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# Immobilization Effect of Cow Dung on Lead and Chromium in Soil Cultivated With Oil Palm

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## ABSTRACT

*Soils may become contaminated from the accumulation of heavy metals through application of fertilizer, animal manures, biosolids (sewage sludge), compost, pesticides, etc. These heavy metals and metalloids may accumulate at such levels that may pose risks to human and ecosystem health. In this study, the chemical speciation, plant uptake and remediation of Pb and Cr in a soil collected from NIFOR Experimental Field under various conditions of ex-situ experimental contamination by amendments with the metal nitrate salts and cow dung was evaluated. Physico-chemical analysis of the soil showed soil to be acidic with pH 5.20. The initial properties of the soil were 1.70 %C, 0.24 %N, 17.80 mg* $kg^{-1}$ *P, 0.60 cmol* $kg^{-1}$*K and 12.90 cmol* $kg^{-1}$ *CEC. Pb and Cr were not detected in the soil.*

*Fractionation studies conducted on soils amended by spiking directly with single metal nitrate salts and different amounts (2, 5, 10 and 20 %) of cow dung, before and after planting maize, showed that Pb and Cr were bound mostly to the residual fraction with higher amount of metals in the residual fraction after planting. After two months, 8.80, 5.80, 5.63, 5.59 and 5.46mg* $kg^{-1}$ *Pb and 6.40, 4.90, 4.78, 4.67 and 4.28 mg* $kg^{-1}$ *Cr were taken up by maize from 0 % (control soil), 2 %, 5 %, 10 % and 20 % amended soil respectively.*

***Keywords:*** *Immobilization, Cow dung, Uptake, Maize, Lead, Chromium*

## INTRODUCTION

Soil is an important component of the environment [1]. It constitutes a dynamic and active system receiving inputs and releasing outputs of both energy and matter from many chemical, physical and biological activities. Uncontrolled human activities from industrialization, urbanization, agriculture and the poor management of liquid and solid wastes may cause soil to become contaminated.

Humans, other higher animals, plants and microorganisms may be exposed to contaminants through the direct ingestion of contaminated soils, consumption of crops and vegetables grown on contaminated soils or drinking water that has percolated through such soils [2, 3]. These contaminants have been categorized into three groups: biological agents, chemical and physical hazards [4 – 7].

Heavy metal contamination of soil can limit the usability of land for agricultural production due to the potential of adversely affecting food quality, crop growth through phyto-toxicity or environmental health through ecotoxicity to plant and animals [2, 3, 8]. As chemical hazards, heavy metals are particularly notorious for their toxicity, mutagenicity and carcinogenicity in biological systems owing to their chemical reactivity, radioactivity and their cumulative tendency in such systems [9].

Heavy metals present in soil are redistributed into different chemical forms with varying bioavailability, mobility and toxicity [10]. This distribution is believed to be controlled by reactions of heavy metals in soils such as mineral precipitation and dissolution, ion exchange, adsorption and desorption, aqueous complexation, biological immobilization and mobilization, and plant uptake. Knowledge of the total contents of heavy metals present in soil provides limited information about their potential behaviour and bioavailability [11, 12].

Speciation of metal contaminated soils is important in developing viable and cost effective remediation strategies and in predicting mobility and bioavailability of the metals [13, 14]. Water-soluble and exchangeable forms are considered readily mobile, while metals incorporated into crystalline lattices of clays appear relatively inactive. The other forms - precipitated as carbonate, occluded in Fe, Mn, and Al oxides or complexed with organic matter – could be considered relatively active or firmly bound, depending upon the actual combination of physical and chemical properties of soil. Thus, soil texture (clay content), pH, organic matter and Fe-Mn oxides have been found to be the most important soil properties and components influencing the lability and biological uptake of heavy metals [11, 12, 15, 16].

Since the mobility and bioavailability of metals are related to the solubility of their geochemical forms, an evaluation of the speciation of heavy metals in the contaminated soils is, thus, required to assess the effects of the various anthropogenic activities on the partitioning of the heavy metals in the soil and provide insight into their bioavailability and a comprehensive technical basis to select remediation techniques for the contaminated sites in order to clean up the soil environment and consequently enhance food/water quality and achieve a healthy population.

In order to enhance crop yield, inorganic fertilizers and pesticides are consciously applied on the cultivated lands. These inorganic fertilizer and pesticides contain heavy metals in varying quantity. The addition of fertilizer to soils of oil palm plantation in Benin City, Edo State, Nigeria have been least characterized in terms of heavy metal speciation, bioavailability and effect on plants by heavy metal contaminated soil after fertilizer application. Though alarming cases of heavy metal contamination have not been identified and properly documented for these soils at present, prevailing anthropogenic and agricultural activities, no doubt, signifies a potential of their eventual accumulation with reduction in the soil quality. An assessment of the possible adverse effects of heavy metals and their transformations, thus, becomes necessary.

Hence the present study sets out to undertake speciation and phytoavailability based evaluation of selected heavy metals in an indigenous soil samples collected from an oil plantation in Benin City under different scenarios of experimental enrichment with the heavy metal salts and/or residuals (cow manure). Maize (*Zea mays*) was used for this study because of its fast germination and growing rate and its intercropping tendency with the oil palm (*Elaeis guineensis*)

## MATERIALS AND METHODS

### Study site

Soil samples were collected from five different locations using plastic spade at 0 – 15cm depth from Field 16 in NIFOR main station. The soil samples were collected in polythene bags and taken to the laboratory. In the laboratory, soil samples were bulked by mixing thoroughly equal amounts of soil from each location. The bulked soil samples were air dried, and crushed to pass through a 2mm sieve and stored in a polythene bag.

### Pot experiment

The parent soil was spiked singly with Pb and Cr salt. 500ml of 5000mg/l of the single Pb and Cr stock was added to 5kg parent soil to furnish 500mg/kg target concentrations. The mixture was thoroughly mixed, split into five parts of a kg/bucket and 0 %, 2 %, 5 %, 10 % and 20 % of cow dung was added to the contaminated soil, mixed thoroughly and left for 2 weeks. Amended contaminated soil samples were collected before maize was planted. The experiment was replicated three times.

### Soil, cow dung and plant analysis

Physico-chemical analysis of the bulked soil, cow dung and plant samples were conducted using standard analytical methods [17]. Cation Exchange Capacity was estimated by summing the exchangeable cations determined by flame photometric method with the exchangeable acidity determined by titration method [18]. The soil pH was determined using a 1:2 soil /water ratio by a Suntex digital pH-meter [19]. Particle size was carried out according to Bouyoucos method [20]. Total organic carbon was determined by the Walkley-Black rapid oxidation method [21]. Available phosphorus was determined by the Bray and Kurtz method [22] while nitrogen was analysed using a micro-Kjeldahl method [17].

Cow dung was collected with a plastic spade inside a polythene bag from NIFOR cattle yard. All non-compostable materials in the waste were sorted out and thrown away. The nutrient content of the cow dung was determined using standard methods [17] and the results were found to be; N = 0.44 ± 0.10 %, P = 0.43 ± 0.06%, K = 0.49 ± 0.20 %, Ca = 1.44 ± 0.40 %, and Mg = 0.06 ± 0.00%.

Maize plants were harvested after two (2) months of germination or emergence and washed thoroughly with running tap water. Plant tissues were cut into small pieces, dried for 72hrs at $60^{0}$C, weighed and ashed in a Muffle furnace at $500^{0}$C for 4hrs. The ash was dissolved in 20% nitric acid. The metal (Pb and Cr) content was determined with Atomic Absorption spectrophotometer, Bulk Scientific VGP 210.

### Fractionation

2g of each of the soil samples was used for total heavy metal determination in accordance with USEPA method described by Khodadoust, *et al*., [23] while fractionation studies was based on the method suggested by Community Bureau of Reference (BCR) with slight modification [24].

**(i) Extractable fraction:** This fraction extracts trace elements which are affected by sorption-desorption processes caused by changes in water ionic composition. This involves extraction of water soluble, exchangeable and carbonate bound metals. The soil was extracted at room temperature for 16 hours with 40mL of 0.1M acetic acid solution (pH 7.0)

**(ii) Reducible fraction (Fe-Mn oxides bound metals):** This fraction extracts the iron and manganese oxides fractions of the soil, which exist as nodules, concretions, cement between particles or simply as a coatings on particles; these oxides are excellent scavengers for trace metals and low redox potential. The residue from (i) was extracted with 40mL of 0.5M hydroxylamine hydrochloride (pH 1.5) with occasional agitation for 16 hours at room temperature

**(iii) Bound to Organic Matter fraction:** This fraction extracts trace elements bounded to various forms of organic matter: living organisms, detritus, coatings on mineral particles, etc which are bio-accumulated by complexation and peptization properties of natural organic matter (humic and fulvic acid). To the residue from (ii), was added 10mL of 8.8M hydrogen peroxide solution and the mixture agitated for 1hr at room temperature and then agitated for another 1hr at 85 ± $2^{0}$C. An additional 50mL aliquot of 1M ammonium acetate (pH 2) was added and the mixture was agitated for 16hrs at room temperature.

**(iv) Residual fraction:** This fraction extracts primary and secondary minerals, which holds trace metals within their crystal lattices. The residue from (iii) was digested with 10mL of 7M nitric acid for 6hrs. The tube was cooled and the side rinsed with distilled water and filtered through a Whatman 1 filter paper into a 100mL volumetric flask. The volume was made to mark with water.

After each extraction, soil was washed with 10ml distilled water, shaken vigorous by hand shaking and then centrifuged for 30mins before the next extraction step. The washes were added to the supernatant from the previous fraction and then made up to 100ml mark with distilled water. The concentrations of Pb and Cr in every extract were measured by atomic absorption spectrophotometer (Buck Scientific VGP 210 model)

### Statistical Analysis

The analytical results were compiled to form a multi-element data base using excel and statistica.

## RESULT AND DISCUSSION

The physicochemical properties of the soil samples (control and soil with different amounts of amendment) are given in Table 1. The pH and nutrient contents increased with increase in amount of amendment. The use of amendment especially at 20 % level raised the pH level of the soil sample from 5.20 to 6.90. It has been established that near neutral pH generally results in micronutrient cations to be soluble enough to satisfy plant needs without becoming soluble enough as to be toxic [25]. The total amount of Pb and Cr in the control and amended soil samples are given in Table 2 and 3.

The application of amendments to soils that can immobilize heavy metals in situ may provide a cost effective and sustainable solution for remediation of contaminants in soils. In this study, the ability of cow dung in reducing Pb and Cr availability in a heavily contaminated soil and allow regeneration was carried out. The results of the fractionation of Pb in the contaminated soil before maize was planted are given in Table 2.

The chemical forms or species, in which a metal is found in the environment, provides predictive insights on the bioavailability, mobility, and fate of the metal contaminant. Fractionation of metals in soils affects their solubility which directly influences their bioavailability [26]. Therefore, metals in extractable fractions would be readily bioavailable to the environment, whereas the metals in the residual fraction are tightly bound and would not be expected to be released under natural conditions [27]. Metal species associated with organic, Fe-Mn oxide fractions are also not readily bioavailable. They are tightly held and bound. Their release into the soil solution depends on strong depletion of minerals content of the soil solution, decomposition and oxidation of organic matter. The identification and quantification of the forms in which a metal is present in soil helps to establish its potential and actual mobility and toxicity in the soil.

**Table 1: Physicochemical properties of control and amended contaminated soil**

| Parameters | Without amendment | With 2% amendment | With 5% amendment | With 10% amendment | With 20% amendment |
|---|---|---|---|---|---|
| **pH** | 5.20 ± 0.01 | 5.50 ± 0.04 | 5.90 ± 0.01 | 6.40 ± 0.01 | 6.90 ± 0.00 |
| **OC (%)** | 1.70 ± 0.60 | 2.10 ± 0.60 | 2.70 ± 0.90 | 3.60 ± 0.50 | 4.80 ± 0.30 |
| **OM (%)** | 2.92 ± 0.60 | 3.61 ± 0.60 | 4.64 ± 0.90 | 6.19 ± 0.50 | 8.26 ± 0.30 |
| **Ca (cmol/kg)** | 8.20 ± 1.00 | 8.70 ± 1.00 | 9.50 ± 0.70 | 10.30 ± 0.00 | 11.80 ± 1.80 |
| **Mg (cmol/kg)** | 3.80 ± 0.10 | 4.60 ± 0.60 | 5.80 ± 0.70 | 6.80 ± 1.00 | 7.90 ± 1.00 |
| **Na (cmol/kg)** | 0.30 ± 0.10 | 0.37 ± 0.00 | 0.43 ± 0.10 | 0.50 ± 0.10 | 0.58 ± 0.50 |
| **K (cmol/kg)** | 0.60 ± 0.10 | 0.64 ± 0.10 | 0.69 ± 0.30 | 0.78 ± 0.60 | 0.90 ± 0.30 |
| **CEC (cmol/kg)** | 12.90 ± 0.10 | 14.31 ± 0.44 | 16.42 ± 0.46 | 18.38 ± 0.40 | 21.18 ± 0.90 |
| **P (mg/kg)** | 17.80 ± 3.73 | 19.60 ± 2.70 | 22.00 ± 4.10 | 27.60 ± 0.00 | 34.90 ± 2.20 |
| **N (%)** | 0.34 ± 0.08 | 0.45 ± 0.14 | 0.49 ± 0.50 | 0.54 ± 0.10 | 0.72 ± 0.10 |

**Table 2: Fraction of Pb (mg/kg) in cow dung amended soil**

| | Without amendment | With 2% amendment | With 5% amendment | With 10% amendment | With 20% amendment |
|---|---|---|---|---|---|
| **Extractable** | 69.70 ± 3.00 (15%) | 65.60 ± 1.60 (14%) | 61.40 ± 3.00 (13%) | 54.30 ± 1.10 (12%) | 54.10 ± 1.90 (12%) |
| **Fe-Mn oxide** | 99.40 ± 2.00 (22%) | 98.30 ± 1.40 (22%) | 99.30 ± 1.30 (22%) | 98.00 ± 3.00 (21%) | 99.00 ± 2.50 (21%) |
| **Organic** | 124.40 ± 2.60 (27%) | 123.80 ± 3.20 (27%) | 124.80 ± 2.00 (27%) | 129.80 ± 2.30 (28%) | 131.00 ± 3.00 (28%) |
| **Residual** | 166.20 ± 4.10 (37%) | 167.20 ± 3.10 (37%) | 173.20 ± 4.00 (38%) | 175.20 ± 4.10 (38%) | 178.30 ± 5.00 (39%) |
| **Sum of fractions** | 459.70 | 454.90 | 458.70 | 457.30 | 462.40 |
| **Total** | 499.30 ± 12.00 | 497.80 ± 21.40 | 498.00 ± 10.00 | 498.30 ± 10.00 | 498.10 ± 11.30 |

**Table 3: Fraction of Cr (mg/kg) in cow dung amended soil**

| Fraction | Without amendment | With 2% amendment | With 5% amendment | With 10% amendment | With 20% amendment |
|---|---|---|---|---|---|
| **Extractable** | 84.10 ± 11.20 (18%) | 80.50 ± 20.00 (17%) | 78.40 ± 13.00 (16%) | 76.20 ± 12.00 (16%) | 73.20 ± 18.20 (16%) |
| **Fe-Mn oxide** | 93.70 ± 17.00 (20%) | 96.80 ± 21.10 (20%) | 98.90 ± 20.00 (21%) | 94.90 ± 11.10 (20%) | 96.80 ± 19.00 (20%) |
| **Organic** | 129.80 ± 20.90 (27%) | 127.10 ± 14.00 (27%) | 128.20 ± 21.80 (27%) | 122.30 ± 20.80 (26%) | 128.30 ± 13.00 (27%) |
| **Residual** | 164.80 ± 12.00 (35%) | 168.80 ± 22.00 (36%) | 169.50 ± 20.00 (36%) | 172.90 ± 23.60 (36%) | 177.80 ± 21.00 (37%) |
| **Sum of fractions** | 472.40 | 473.20 | 475.00 | 476.30 | 476.10 |
| **Total** | 499.10 ± 16.70 | 497.70 ± 20.00 | 498.10 ± 18.40 | 498.20 ± 10.50 | 498.70 ± 18.60 |

**Table 4: Fraction of residual Pb (mg/kg) in cow dung amended soil**

| | Without amendment | With 2% amendment | With 5% amendment | With 10% amendment | With 20% amendment |
|---|---|---|---|---|---|
| **Extractable** | 55.80 ± 12.70 (12%) | 39.70 ± 10.90 (9%) | 31.60 ± 6.30 (7%) | 25.10 ± 3.50 (5%) | 16.70 ± 2.70 (4%) |
| **Fe-Mn oxide** | 97.30 ± 10.20 (22%) | 98.80 ± 20.30 (22%) | 98.20 ± 11.70 (22%) | 98.60 ± 9.30 (22%) | 91.60 ± 10.40 (20%) |
| **Organic** | 132.90 ± 11.20 (30%) | 124.80 ± 11.10 (28%) | 126.90 ± 14.50 (28%) | 127.20 ± 11.30 (28%) | 129.50 ± 11.00 (28%) |
| **Residual** | 160.70 ± 11.80 (36%) | 186.90 ± 12.10 (41%) | 197.80 ± 21.00 (43%) | 205.40 ± 11.70 (45%) | 222.90 ± 12.00 (48%) |
| **Sum** | 446.70 | 450.20 | 454.50 | 456.30 | 460.70 |
| **Total** | 490.50 ± 12.40 | 493.00 ± 13.90 | 494.10 ± 13.00 | 495.30 ± 11.30 | 496.00 ± 11.40 |

## Table 5: Fraction of residual Cr (mg/kg) in cow dung amended soil

| | Without amendment | With 2% amendment | With 5% amendment | With 10% amendment | With 20% amendment |
|---|---|---|---|---|---|
| **Extractable** | 74.30 ± 10.60 (16%) | 72.50 ± 21.80 (15%) | 66.90 ± 13.00 (14%) | 57.90 ± 16.30 (12%) | 41.50 ± 21.20 (9%) |
| **Fe-Mn oxide** | 94.60 ± 24.00 (20%) | 98.70 ± 16.00 (21%) | 96.60 ± 11.20 (21%) | 94.50 ± 14.40 (20%) | 96.60 ± 20.10 (20%) |
| **Organic** | 133.70 ± 16.20 (29%) | 125.60 ± 14.30 (27%) | 128.70 ± 10.10 (27%) | 133.40 ± 20.40 (28%) | 136.40 ± 30.00 (29%) |
| **Residual** | 163.80 ± 12.00 (35%) | 173.70 ± 22.60 (37%) | 179.70 ± 20.00 (38%) | 186.80 ± 30.20 (40%) | 199.60 ± 10.20 (42%) |
| **Sum of fractions** | 467.40 | 470.50 | 471.90 | 472.60 | 474.10 |
| **Total** | 492.70 ± 10.00 | 493.80 ± 11.60 | 494.40 ± 11.10 | 494.80 ± 10.20 | 495.80 ± 10.00 |

## Table 6: Metal uptake after 2 months on amended contaminated soil

| | Plant on contaminated soil without amendment | With 2% amendment | With 5% amendment | With 10% amendment | With 20% amendment |
|---|---|---|---|---|---|
| **Cr (mg/kg)** | 6.40 ± 1.30 | 3.90 ± 0.00 | 3.70 ± 1.00 | 3.40 ± 0.50 | 2.90 ± 0.60 |
| **Pb (mg/kg)** | 8.80 ± 1.00 | 4.80 ± 0.40 | 3.90 ± 0.50 | 3.00 ± 0.80 | 2.10 ± 1.40 |

From the results, Pb was found to be mostly bound to the residual fraction of the amended and non-amended contaminated soils. The high amount of lead in this fraction (37 – 39 %) may be due to the presence of acid resistant mineral and organic materials [28]. Heavy metals interact with organic matter through various mechanisms, which affect their bioavailability. In this study, lead was next bound to the organic fraction of the amended contaminated soil. In the extractable fraction of the soils analyzed, 12 – 15 % Pb was found.

The result of the fractionation of Cr in the cow dung amended metal contaminated soil samples are given in Table 3. From the result, it was observed that Cr was mostly bound to the residual fraction of the amended contaminated soil before maize was planted. Considering the sandy nature of the parent soil, the Cr may have co-precipitated with various silicate species consequent to their adsorption into the mineral lattice [29, 30]. It was observed that the amount of Cr in the extractable fraction of the soil samples were the lowest.

Tables 4 and 5 show the different forms of Pb and Cr respectively in the amended contaminated soil samples after two months of growing maize. The results obtained indicate that after two months of germination, the species of the metals associated with the extractable fraction in the amended soils reduced. These reductions are significant because these are the fractions that have positive influence on metal bioavailability. It is pertinent to note that their rate of reduction increased as the percentage of amendment in the soil increased. Plant uptake is dependent on movement of element from the soil to the plant root through the soil solution; the limiting step for elemental concentration in soil is usually from the soil to the root. The relative mobility and bioavailability of trace metals associated with different fractions has a lot of influence on plant uptake of metals.

These reductions are more noticeable with Pb (Table 4). This reduction trend cannot be said of the Fe- Mn oxide, organic and residual fractions. In this study, the amounts of Pb and Cr in the residual fraction of the soil sample after growing maize were found to be higher. This may be due to the incorporation of carbon-rich composts into soils which has been shown to increase metal solubility through formation of soluble metal organic complexes [31]. The amendment apart from enriching the soil a key to effective phytoremediation, also immobilizes heavy metals in soils thereby reducing mobility of metals in the soil [32]. .

Table 6 shows the uptake of metals by plants. Generally more metals were taken up by plants in the control pots than in the pots with amendments. This is true for both Pb and Cr. Cationic trace elements in soils reacts with certain organic molecules to form organo-metallic complexes called chelates. If these complexes are not soluble, the metals are tightly held and bound. They are thus not bioavailable to plants. They could only be slowly released through decomposition. Pb and Cr in the organic and Fe-Mn oxide fraction recorded little change in their amount. Bounding of metals is also a method of soil remediation called phytostabilization. The slow release through decomposition of these toxic metals to plants also gives the plants a longer lease of life when compared with the control plants. The control plants were found to be dying before the expiration of the two months experimental period.

The control plant growth was generally retarded with stunted growth, yellowing of leaves, reduced leaf expansion and dying before the expiration of the 2 months duration when compared to the plants on amended contaminated soil. The increase in the values of pH, CEC and OM especially at 20 % amendment level (Table 1) occasioned by the addition of cow dung amendment did more to bound the metals than make them available for plant uptake. The metals bioavailability of soil depends to a large extent on their distribution between solid and solution phase, which in turn is dependent on soil processes like CEC, OM, and pH. The effect of organic matter amendments on heavy metal solubility depend greatly upon the degree of humification of their OM and their effect upon soil pH [33]. Generally, the concentration of an element in the soil solution is believed to depend on the equilibrium between the soil solution and solid phase, with pH playing the decisive role [34]. The soil's ability to immobilize heavy metals increases with rising pH and peaks under slightly alkaline conditions.

## CONCLUSION

The study revealed that in cases of metal contamination accumulation of heavy metals from regular application of inorganic fertilizer to soils cultivated with oil palm, cow dung can be used to immobilize the heavy metals in the contaminated soil.

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