Immobilization and Phytoavailability of As, Cr and Cu in CCA Contaminated Soil, Effect of Poultry Manure Addition

D. O. Emwanta and F. E. Okieimen*
University of Benin, GeoEnvironmental & Climate Change Adaptation Research Centre, Benin City

ABSTRACT

The objectives of this study were to evaluate the ability of poultry manure to reduce the levels of soluble, bioavailable and mobile metals forms of As, Cr and Cu in contaminated soil and thus the transferability of the metal to okra (Abelmoschus esculentus) plant, and the long term stability of poultry manure-treated soil. The contaminated soil sample (As 19.80±1.90 mg.kg^{-1}*, Cr 190.00±2.10 mg.kg*$^{-1}$ *and Cu 108±1.00 mg.kg*$^{-1}$*) was treated with various amounts (0, 5, 10, 20 and 30wt%) of poultry manure (pH 6.81 ±0.04, organic matter 5.99 ± 0.11%, P 1522.00 ±4.30 mg.kg*$^{-1}$ *and N 0.40 ± 0.10%), re-wetted to 60% of its water holding capacity and incubated at ambient temperature (28-31*^{o}*C) for 2 weeks. Single extractions of test soil samples with deionised water and 0.01M* $CaCl_2$ *solution and sequential extractions following the BCR protocol of the test soil samples revealed reductions of up to 40% and about 60% in the bioavailable and water-soluble metal fractions respectively and in the mobile metal pools. The level of metal uptake by okra decreased with increase in the level of poultry manure application and varied in the order Cu > Cr > As. The transfer coefficient of the metals from the soil to okra plant varied from 0.14 to 0.06 for As, 0.20 to 0.007 for Cr and 0.06 to 0.04 for Cu in unamended and poultry manure amended soil samples respectively, while the translocation factor was about the same order of magnitude; Cr (50%) and Cu (30%) in unamended and poultry manure amended soils, but varied from 54% in unamended soil to 85% in poultry manure amended soil for As. Post-harvest acid treatment of the test soil samples did not reveal marked release of the metal fraction fixed in the soil matrix following poultry manure addition to the contaminated soil*

INTRODUCTION

The contamination of soils due to the presence of toxic metals can result in negative consequences, such as the loss of ecosystem and of agricultural productivity, the deterioration of the food, human and animal health problems, etc[1]. Unlike organic contaminants, most metals in soil environment do not undergo microbial or chemical degradation and therefore the total; concentrations and excotoxicity of metals persist in soils for a long time after their introduction to soil.

Currently, several technologies can be employed to clean up the soils contaminated by toxic metals, including thermal, biological and physical/chemical procedures or their appropriate combinations. These techniques are generally expensive, can be environmentally invasive, can be deleterious to normal soil properties and may produce secondary wastes[2,3]. Recently, more attention has been focused on the development of *in situ* immobilization methods of metals in soils which are considered to be generally less expensive and less-disruptive to the natural landscape, the hydrological conditions and the ecosystem than the conventional soil remediation techniques. The *in situ* immobilization of metals in soils using inexpensive amendments, such as minerals (apatite, zeolite or clay) or waste by-products (steel shot, berringite, iron-rich biosolids) is considered a promising alternative to the currently available remediation methods[4,5]. The main goal of *in situ* remediation technique is to reduce the fraction of toxic metals or compounds which are potentially mobile and bioavailable.

In this paper the effect of poultry manure application on the soluble, bioavailable and mobile fractions in chromated-copper-arsenate (CCA) contaminated soil and on metal uptake by okra (*Abelmoschus esculentus*) was examined together with the long-term stability of the poultry manure treated soils under acidic stress conditions. Previous studies[6,7] revealed that the soil is moderately-to-highly contaminated with respect to As, Cr And Cu.

EXPERIMENTAL

CCA contaminated soil samples were collected from ten locations in a wood treatment site in Benin City. The samples were pooled, air-dried and sieved through a 2mm screen. The physicochemical properties and pseudo-total levels of As, Cr and Cu in the soil sample are given in Table 1. Poultry manure was collected from a livestock farm in Kwale, Delta State, Nigeria. The physico-chemical properties of the poultry manure used in this study are given in Table 2.

Table 1: Physicochemical characteristics of CCA contaminated soil

Parameters	CCA contaminated soil
pH	5.60±0.03
Clay (%)	18.90±1.30
Silt (%)	1.60±0.40
Sand (%)	79.50±0.30
Carbon (%)	0.56±0.10
Organic matter (%)	0.55±0.04
Nitrogen (%)	0.97±0.04
Sodium (meq/100g)	0.10±0.03
Potassium (meq/100g)	0.40±0.00
Magnesium (meq/100g)	9.40±0.20
Calcium (meq/100g)	14.80±0.90
CEC (meq/100g)	24.70±0.50
Phosphorus (mg.kg^{-1})	38.70±1.10
As (mg.kg^{-1})	19.80±1.90
Cr (mg.kg^{-1})	190.00±2.10
Cu (mg.kg^{-1})	108.30±1.00

Application of poultry manure to the contaminated soil:

The poultry manure was added to the soil (three replicates) at 0, 5, 10 and 20wt% based on the dry weight of the soil. The amendment was thoroughly mixed with the soil sample (1kg) in 2L plastic bowls and rewetted to 60% of the water holding capacity of the soil. The bowls were kept at ambient temperature (28-31°C) for two weeks and mixed at frequent intervals.

Determination of soluble, bioavailable and mobile metal fractions in the test soil samples:

The water soluble As, Cr and Cu fractions in the unamended and poultry manure treated soil samples were determined as described previously[8]. A known amount of the soil was agitated in distilled/deionised water at soil:water ratio of 1:5 for 6h and then centrifuged at 1500rpm. The amounts of the metals in the supernatant determined by AAS (Buck Scientific VGF model 210A) are reported as the water soluble metal fractions.

The bioavailable fractions of As, Cr and Cu in the test soil samples were determined following the procedure described by Oliver *et al* [9]. In a typical experiment, a known amount of the test sample was mixed with 0.01M $CaCl_2$ solution in a plastic bottle, agitated for 4h and then centrifuged at 1500rpm. The amounts of As, Cr and Cu in the supernatant determined by AAS are reported as the bioavailable metal fractions.

Table 2: Physicochemical properties of poultry manure

Parameters	Poultry manure
pH	6.81±0.04
Organic matter (%)	5.99±0.11
Carbon exchange capacity (meq/100g)	8.90±0.40
Ca^{2+}	1.20±0.10
Mg^{2+} ($mg.kg^{-1}$)	0.60±0.10
N (%)	0.40±0.10
P ($mg.kg^{-1}$)	1522.0±4.30
K ($mg.kg^{-1}$)	0.44±0.10
As ($mg.kg^{-1}$)	0.60±0.10
Cr ($mg.kg^{-1}$)	0.20±0.01
Cu ($mg.kg^{-1}$)	1.70±0.30

Sequential extractions proposed by the European Communities Bureau of Reference (modified BCR Method)[10] performed on 1-g portions of the test soil samples allowed the distribution of As, Cr and Cu among operationally defined pools: exchangeable and associated with carbonates (B_1), associated with easily and moderately reducible Fe- and Mn-oxides (B_2) associated with organic matter (B_3) and residual fraction (R) to be determined. The sequential extraction scheme is given in Table 3 together with the operational conditions. The extractable (B_1) metal pool represents the mobile metal fraction in the testy soil samples

Plant uptake studies:

Pot experiments similar to those described by Nolan *et al.*[11] with modifications[12] were used to assess the effect of poultry manure application on the uptake of As, Cr and Cu by okra plant from the test soil samples. Four viable seeds of okra were sown in each pot at a depth of about 0.5cm. No nutrients were added. The pots were watered on alternate days and the okra plant were harvested 30days after germination by cutting the shoots 0.5cm above the soil surface and carefully removing the roots from the soil. The roots and shoots were rinsed thoroughly with deionised water, dried at 70°C until constant weight and then ground. Subsamples of the ground shoots (200mg) and roots (100mg) were digested in a mixture of concentrated HNO_3 and $HClO_4$ acids (4:1 by volume) and the As, Cr and Cu in the digestate solutions were determined by AAS. Reagent blank and analytical replicates were used to ensure accuracy and precision of analysis. The data reported here are average values of triplicate determinations.

Table 3: BCR sequential extraction procedure used for heavy metal fractionation

Step	Metal pools	Extractant	Agitation time
B_1	Extractable	40mL of 0.11M CH_3COOH	16h at room temperature
B_2	Reducible	40mL of 0.5M $NH_2OH.HCl$ (pH 2)	16h at room temperature
B_3	Organic-bound	10mL of 8.8M H_2O_2	1h at room temperature then 1h at 85°C
		Cool + 50mL of 1M CH_3COONH_4 (pH 2)	16h at room temperature
R	Residual	Aqua regia digestion (21mL concentration HCl + 7mL concentration HNO_3)	16h at 180°C

Post-harvest stability of poultry manure treated soil samples:

Test soil samples were trerated with 0.001M HCl solution at soil:acid solution ratio of 1:2 and then air-dried. Chemical fractionation using the BCR sequential extraction procedure as described previously was used to determined the mobile metal pools in the acid-treated test soil samples.

RESULTS AND DISCUSSION

Effect of poultry manure application available metal fractions in contaminated soil

The results of the effect of poultry manure application on the water soluble and bioavailable metal fractions in the test soil samples are given in Table 4. the results show that the application of poultry manure was accompanied by appreciable decrease 28% in As, 29% in Cr and 39% in Cu in the bioavailable forms of the metals in the contaminated soil and corresponding decrease in water-soluble metals fractions; 30% in As, 34% in Cr and 58% in Cu. Soil heavy metal environmental risk is related to the bioavailable forms of the metals. Chemical alterations of soil heavy metals to less soluble and less bioavailable forms correspond to pollution abatement/amelioration and an output of soil remediation.

Contaminant mobility is increasingly being used as key indicator of their potential risk to environmental receptors. The mobility of metals in soil assessed on the basis of absolute or relative content of the fraction that is most weakly bound to the soil components and correspond, to the exchangeable (B_1 fraction) metal pools from the BCR sequential extraction procedure. The distribution pattern of As, Cr and Cu in the poultry manure amended soils is shown in Fig. 1. Many limitations surround sequential fractionations since they are semi-quantitative and not completely selective, open to re-distribution and only operationally defined. They are however considered the best available method for gaining knowledge on the forms in which metals are present in soil[13]. The fractionation of the test soil samples revealed (Table 5) that the metals in the residual fraction (As 31.55%, Cr 29.30% and Cu 31.75%) and in the exchangeable forms (As 28.88%, Cr 27.02% and Cu 29.68%) and lowest in the reducible (Fe- Mn-oxide bound) forms (As 17.65%, Cr 20.02% and Cu 16.47%).

Table 4: Effect of poultry manure application on water-soluble and bioavailable fraction of As, Cr and Cu in contaminated soil

Level of poultry manure application (%)	Soluble metal forms (mg.kg^{-1})	Available metal forms (mg.kg^{-1})
0	3.70±0.40; 33.60±0.90^a (18.40±0.30)	5.30±0.10; 42.10±1.00^a (32.00±1.00)
5	3.40±0.40; 31.10±0.70^a (14.40±0.80)	5.00±0.40; 40.40±1.40^a (30.70±1.00)
10	3.10±0.10; 29.60±0.90^a (12.70±0.10)	4.60±0.20; 36.60±1.20^a (26.80±1.00)
20	3.00±0.06; 25.30±0.80^a (10.80±0.70)	4.10±0.10; 33.40±1.00^a (22.10±1.10)
30	2.60±0.08; 22.20±0.10^a (7.80±0.90)	3.80±0.09; 29.80±1.30^a (19.60±1.00)

a = values for Cr
values in parentheses are for Cu

The metal fraction of direct environmental concern is the exchangeable fraction by which metal mobility index is defined. The results in Table 5 show appreciable reductions in the mobile metal fractions following the addition of poultry manure to the contaminated soil and corresponding increases in the amounts present in the organic bound (oxidisable) and residual forms. The organic matter and phosphorus contents of organic amendments are generally considered to be responsible for transforming the more labile metal pools into the more recalcitrant forms. There appears to be no consensus in published data on the data on the effect of organic matter in the immobilization of As in contaminated soil. Soluble organic matter is reportedly capable of competing with As for sorption sites and may displace both As(V) and As(III) from iron oxides[14]. On the other hand compost derived organic matter has shown to reduce metal accumulation by vegetables grown on CCA contaminated soil[15]. Organic matter can also alter As speciation by reducing As(V) to the more toxic and mobile As(III) and thereby increase risk to human and environmental receptors[16]. The mobility of Cr in soil depends on its oxidation state, the more toxic and mobile Cr(III) being less stable in natural environments.

Table 5: Distribution pattern of As, Cr and Cu in poultry manure amended contaminated soil.

Fractions	**Levels of poultry manure application (wt%)**				
	0	**5**	**10**	**20**	**30**
Exchangeable (%) (B_1)					
As	28.88	27.66	20.83	23.12	20.95
Cr	27.02	23.87	22.21	20.86	19.47
Cu	29.68	27.97	25.82	23.71	21.11
Reducible, (B_2) Fe- Mn oxides- bound (%)					
As	17.65	17.02	16.67	17.58	17.91
Cr	20.02	19.79	20.42	20.59	20.78
Cu	16.47	15.71	16.03	16.62	18.05
Oxidisable (B_3) Organic-Bound (%)					
As	21.93	24.47	25.64	26.13	27.36
Cr	26.37	26.43	27.05	27.72	27.66
Cu	22.09	21.93	22.74	23.23	23.30
Residual (%)					
As	31.55	30.85	31.77	33.16	33.33
Cr	29.30	29.91	30.31	30.83	32.08
Cu	31.75	34.39	35.41	36.44	37.54

In the presence of organic matter and divalent iron, (Cr(VI) is readily converted to the oxide of Cr(III) or co-precipitated with iron hydrous oxide that have low mobility and bioavailability in soils[17]. Stability of copper is pH dependent, with mobility increasing with decrease in pH. Carbonates, phosphates and clay can keep Cu mobility in soil low by chemisorption[18]. The well-known affinity of Cu for organic matter has two effects; increased Cu mobility with soluble organic matter and reduced solubility with high molecular weight organic acids[19,20]. The phosphorus contents of the organic amendments are relatively high: 1522.0±4.30mg.kg^{-1} and 5.99±0.11% in poultry manure. Phosphate-based materials are well known metal fixing agents in soil through adsorption by phosphate, phosphate anion induced adsorption and precipitation of metals as phosphates. It would seem from these results that organic matter and phosphorus contents of the poultry manure would account for immobilization of metals in the contaminated soil and would provide plausible explanation for the observed effect of organic amendments on the water-soluble, bioavailable and mobile pools of As, Cr and Cu in the contaminated soil.

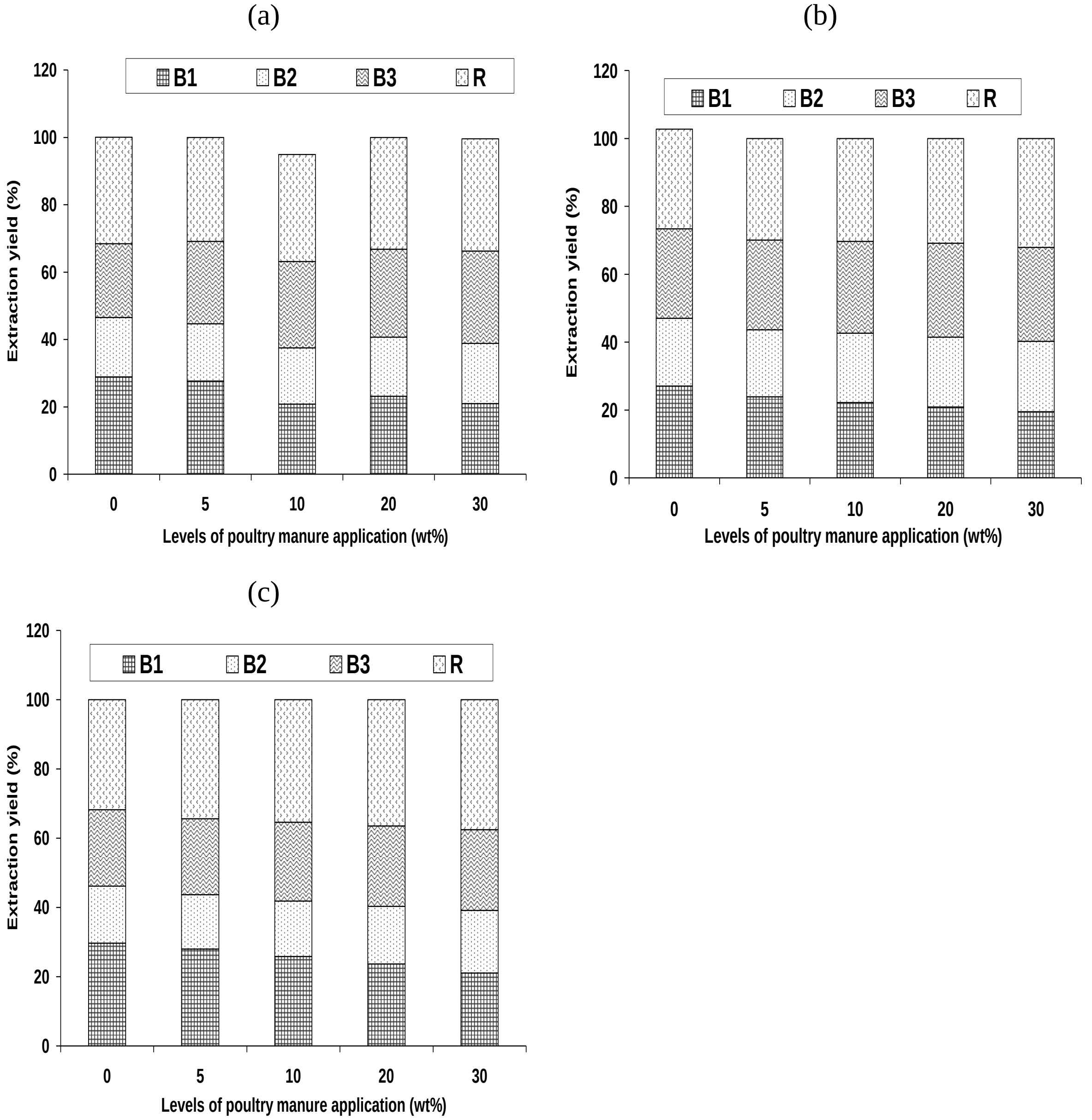
(a) (b) (c)

Figure 1: Distribution pattern of (a) As, (b) Cr and (c) Cu in poultry manure amended contaminated soil.

Effect of organic amendments on metal uptake by okra (*A. esculentus*) plant:

The analysis of the results of metals uptake by okra plant grown on poultry manure amended CCA contaminated soil is given in Table 6. The results show that metal uptake was in the order Cu > Cr > As, and that poultry manure application appreciably reduced metal uptake level by okra. For instance at 30wt% poultry manure application, levels of metal uptake by okra plant was reduced by 57% for As, 66% for Cr and 32% for Cu.

Generally, the concentrations of metals in the shoots were lower than in roots. In this study, the levels of metals in okra shoots were about 2-3 fold lower than in the roots. The transport of metals from the roots to shoots involves long distance translocation in the xylem and storage in the vacuole of the leaf cells, processes that are affected by factors such as the concentration of available metals in soil solubility sequences and nature of the plant species[21]. Transfer coefficient (TC) and translocation factor (TF) are indices that relate to metal transferability to plants in contaminated soil. Metal transfer coefficients, given as the ratio of metal concentration in plant shoots to the pseudo total concentration in soil for As, Cr and Cu in the test soil samples are given in Fig. 2(a), while the translocation factors, the fraction of the metals in the roots that is taken up in the shoots are shown in Fig 2(b).

The results show that poultry manure application to the contaminated soil is accompanied by reductions of an order of magnitude in the values of TC. Reductions in bioavailable metal forms and in metal transferability will ameliorate metal phytotoxicity and encourage re-vegetation of contaminated site. The results in Fig 2(B) show that the fraction of the metals in the plant roots that is taken up in the shoots was about the same order of magnitude Cr about 50% and Cu (about 30%) in unamended and poultry manure amended soils, but varied from 54% in unamended soil to 85% in poultry manure amended soil for As. These relatively high levels of metal transfer from the roots zone preclude metal-induced root damage and the resulting phytotoxic plant growth inhibition[22].

Table 6: Effect of poultry manure application of metal uptake by okra plant in CCA contaminated soil

Level of amendment application (%)	**Level of metal uptake (mg.kg^{-1} dw)**					
	Shoots			**Roots**		
	As	**Cr**	**Cu**	**As**	**Cr**	**Cu**
0	2.80±0.20	3.90±0.04	6.90±0.80	5.20±1.00	7.70±1.20	22.40±3.00
5	2.40±0.18	3.6±0.90	5.60±0.40	3.70±0.04	5.90±1.10	19.60±1.40
10	1.80±0.10	2.30±0.40	5.20±1.00	2.80±0.60	3.70±0.50	16.30±1.00
20	1.20±0.40	1.30±0.20	4.70±0.40	1.40±0.70	2.60±0.90	12.80±0.90

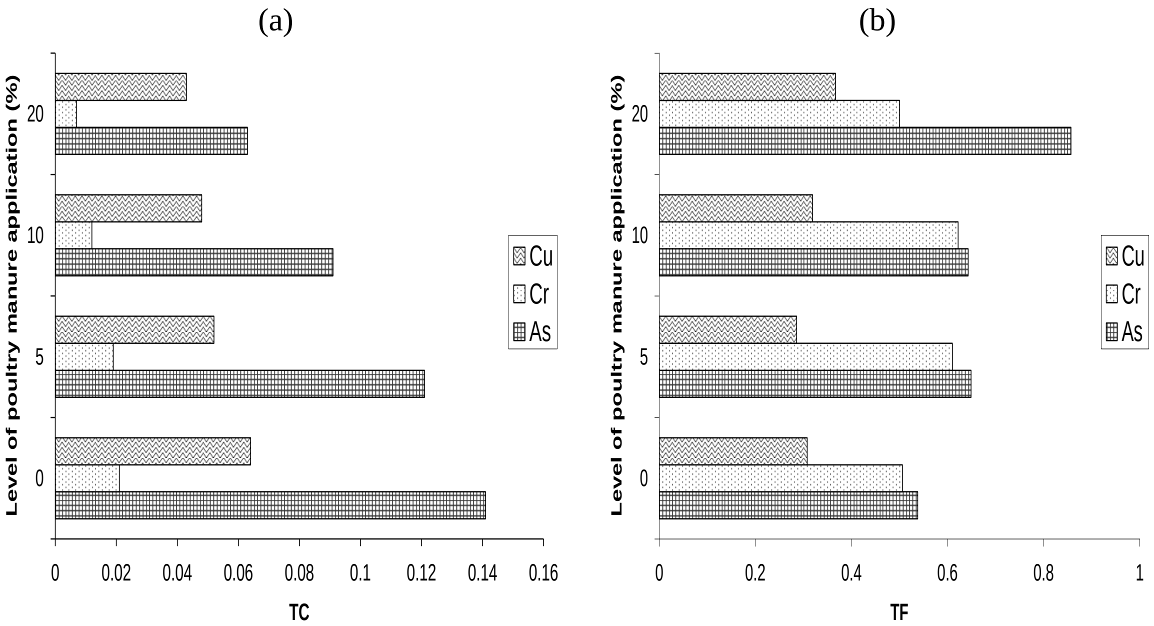

Fig. 2: Effect of poultry manure application on (a) transfer coefficient (TC) and (b) translocation factor (TF) in okra grown in CCA contaminated soil

Post-harvest stability of poultry-manure treated soil

Post-harvest stability test was carried out on the poultry manure treated soil to determine the effect of induced environmental acidification on the release (remobilization) of metals in the contaminated soil. It would appear from the results (Table 7) that apart for Cr for which the mobile fraction increased in the poultry manure amended soil relative to the unamended soil, As and Cu remained largely in the recalcitrant (oxidizable or organic matter bound and reducible or Fe-Mn-oxide bound) soil fractions, suggesting the retention of these metals in the 'fixed' state. It is also pertinent to note the apparent re-distribution of the metals that occurred following acid treatment of the soil; reduction in the residual fraction of Cu and Cr in the tests samples, and concomitant increase in oxidisable and reducible forms.

CONCLUSION

This paper examined the effectiveness of the poultry manure as a metal fixing additive for the remediation of soil contaminated with As, Cr and Cu. Although chemical alterations of metals in multi-element contaminated soil present challenges both in terms of the kinetics and dynamics of metal interactions multiple reactions sites in the soil matrix, the results from this study indicate the potential for the application of poultry manure in reducing bioavailable and toxicity to plants of metal pollutants in contaminated soils.

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Table 7: Post-harvest distribution pattern of As, Cr and Cu in poultry manure amended contaminated soil after treatment with acid

Fractions	Levels of poultry manure application (wt%)				
	0	5	10	20	30
Exchangeable					
As	16.18	6.55	10.29	9.64	9.37
Cr	16.52	20.63	18.39	15.17	16.96
Cu	4.55	4.35	2.96	1.52	2.23
Reducible, (Fe- Mn oxides- bound)					
As	30.88	39.34	39.71	37.35	35.94
Cr	28.47	26.76	29.72	33.01	35.51
Cu	40.53	39.49	43.33	44.11	44.24
Oxidizable Organic acid bound					
As	27.94	29.50	30.88	32.53	29.69
Cr	34.97	34.01	38.21	40.87	41.79
Cu	43.18	46.01	45.93	49.05	50.93
Residual (%)					
As	25.00	24.59	19.17	20.48	25.00
Cr	20.04	18.59	13.68	10.95	5.41
Cu	11.74	10.14	7.78	5.32	2.60

REFERENCES

1. Raicevic, S., Kaludjerovic-Radoicic, T. and Zouboulis, A.I. J. *Hazard. Mater.*, **B117**, (2005) 41-53.

2. Dermont, G., Bergeron, M., Mercier, G. and Richer-Lafleche, M. *J. Hazard. Mater.*, **152**, (2008) 1-31.

3. Khan, F.I., Hussain, T. and Hejazi, R. J. *Environ. Manage.*, **71**, (2004) 95-122

4. Chen, T.C., Wright, J.V., Conca, J.L. and Peurrung, L.M. *Water Air Soil Pollut.*, **98**, (1997) 57-78.

5. Ma, Q.Y., Traina, S.J., Logan, T.J. and Ryan, J.A. *Environ. Sci. Technol.*, **28**, (1995) 1219-1228.

6. Uwumarongie, E.G. (2009) Assessment of heavy metal mobility in chromated copper arsenate (CCA) contaminated soil. PhD thesis, University of Benin, Benin City.

7. Okieimen, F.E. and Uwumarongie-Ilori, E.G. (2012) Distribution pattern of As, Cr and Cu in soil samples in the vicinity of wood treatment site in peri-urban area of Benin City. *Int. J. Chem. Sci.* (in press)

8. Tsetimi, G.O. and Okieimen, F.E. *J. Environ. Chem. Ecotox.*, **3(8)**, (2011) 214-224.

9. Oliver, D.P, Tiller, K.G., Alston, A.M., Naidu, R. and Cozens, G.D. *Aust. J. Soil Res.*, **37**, (1999) 1123-1138.

10. Tokalioglu, S., Kartal, S. and Gultekin, A. *Intern. J. Environ. Anal. Chem*., **88(6)**, (2006) 417-430.

11. Nolan, A.L., Zhang, H. and McLaughin, M.L. *J. Environ. Qual.,* **34(2)**, (2005) 496-507.

12. Wuana, R.A., Okieimen, F.E. and Imborungu, J.A. *Int. J. Environ. Sci. Techn*., **7**, (2010) 485-496.

13. Templeton, D.M., Ariese, F., Cornelis, R., Danielson, L.G., Muntau, H., van Leeuwen, H.P. and Lobinski, R. *Pure Appl. Chem*., **72(8)**, (2000) 1453-1470.

14. Redman R. S., Sheehan K. B., Stout R. G., Rodriguez R. J., Henson J. M. *Science* **298**, (2002) 1581.

15. Cao, R.X. and Ma, I.Q. *Environ. Pollut*., **132(3)**, (2004) 435-442.

16. Balasiou, C.F., Zasgury, G.J. and Deschen, I. *Sci. Total Environ*., **208(1-3**), (2001) 239-255.

17. Fendorf, S.E. *Geoderma*, **67**, (2005) 55-71.

18. Kumpiene, J., Lagerkvist, A. and Maurice, C. *Waste Manage*., **28**, (2008) 215-225.

19. Hsu, J.H. and Lo, S.I. *J. Environ. Qual*., **29(2)**, (2000) 447-453.

20. Kizilkaya, R. *Ecol. Engg*., **22(2)**, (2004) 141-151.

21. Yang, X.E., Baliger, V.C., Foster, J.C. and Martens, D.C. *Plant Soil*, **197** ,(1997) 271-276.

22. Heiss, S., Wachter, A., Bogs, J., Cobbett, C. and Rausch, T. *J. Exp. Bot*., **54**, (2003) 1833-1839.