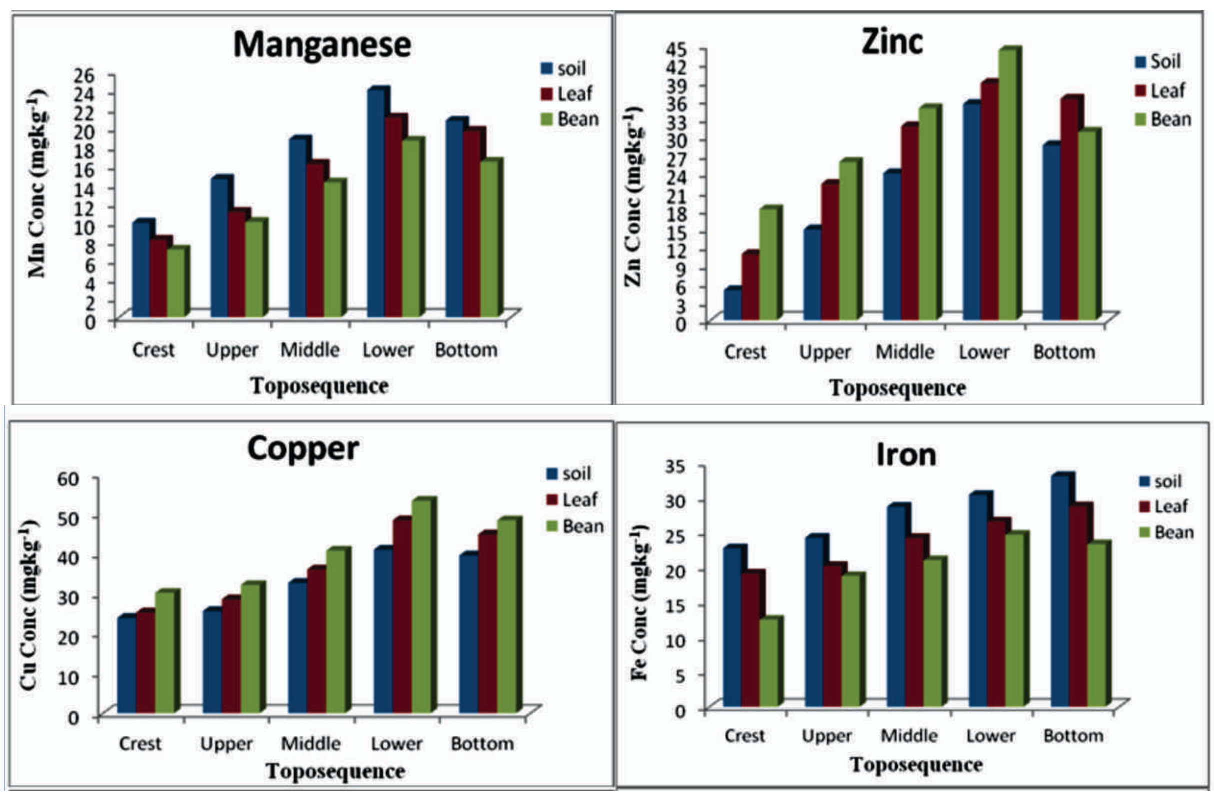

Table 1: Correlation coefficient (r) matrix among Cu, Pb, Mn and Fe in soil, and leaf and cocoa bean seed of the study area (N=10)

	Cocoa Bean				Cocoa Leaf				Cocoa Soil			
	Zn	**Mn**	**Fe**	**Cu**	**Zn**	**Mn**	**Fe**	**Cu**	**Zn**	**Mn**	**Fe**	**Cu**
Soil Cu	0.597*	0.443	-0.592*	0.726**	0.338	-0.523*	-0.613*	0.498	0.752**	-0.276	0.654*	1.000
Soil Fe	0.352	0.523*	0.832**	0.575*	0.510*	-0.578*	0.907**	-0.769**	-0.488	0.585*	1.000	
Soil Mn	0.466	0.758**	0.331	0.533*	0.404	0.742**	-0.445	-0.324	0.668*	1.000		
Soil Zn	0.819**	0.521*	0.679*	0.496	0.822**	-0.347	0.618*	-0.781**	1.000			
Leaf Cu	0.466	0.406	0.584*	0.963**	0.551*	0.669*	0.367	1.000				
Leaf Fe	-0.681*	0.576*	0.977**	0.397	0.445	0.783**	1.000					
Leaf Mn	0.332	0.813**	0.453	0.669*	-0.785**	1.000						
Leaf Zn	0.955**	-0.586*	-0.669*	0.588*	1.000							
Bean Cu	0.618*	0.486	0.237	1.000								
Bean Fe	0.328	0.847**	1.000									
Bean Mn	0.578*	1.000										
Bean Zn	1.000											

*. Correlation is significant at the 0.05 probability level (2-tailed).
**. Correlation is significant at the 0.01 probability level (2-tailed).

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