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Characterization of Heavy Metal Fractions in Agricultural Soils Using Sequential Extraction Technique

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Abstract: The accumulation of copper in cocoa plantations due to the application of copper-based fungicides in the control of black pod disease is of environmental concern. It is well known that, heavy metals have affinity for different compartment in soil. The risk associated with the presence of metals in soil is their potential eco-toxicity and ability to enter the food chain. Total metal content of a soil is inadequate for predicting the toxicity of heavy metals in soil. Hence, sequential extraction was used to fractionate Cu, Pb, Zn and Cd in soils collected from selected cocoa plantations in Cross River State in order to determine the fraction of the total metal content that is bioavailable. Result shows that, copper and zinc were predominantly associated with the organic fraction, Pb was mostly associated with the exchangeable fraction while Cd was extractable fraction. The apparent mobility and bioavailability of the four heavy metals in the studied soils were in the order: Pb>Zn>Cd>Cu. This implies that lead has the highest mobility in the studied soils.

Key words: Black pod · Chemical Speciation · Cocoa · Cross River · Heavy metals and Sequential extraction technique

INTRODUCTION

There is increasing awareness that, heavy metals present in soil may have negative consequences on human health and environment [1, 2]. From environmental point of view, all heavy metals are important because they are non biodegradable and are largely immobile in the soil system. They tend to accumulate and persist in soils for a long time [3]. The most frequently reported heavy metals with regards to potential hazards and occurrence in contaminated soils are; Cd, Cr, Pb, Zn Fe and Cu. Fertilizer and pesticide applications are important agricultural activities which contribute to the input of heavy metals into the terrestrial environment. The attack of black pod disease is a major challenge confronting Nigerian cocoa farmers. The disease incidence caused by *Phytophthora sp.* is a strong factor which has negatively affected the production of cocoa in Nigeria for several years. The impact is high in Cross River State due to high precipitation and humidity. The most effective and popular method of controlling the menace of black pod disease in Nigeria is the use of copper-based fungicides on a regular bases especially during the wet season when the disease is prevalent. However, larger portion of the applied fungicide end up in the top soil due to the fact that, 15% of applied pesticides get to the target while the rest is distributed and confined to upper 5 cm layer of the soil [4].

Metals in soil can be divided into two fractions [5]: (i) Inert fraction assumed as the non-toxic fraction and (ii) the labile fraction assumed to be potentially toxic. To assess the availability of heavy metals, only the soil labile fraction is taken into account because this fraction is often called by extension, the bio-available fraction [6]. The availability of metals for plants and micro-organisms in soil depends on the composition of the compartments of the soil such as carbonates, oxy metal hydroxides, organic matter and silica. However, the bioavailable fraction can differ from one metal to another and from one receptor to another. According to Salmons and Forstner, [7] accumulation mechanisms of heavy metal in soil can be classified into six categories (a) Water soluble (b)

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Extractable © carbonate bound (d) metal bound to Fe-Mn oxides (e) metal bound to organic matter (f) Lattice metal (residual). Heavy metals present in these categories have different remobilization behaviors under changing environmental conditions [8]

The total heavy metal content in soils provide a convenient means of expressing a measure of pollution but several documented reports have shown that, such measures are inadequate in predicting the toxicity of metal pollutants [9]. Heavy metals may be distributed among many components of the soil or sediment and may be associated with them in different ways [10]. The nature of this association is known as speciation. The chemical form of heavy metals in soil is of great significance in determining the potential bioavailability and translocation of the metals to other environmental compartments like water, plant and microorganisms when physicochemical conditions are favorable.

MATERIAL AND METHODS

Twelve surface soil samples (0-15cm) were collected with soil auger under cocoa plantations area (two samples for each location) in; Okundi (5°57N and 8°46 E), Efraya (5°53N and8°45 E), Ajassor (5°52N and 8°48 E), Boki-Biakwan (6°03N and 8°53 E), Bendege (6°N and 8°49E) and Adiginpo (5°59N and 8°44 E) all in Cross River State of Nigeria. All the selected cocoa plantations had received Cu-based fungicides for more than ten years. Collected soil samples were air-dried and (sieved with 2 mm sieve prior to analysis). The procedure of Tessier *et al*. [11] used in this study was designed to separate heavy metals into six operationally defined fractions: water soluble, exchangeable, carbonate bound, Fe-Mn oxides bound, organic bound and residual fractions. A summary of the procedure is as follows:

One gram of each soil was weighed into 30 ml polypropylene sample bottle and the following fractions obtained.

- (F1) water soluble: Soil sample extracted with 15 ml of deionized water for 2 hours.
- (F2) Exchangeable: The residue from water soluble fraction was extracted with 8 ml of 1M $MgCl_2$ (pH 7.0) for 1 hour.
- (F3) Carbonate –Bound: The residue from exchangeable fraction was extracted with 8 ml of IM Ammonium acetate (adjusted to pH 5.0 with Acetic acid) for 5 hours.
- (F4) Fe-Mn oxides-bound: The residue from carbonate fraction was extracted with 0.04M NH_2 OH. HCl in 25% (v/v) Acetic acid at 96°C with occasional agitation for 6 hours
- (F5) Organic- Bound: The residue from Fe-Mn oxides bound fraction was extracted with 3 ml of 0.02M Nitric acid and 5 ml of 30% H_2O_2 (adjusted to pH 2 with HNO_3) was added and the mixture heated to 85°C for 3 hours, with occasional agitation. A second 3 ml aliquot of 30% H_20_2 (pH 2 with HNO_3) was added and the mixture heated again to 85°C for 3 hours with intermittent agitation. After cooling, 5 ml of 3.2M NH_4OAc in 20% (v/v) HNO_3 was added and the samples was made up to 20 ml with deionized water and agitated continuously for 30min.
- (F6) Residual: The residue from organic fraction after drying was digested in a conical flask with 10 ml of 7M HNO_3 on a hot plate for 6 hours. After evaporation, 1 ml of 2M HNO_3 was added and the residue after dissolution was diluted to 10 ml. the residue was washed with 10 ml of deionized water. After each successive extraction separation was done by centrifuging (Beckman Model J2-21) at 4000 rpm for 30 min. The supernatants were filtered and analyzed for heavy metals. The residue was washed with 8 ml of deionized water followed by rigorous hand shaking and then followed by rigorous hand shaking and then followed by 30 min of centrifugation before the next extraction.

Quality Assurance: All chemicals used were of reagent grade and pure deionized water was used throughout the experimentation. All plastic ware soaked in 10% $HNO_{3.}$ Procedural blanks preparations of standard solutions under clean laboratory environment, calibration of the Buck 210A Atomic Absorption spectrophotometer (AAS) using certified standards and the analysis of calibrated standards after 10 samples to ensure that the instrument remained calibrated.

Statistical Analysis: All the data generated from the experiments were subjected to statistical analysis using SPSS version 17. All values are means of three replicates.

RESULTS

Table 1 present the results of soil physicochemical properties of sample obtained from Cross River State. Soil manganese ranged from 12.30 to 37.20 mg kg^{-1} with a mean value of 28.39 mg kg^{-1}. Soil from Boki–Biakwan 2

Table 1: Physicochemical properties of the studied soils obtained from Cross River State.

Location	Mn	Fe mg kg^{-1}	Zn	CEC	pH	C	Sand %	Silt	Clay
Okundi 1	36.18±0.87	14.22±0.21	9.18±0.45	6.67±0.73	4.17±0.11	1.36±0.38	22.28±0.65	19.30±0.44	58.42±0.23
Efraya 1	37.20±0.52	16.80±0.83	7.56±0.82	4.95±0.13	4.82±0.23	2.40±0.84	25.20±0.83	22.00±0.39	52.80±0.35
Ajassor 1	34.26±0.32	11.76±0.44	8.04±0.11	3.90±0.42	5.02±0.38	2.40±0.34	24.20±0.16	19.30±0.54	56.50±0.08
Boki Biakwa 1	24.42±0.92	12.78±0.34	4.68±0.82	3.83±0.36	4.29±0.94	1.12±0.84	28.70±0.76	23.31±0.65	47.99±0.29
Okundi 2	31.80±0.34	12.12±0.25	10.92±0.34	5.41±0.22	4.79±0.94	2.07±0.34	22.38±0.33	21.56±0.15	56.06±0.60
Efraya 2	34.26±0.43	9.18±0.30	8.16±0.63	7.10±0.73	4.82±0.37	1.60±0.83	23.00±0.34	16.00±0.09	61.00±0.41
Ajassor 2	36.18±0.12	13.86±0.82	5.94±0.32	6.84±0.44	4.35±0.87	3.27±0.93	24.30±0.41	19.10±0.34	56.60±0.80
Boki Biakwa 2	12.30±0.82	8.88±0.88	10.44±0.54	6.43±0.16	4.64±0.17	2.55±0.27	23.80±0.32	18.30±0.10	57.90±0.82
Bendege 1	32.88±0.42	9.78±0.82	3.24±0.92	8.10±0.73	4.14±0.l8	1.52±0.74	35.60±0.65	21.30±0.31	43.10±0.45
Adiginpo1	13.08±0.10	7.98±0.34	2.82±0.32	7.62±0.89	5.26±0.29	1.68±0.24	30.36±0.83	20.79±0.73	48.85±0.48
Adiginpo 2	16.34±0.73	8.20±0.45	2.10±0.45	6.42±0.83	5.11±0.87	1.72±0.74	25.20±0.44	22.00±0.63	52.80±0.78
Bendege 2	31.43±0.32	8.70±0.81	3.27±0.23	7.34±0.27	4.35±0.43	1.60±0.43	32.62±0.28	17.00±0.90	50.38±0.57
Mean	28.36	11.18	6.36	6.24	4.65	1.94	26.47	19.99	53.53
STD	9.35	2.83	3.10	1.40	0.35	0.61	4.34	2.20	5.18

had the least value while soil from Efraya 1 had the highest value. Soil extractable iron (Fe) ranged from 7.98 to 16.80 mg kg^{-1} with a mean value of 11.18 mg kg^{-1}. Soil from Adiginpo 1 had the least value while Efraya 1 had the highest value. Zn ranged from 2.10 to 10.92 mg kg^{-1} with a mean value of 6.36 mg kg^{-1}. Soil from Adiginpo 2 had the least value while soil sample from Okundi 2 had the highest value. The cation exchange capacity of the soil ranged from 3.83 to 8.10 cmol kg^{-1} with a mean value of 6.24 cmol kg^{-1}. Soil from Boki-Biakwan 1 had the least value while soil from Bendege 1 had the highest CEC value. Soil pH ranged from 4.17 to 5.26 with a mean value of 4.65 oil obtained from Okundi 1 had the least pH value while soil obtained from Adiginpo 1 had the highest pH value. Soil organic carbon ranged from 1.12 to 3.27% with a mean value of 1.94%. The least value was obtained in soil from Boki-Biakwan 1 while the highest value was obtained in soil from Ajassor 2. Silt content ranged from 16 to 23.31% with a mean value of 19.99%. The minimum value was obtained in soil from Efraya 2 while the maximum value was obtained in soil from Boki- Biakwan 1. Sand content ranged from 22.28 to 35.60% with a mean value of 26.47%. Soil from Okundi 1 had the minimum while soil from Bendege 1 had the maximum value. On the other hand, clay content ranged from 43.10 to 58.42% with a mean value of 53.53%. The minimum clay content was in soil from Bendege 1 while the maximum value was obtained in soil from Okundi 1. Result of copper speciation in soil samples collected from selected cocoa plantations in Cross River State is presented in Figure 1. Water soluble Cu in soils ranged from 0.39 to 5.99%. The minimum value was obtained in soil from Efraya 2 while the maximum value was in soil from Adiginpo 2. However, soil samples from Bendege 1, Ajassor 2, Boki-Biakwan 1, Adiginpo 1, Okundi 2 and

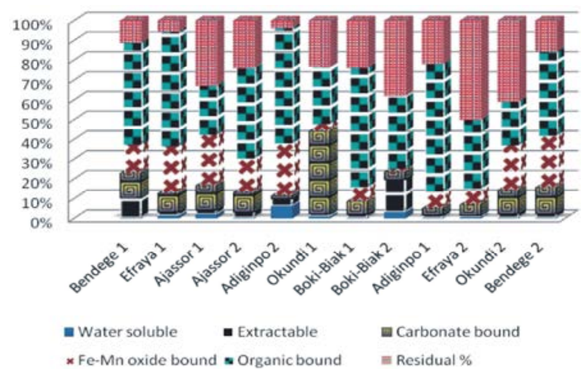

Fig. 1: Percent of copper in various fractions.

Bendege 2 had no water soluble Cu within the limit of detection. Exchangeable Cu content of soils samples ranged from 0.86 to 19.28%. Soil from Efraya 2 had the least value while from Bendege 1 had the maximum value. Carbonate bound Cu in studied soils ranged from 3.68 to 49.72%. Soil obtained from Adiginpo had the least value while soil from Bendege had the highest carbonate bound Cu in the soils from Cross River State. Fe-Mn hydrous oxide bound Cu ranged from 1.79 to 27.30%. Soil from Boki-Biakwan 2 had the least value while soil from Efraya 1 had the highest Fe-Mn oxide bound Cu. Copper bound to organic fraction of the soil ranged from 22.14 – 64.47%. Soil from Okundi 2 had the minimum while soil from Adiginpo 1 had the maximum value. Copper bound to residual fraction of the soil ranged from 3.81 to 50.02%. Soil obtained from Okundi 1 had the least value while soil from Okundi 2 had the highest value. Meanwhile, only 2% of the total Cu on average was resident in water soluble fraction while 2.24%, 9.01%, 15.00%, 43% and 29% was associated with the exchangeable, carbonate, Fe-Mn oxide, organic and residual fractions respectively.

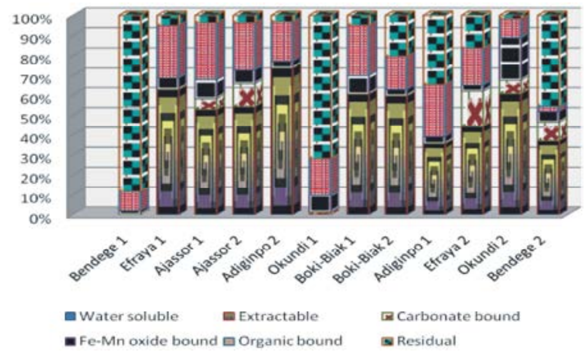

Fig. 2: Percent of lead in different pools.

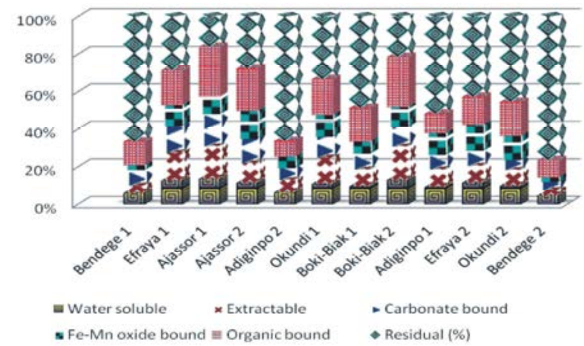

Fig. 4: Percent of cadmium in different pools.

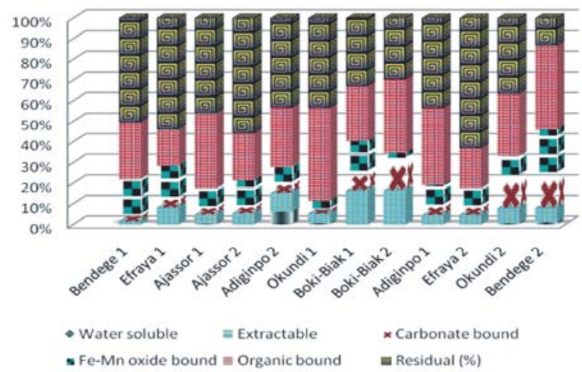

Fig. 3: Percent of zinc in different pools.

Figure 2 showed the result of chemical speciation of lead (Pb) in the studied soil. Lead concentration in the water soluble was below detection limit in all the samples. The exchangeable Pb ranged from 30.85 to 73.79%. Soil from Bendege 2 had the least value while soil sample from Okundi 2 had the highest value. Carbonate bound Pb in the samples ranged from 1.39 to 12.44%. Soil from Ajassor 1 had the minimum value while sample from Efraya 2 had the maximum value. Fe-Mn oxide bound Pb in the soil samples ranged from 1.96 to 21.37%. Soil obtained from Bendege 1 had the least value while soil from Okundi 2 had the highest value. Lead (Pb) associated with the organic fraction ranged from 2.75 to 28.41%. Soil from Bendege 2 had the least value while soil from Adiginpo 1 had the highest value. Lead (Pb) associated with the residual fraction ranged from 1.68 to 88.24%. Soil sample from Okundi 2 had the least value while soil sample obtained from Okundi 1 had the highest Pb content. The chemical speciation of Zinc in soils from selected cocoa plantations in Cross River State is presented in Figure 3. Concentration of water soluble Zn ranged from 0.40 to 6.16%. Soil from Okundi 2 had the least value while soil from Adiginpo 2 had the highest. However, soil sample from Bendege 1, Efraya 1, Ajassor 2, Boki–Biakwan 1 and 2, Adiginpo 1 and Efraya 2 had Zn concentration below detection limit. Zinc bound to the exchangeable fraction ranged from 1.49 to 16.18%. Soil from Bendege 2 had the least Zn value while soil from Efraya 1 had the highest value. Zinc associated with the carbonate fraction ranged from 2.70 to 17.38%. Soil sample from Okundi 1 had the minimum Zn content while the maximum was in soil from Bendege 2. Zinc associated with Fe-Mn oxides ranged from 2.17 to 20.69%. Soil from Boki –Biakwan 2 had the least value of Zn while soil from Bendege 1 had the highest value. Zinc associated with organic fraction ranged from 17.50 to 45.13%. Soil obtained from Boki-Biakwan 2 had the least value while soil from Adiginpo 1 had the highest value. Zn bound to the residual fraction ranged from 13.13 to 63.22%. Soil obtained From Boki- Biakwan 2 had the least value while from Efraya 2 had the highest value. An average of 1.60% of the total soil zinc was associated with the water soluble fraction while 6.01%, 6.54%, 10.91%, 30.22% and 45.61% was associated with the exchangeable, carbonate, Fe-Mn oxide, organic and residual fractions respectively.

Chemical speciation of cadmium in the studied soils is presented in Figure 4. Water soluble cadmium ranged from 4.68 to 13.58%. Soil from Bendege 2 had the least value of Cd while soil from Adiginpo 2 had the highest value. Cadmium associated with the exchangeable fraction ranged from 2.94 to 17.92%. Soil sample from Bendege 2 had the least value while sample from Ajassor 1 had the highest value. Carbonate bound Cd ranged from 4.37 to 17.17%. Sample from Okundi 2 had the least value while the highest value was from Ajassor 1. Cadmium associated with Fe-Mn oxide ranged from 2.46 to 13.94%. Soil obtained from Bendege 1 had the least value while soil from Adiginpo 1, had the highest Cd value associated with Fe-Mn oxide fraction. Cadmium bound to organic fraction of the soil ranged from 8.26 to 26.42%. Soil from Adiginpo 1, had the least value while soil from Ajassor 1,

Table 2: Mobility factors of the various metals in the studied soils.

Soil sample	Cu	Pb %	Zn	Cd
Bendege 1	22.96±2.31	10.00±2.32	4.99±0.34	18.33±1.34
Efraya 1	12.77±154	62.93±3.92	13.45±1.82	41.67±2.16
Ajassor 1	16.62±1.83	58.38±2.32	8.94±0.42	48.11±2.06
Ajassor 2	13.12±1.93	72.92±3.74	9.30±0.88	34.82±1.03
Adiginpo 2	11.46±1.73	73.79±3.71	20.67±0.93	19.31±2.02
Okundi 1	22.20±0.34	1.39±0.21	7.86±0.36	36.00±2.34
Boki-Biakwan1	8.36±0.72	69.69±2.84	25.98±1.77	27.07±1.83
Boki-Biakwan 2	9.74±0.39	67.42±2.37	32.36±1.32	41.25±2.45
Adiginpo1	4.63±0.82	35.65±2.11	9.69±0.99	26.31±1.43
Efraya 2	6.98±0.53	73.50±3.02	9.22±0.54	28.48±2.03
Okundi 2	14.20±1.16	67.57±2.84	24.32±1.24	23.62±1.93
Bendege2	16.25±1.24	47.64±2.67	25.33±1.21	11.98±1.04
Mean	13.27	57.35	16.01	29.74
STD	5.63	22.04	9.14	10.82

Table 3: Correlation between fractions of heavy metals (Cu and Pb) and physicochemical properties of the studied soils.

	Copper						Lead				
	Wat.	Exc	Carb	Fe/Mn	Org.	Res.	Exc	Carb	Fe/Mn	Org.	Res.
Mn	-.52*	.23	.7**	.44	-.43	-.03	-.22	.46	.27	-.10	.24
Fe	-.23	-.15	.26	.06	-.34	-.25	-.12	-.10	.30	.24	-.20
Zn	.29	-.36	.33	-.13	-.28	.60*	.28	.22	.53*	.18	-.18
CEC	-.10	.53*	.09	-.08	.33	.12	-.45	.18	-.37	-.29	.7**
pH	.15	-.11	-.51	.09	.12	-.05	.35	-.11	.05	.28	.44
C	.25	.11	.04	.16	.54	-.01	.47	.17	.09	.26	-.49
Sand	-.41	.69*	-.01	.14	.31	-.47	-.43	-.26	-.39	-.36	.39
Silt	-.12	.04	-.55*	-.26	-.27	-.7**	.01	-.7**	.18	.08	-.44
Clay	.39	-.6*	.24	-.01	-.14	.69*	.36	.52*	.25	.27	-.14
Wat.	1.0	.25	-.32	-.33	-.02	.22	1.0	.31	.42	.17	-.8**
Exch	-.25	1.0	.13	-.02	-.21	-.35	.31	1.0	.20	-.05	.25
Carbona.	-.32	.13	1.0	.55*	-.16	.20	.42	.20	1.0	-.11	-.34
Fe-Mn	-.33	-.02	.55*	1.0	-.04	-.24	.17	-.05	-.11	1.0	-.17
Organic	-.02	-.21	-.16	-.04	1.0	24	-.8**	.25	-.34	-.17	1.0
Residual	.22	-.35	.20	-.24	.24	1.0	-.22	.46	.27	-.10	.24

Table 4: Correlation between fractions of heavy metals (Cd and Zn) and physicochemical properties of the studied soils.

	Cadmium						Zinc					
	Wat.	Exc	Carb	Fe/mn	Org.	Res.	Wat.	Exc	Carb	Fe/Mn	Org.	Res.
Mn	-.47	-.27	.06	-.18	.08	.38	.16	.12	-.15	.42	.42	.05
Fe	.05	-.29	.44	-.06	.18	-.32	-.01	.28	-.41	.42	-.24	.19
Zn	-.12	.20	.10	.21	.33	.10	.42	.03	-.55*	-.22	.15	-.09
CEC	.17	-.16	.30	-.21	-.16	-.30	-.14	-.14	.33	-.02	.21	-.10
pH	-.59*	.01	.22	.22	-.05	.57	.25	.20	-.07	-.43	.04	.27
C	.39	.02	.36	-.13	.59*	-.27	.02	.51*	-.17	-.41	-.50	-.14
Sand	-.27	.13	.16	-.22	.63*	-.58*	-.27	.09	.54*	.33	.25	.13
Silt	.13	-.02	-.31	.02	-.8*	-.36	.05	.15	-.19	.12	-.45	.32
Clay	.51	-.28	.08	.51	-.12	.81	.20	-.14	-.37	-.33	-.02	-.25
Wat.	1.0	-.24	.36	.59*	.13	-.51	1.0	.22	-.02	-.22	.02	-.18
Exch	-.24	1.0	-.26	-.42	-.13	.56	.22	1.0	.06	-.11	-.34	-.05
Carbona.	.36	-.26	1.0	.41	.72*	-.33	-.02	.06	1.0	.35	.04	-.39
Fe-Mn	.59*	-.42	.41	1.0	.41	-.68*	-.22	-.11	.35	1.0	.23	.41
Organic	.13	-.13	.72*	.41	1.0	-.05	.02	-.34	.04	.23	1.0	.37
Residual	-.51	.56	-.33	-.68	-.05	1.0	-.18	-.05	-.39	.41	.37	1.0

had the highest value. Cadmium bound to the residual fraction ranged from 16.04 to 76.51%. Soil sample from Ajassor 1 had the least value while soil from Bendege 2 had the highest value. An average of 8.97% of the total soil cadmium was associated with the water soluble fraction while, 9.03, 8.52, 8.38, 15 and 50% of the total cadmium was associated with exchangeable, carbonate, Fe -Mn oxide, organic and residual fraction respectively.

Table 2 presents the mobility factor of heavy metals in soils collected from selected cocoa plantations in Cross River State. Mobility index of copper ranged from 4.63 to 22.96% with an average factor of 13.27%. Soil sample from Adiginpo 1 had the least Cu factor while soil from Bendege 1 had the highest Cu factor. Mobility factor of Pb ranged from 1.39 to 73.79% with an average factor of 57.35%. Soil from Okundi 1 had the minimum factor while soil from Adiginpo 2 had the highest factor. Zinc mobility factor in all the studied soils ranged from 4.99 to 32.36% with a mean factor of 16.01%. Soil from Bendege 1 had the minimum Zn factor while soil from Boki-Biakwan 2 had the highest factor. Cadmium mobility factor ranged between 11.98% and 48.11% with an average factor of 29.74%. Soil sample obtained from Bendege 2 had the least factor while soil from Ajassor 1 had the highest factor. Table 3 showed that, exchangeable Pb had negative correlation with residual Pb at significant level (-0.80; $P < 0.01$). Carbonate bound Pb had negative correlation with silt at significant level (-0.70; $P < 0.01$) and positive correlation with soil clay at significant level (0.52; $P < 0.05$). Lead associated with Fe-Mn oxide had positive correlation with soil Zn at significant level (0.53; $P < 0.05$). Residual lead had positive correlation with cation exchange capacity at significant level (0.70; $P < 0.05$) and negative correlation with exchangeable lead at significant level (-0.80; $P < 0.01$). The correlation coefficients of copper speciation and physicochemical properties of the studied soils are presented in Table 3. Copper bound to water soluble fraction had a negative correlation with soil manganese at significant level (-0.52; $P < 0.05$). Exchangeable Cu had positive correlation with CEC (0.53; $P < 0.05$) and sand (0.69; $P < 0.05$) at significant level but negatively correlated with soil clay at significant level (0.60; $P < 0.05$). Carbonate bound Cu had positive correlation with Mn at significant level (0.66; $P < 0.01$). Fe-Mn oxide bound Cu positively correlated with carbonate bound Cu at significant level (0.55; $P < 0.05$). Organic fraction Cu had negative correlation with most of the soil physicochemical parameters except soil pH, CEC, soil organic carbon and sand content of soil. Residual fraction Cu had significant positive correlation with soil Zn (0.60; $P < 0.05$) and soil clay content (0.69; $P < 0.05$). It however, had negative correlation with silt at significant level (-0.70; $P < 0.05$).

Table 4 showed that, exchangeable Zn had significant negative correlation ($P < 0.05$) with soil extractable Zn in soils obtained from Cross River State. Carbonate Zn and extractable Zn had a significant negative correlation with sand content of the soils Data in Table 4 show that water soluble Cd had a significant negative correlation with soil pH (-0.59; $P < 0.05$) and a positive correlation with Cd bound to Fe-Mn oxide. Cadmium associated with the carbonate fraction had a positive correlation with Cd bound to organic fraction at significant level. Fe-Mn bound Cd also had significant correlation with water soluble Cd. Organically bound Cd had significant positive correlation with organic carbon and sand.

DISCUSSION

Copper was predominantly associated with the organic fraction. This might be due to the fact that, metal ions form complex with organic matter thereby, reducing their bioavailability in soil, plants and the terrestrial ecosystem. Cu is often bound to the organic fraction in the soil and soil organic matter can be the most important soil factor in determining copper bioavailability [14]. Pampura *et al*. [12] reported a range of 37 to 91% of the total soil Cu present in organic fraction. Reddy *et al*. [13] found that, the proportion of Cu bound to organic matter in the soil solution increased from 37 to 95% as the pH decreased. Result obtained from this study is in agreement with the report of Harrison *et al*. [14] who found significant amount of Cu in road side soils associated with the organic fraction. Ma and Rao also reported that significant amount of Cu in nine contaminated soils having strong association with organic fraction. Lead was predominantly associated with the exchangeable fraction in most of the studied soils. This pattern is at variance with the report of Kabata-Pendias and Pendias [15] who reported predominance association of Pb with Fe -Mn Oxide. The predominant association of Zn with the residual fraction in soils obtained from Cross River State (45%) may be due to the high clay content of the soils which gave room for chemisorptions of Zn unto the clay edges in the inner lattice of the soil minerals. This is in agreement with the report of Mohamed who found zinc mostly associated with the residual fraction. Aydinalp [16] also reported that, zinc was mainly associated with the residual fraction in cultivated soils in Turkey. The predominant association of Cd to the residual fraction is an indication of its stability in the studied soil which is good for quality of cocoa from this area. Mobility of metals may be assessed on the basis of absolute and relative content of fractions weakly bound to soil components. The mobility factor was calculated according to Salbu *et al*. [17] and Okunola *et al*. [18]. Some of the studied soils from Cross River State had Cu mobility factor below 10% while others had mobility factor higher than 10%. According to Kabala and Singh, mobility factor not higher than 10% is a symptom of high stability of metal in soil. Sample from Adiginpo 1 (4.63%), Efraya 2 (6.98%), Boki-Biakwan 1 (8.36%) and Boki-Biakwan 2 (9.74%) had mobility factor less than 10% for Cu. This is an indication of relative stability of Cu in the cocoa plantations where the soil samples were collected. The low Cu mobility factor in these soils implies that, copper bioavailability, contamination and eco-toxicity will be lower in cocoa plantations in Adiginpo 1, Efraya 2, Boki-Biakwan 1 and 2 compared to the rest soils that had mobility factor higher than 10%. Yusuf [19] also reported mobility factor less than 10% for Cu in his study on heavy metal speciation of soils from dumping sites in Ojota, Lagos. About 83.33% of the total number of cocoa plantations assessed in Cross River State had Pb in the mobile state which implies that, Pb mobility may be very high in the studied soils. This is an indication of anthropogenic source. The average mobility factor of Pb in the studied soils (57.35%) is higher than the value reported for Pb (21.72%) by Yusuf. Olumatosin *et al.* [20] stated that mobility factor of 5.6% for Pb in valley bottom soils of some urban cities in south-western Nigeria. The low range of Zn mobility factor obtained in the studied plantations may be due to the fact that, Zn is not a component of farmers' inputs in cocoa plantations. Mobility factor of cadmium in the studies soils was least in soil from Bendege 2 while the highest value was obtained in soil from Ajassor 1. The range of Cd mobility factor in the investigated soils from Cross River State (11.98-48.11%) was lower than the range of Cd (10 - 48%) reported by Yusuf in soils of waste disposal sites in Ojota, Lagos. Adaikpoh [21] reported a range of 26.14 – 54.36% for Cd mobility factor in soil samples collected from Umutu oil field in Niger Delta, Nigeria. One possible reason why Cd mobility factor was found low in the studied plantations is the fact that, cocoa farmers in Nigeria do not use agricultural inputs that contain Cd.

CONCLUSION

The use of total metal content of a soil in contamination characterization is a useful parameter. However, the speciation of heavy metals with selective extractants gives additional information on the fundamental reactions controlling the behavior of metals in soils. Fractionation of heavy metals in soils also gives more information on which of the various fractions is bio-available for plant uptake. Copper and zinc were predominantly associated with the organic fraction while lead was largely associated with the extractable and Cd was mostly associated with the residual fraction. Out of the four heavy metals considered in this study, Pb appeared the most potentially bioavailable indicating its possibility of entering the food chain.

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