

Prediction of Metals Available to Maize Seedlings in Crude Oil Contaminated Soil

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Abstract—The study assessed the effect of crude oil applied at rates, 0, 2, 5, and 10% on the fractional chemical forms and availability of some metals in soils from Usen, Edo State, with no known crude oil contamination and soil from a crude oil spill site in Ubeji, Delta State, Nigeria. Three methods were used to determine the bioavailability of metals in the soils: maize (*Zea mays*) plant, EDTA and BCR sequential extraction. The sequential extract acid soluble fraction of the BCR extraction (most labile fraction of the soils, normally associated with bioavailability) were compared with total metal concentration in maize seedlings as a means to compare the chemical and biological measures of bioavailability. Total Fe was higher in comparison to other metals for the crude oil contaminated soils. The metal concentrations were below the limits of 4.7% Fe, 190mg/kg Cu and 720mg/kg Zn intervention values and 36mg/kg Cu and 140mg/kg Zn target values for soils provided by the Department of Petroleum Resources (DPR) guidelines. The concentration of the metals in maize seedlings increased with increasing rates of crude oil contamination. Comparison of the metal concentrations in maize seedlings with EDTA extractable concentrations showed that EDTA extracted more metals than maize plant.

Keywords—Availability, crude oil contamination, EDTA, maize, metals.

I. INTRODUCTION

THE effects of crude oil spill on the environment have been an issue of concern all over the world as it leads to the contamination of soil. Worse hit are countries such as Nigeria, whose major economy depends on crude oil.

In Nigeria, the last century has experience over 550 reported cases of crude oil spill in the Niger Delta area alone. This has led to the release of more than two million barrels of crude oil into the environment [1].

Crude oil is a liquid and contains complex mixtures of hydrocarbons, some organic materials and trace amounts of metals and heavy metals depending on its composition [2]. These substances when introduced into the soil can cause alteration in the environment. A major substance of interest in the crude oil is heavy metals which are chemical elements with density greater than 4g/cm^3 . Heavy metal contamination been of major concern because they are toxic, persistent and non-degradable [3] and as such accumulate in the ecosystem

where they may cause adverse effects on the soil functions, plants and animals [3], [4] which ultimately affect humans and his farmlands [5].

Fractionation of metals in the various chemical forms determines their bioavailability in the ecosystem. Metal content of soils is a critical measurement for assessing the risks of any soil. However, only the chemical fractions of these metals provide predictive insights on the bioavailability, mobility and fate of the metal contaminants [3], [6]. If the biogeochemical composition of the total metal content is known, it will provide vital information about the pollution level of the soil [7] and since they control the bioavailability, it will ultimately control metal soil-plant transfer [3], [6], [8].

Studies have shown that various plants such as grasses, oat and wheat [9] and other agricultural crops like soybean, pea and carrot [10] can tolerate and are capable of growing in soils polluted with hydrocarbons and participate in their degradation through the part of the root which favors the growth of several microorganisms. The organic load of the contaminated soil may directly or indirectly alter the metal status of the soil which may affect the metal mobility [11]. Heavy metal binding in soil and the chemicals forms in soil solution depends on the amount of organic matter in soils. The degradation of organic matter alters soil pH and indirectly affects the availability of metals. The use of various single extractants for the prediction of bioavailability of metals from tannery sludge amended soil and the prediction of bioavailability of metals from the soil receiving tannery waste water have been reported [11]. There is however, no report on the availability of metals from crude oil contaminated soil.

Since crude oil spill is one major route for entry of heavy metals into the environment to cause heavy metal contamination of soil, etc., prediction and comparison of the total concentration, bioavailable concentration and EDTA extractable metals in crude oil contaminated soil to the cereal plant species - maize (*Zea mays*) is very important.

The study evaluated and compared the total, bioavailable and EDTA extractable concentrations of Cd, Pb, Zn, Fe and Cu in crude oil contaminated soil to the cereal plant species - *Zea mays*.

II. MATERIALS AND METHODS

A. Experimental Design

P Holes were dug at five different points within an uncultivated land without known crude oil contamination (Usen) to a depth of 15cm each using plastic spade. Soil was also collected from a community at Ubeji where there was crude oil spill into polythene bags and taken to the laboratory.

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Equal amounts of soil from each point were mixed thoroughly to form a composite of all the samples. The soils were air-dried at room temperature (28-31°C), crushed in a porcelain mortar and sieved through a 2mm sieve. The air-dried < 2mm samples were stored in polythene bags and labeled for future use. The composite soil was weighed into 120 polythene bags such that each bag contained 500g soil and another 30 bags containing the soil collected from crude oil spill site, making a total of 150 bags.

B. Plant Material and Crude Oil

Maize (*Zea mays*) seeds were bought from a local market in Benin City, Edo State, Nigeria and identified as Dmr-Esr-w cultivars, in the Department of Crop Science, University of Benin, Benin City, Nigeria.

Bonny Light Crude Oil, °API (American Petroleum Institute) gravity =37 was obtained from Warri Refinery and Petrochemical Company Delta State, Nigeria.

C. Soil Treatment and Planting of Seeds

The composite soils of 500g were treated with distilled water (control) or 10ml, 25ml and 50ml of crude oil to obtain 0, 2, 5 and 10% v/w crude oil contamination. Flootation method was used to assess seed viability. Seeds were placed in a beaker containing tap water and stirred. Seeds that did not float were regarded as viable seeds while those that floated were regarded as non-viable and discarded. The seeds were planted by a modified version of [12]. Three viable maize seeds were sown in 500g sandy loam soil with a depth of about 1-2 cm and watered daily with distilled water.

D. Plant Analysis

After 21 days post germination, equal amounts of seedlings that germinated were harvested. The plants were then washed thoroughly with running tap water, blotted dry, cut into pieces, mixed thoroughly and oven dried at 80°C.

E. Physicochemical Analyses

The physicochemical analyses of the soil were carried out on the composite soil samples collected. The pH of the soil was determined by Suntex digital pH-meter using a ratio of 1:2 soil weight/water volume [13]. Particle size analysis on the soils was carried out using the methods described by [14]. Total organic carbon was determined by the Walkley and Black rapid oxidation method [15]. Cation exchange capacity was estimated by summing the exchangeable acidity determined by titration method.

F. Single Extraction of Total Metals

EDTA extractable fraction was obtained by mechanical shaking of the sample (5g) at 200rpm with 50ml of 0.05M EDTA solution at room temperature for 6hrs. For the determination of total metal concentrations in the soil, 1g of soil was digested with 5ml of HNO₃ and 1ml of HClO₄ in a digester for 6hrs. The digested solution was then diluted with distilled water and filtered through a Whatman No. 1 filter paper and made up to 100ml. The metals in the solution were determined by AAS Bulk Scientific (VGP210).

G. Metal Accumulation by Plant

1g of oven dried plants was weighted and ashed in a muffle furnace at 500°C for 3hrs. The ash was dissolved in 20% trioxonitrate (v) acid and filtered through a Whatman No. 1 filter paper and made up to 100ml mark. The metal contents were determined with AAS.

H. Soil Fractionation

Fractionation studies were performed on the soil samples to assess the amount of bioavailable metals before planting maize and EDTA extraction. Reference [16], a modified version of [17], was used to investigate the distribution of the metals in the soils into five optional geochemical forms (speciation). The extraction procedure was performed by sequentially extracting 2g of Bonny Light crude oil contaminated soil sample in 50ml polypropylene bottle. The exchangeable fraction was determined through extraction with 20ml of 1M NH₄OAc at pH 7 for 2hrs. The carbonate bound fraction was determined after extraction with 20ml of 1M NH₄OAc adjusted to pH 5 with acetic acid for 2hrs. The fraction bound to Fe/Mn oxides and hydroxides was determined after extraction with 20ml of 0.04M NH₂OH.HCl in 25% HOAc for 6hrs at 60°C. The fraction bound to organic matter was determined after extraction with 15ml of 30% H₂O₂ (adjusted to pH with HNO₃) for 5.5hrs at 80°C and then 5ml of 3.2M NH₄OAc in 20% HNO₃ for 30mins. The residual fraction was determined after digesting 1g of the residue with 5ml of trioxonitrate (v) acid for 6hrs.

I. Statistical Analysis

All analyses were done in triplicate and results expressed as mean ± standard error of mean (SEM). Analysis of variance was used to test for differences in the groups, while Duncan's multiple comparisons test was used to determine significant differences between means.

III. RESULTS

Table I shows the results of the physicochemical analyses. The pH was significantly lower ($P < 0.05$) in the crude oil contaminated soil (5.84 ± 0.03) but higher in the Ubeji soil (6.74 ± 0.01) than control soil (6.44 ± 0.03). Total organic carbon, total organic matter and cation exchange capacity were significantly lower ($P < 0.05$) in control than in crude oil contaminated soil.

The concentration of metals in the crude oil contaminated soil and control soil are presented in Table II. The values of Cd, Pb, Fe, Cu, and Zn for contaminated soil were above the value of control soil but below those of 190mg/kg Cu, 720mg/kg Zn, 36mg/kg Cu and 140mg/kg Zn intervention and target values for soils provided by [18] guidelines. The result shows that the metal concentration in the crude oil contaminated soil is higher than that of control.

Tables III (A)–(E) show the chemical forms in which the metals were distributed in the crude oil contaminated soil before planting of maize and extraction with EDTA. The highest concentrations of Cu, Zn, Pb and Fe were found in the residual fractions of the crude oil contaminated soil. Cadmium

was not detected in all the fractions except the acid soluble fraction.

Table IV shows the results of the concentration of metals that accumulated in the maize seedling, concentrations extracted by EDTA and the residual concentration of the metals in the crude oil contaminated soil after 21 days post germination and EDTA extraction.

The results showed that Cd was not detectable. 0.09-2.17mg/kg Pb, 7.50-11.47mg/kg Zn, 24.95-35.05mg/kg Fe and 0.87-1.14mg/kg Cu was accumulated by maize seedlings 21 days post germination from the crude oil contaminated soil. Iron was the highest metal accumulated by the maize seedlings.

Table V shows the comparison of the concentrations of Cu, Zn, Pb and Fe obtained using several techniques. The least concentration of Fe was found in harvested maize seedlings when compared to their concentration in the total metals. The highest concentrations of the metals were extracted by EDTA. The concentration of Pb accumulated in Ubeji soil by maize seedling was higher than the bioavailable concentration before germination.

IV. DISCUSSION

The low pH value observed in the study indicates that the soil is moderately acidic. The high cation exchange capacity of the crude oil contaminated soil (CCS) may be due to high calcium content.

The high values of the metals (Cd, Pb, Fe, Cu, and Zn) in the crude oil contaminated soil, though below those of intervention and target values for soils provided by [18] guidelines, may indicate contamination with heavy metals from the crude oil.

The high concentrations of the metals (Cu, Zn, Pb and Fe) found in the residual fractions of the crude oil contaminated soil before planting of maize and extraction with EDTA, may be a result of the sandy nature of the soil as well as presence of acid resistant minerals and the co-precipitation of the metals with various silicate species consequent to their adsorption into the mineral lattice. Detection of cadmium only in the acid soluble fraction, may indicate that it will be readily bioavailable and mobile as metals in this fraction are exchangeable ions which occur either as free hydrated ions or as various complexes with organic or inorganic ligands [19]. The percent bioavailability of the metals in the crude oil contaminated soils is relatively high since the mobile phase contains reasonable percent of total extractable fractions.

Even though all the metals were accumulated by the maize plant, the higher accumulation level of Fe by the maize seedlings recorded in the study, may be attributed to findings [20] that crude oil can raise the levels of iron and zinc to toxic levels. It may also be attributed to the sandy nature of the soil which may have aided mobility of Fe.

When the concentration of metals removed by the maize seedlings and EDTA were compared, it was observed that EDTA removed more metal from the crude oil contaminated soil.

Comparing the concentrations of the metals (Cu, Zn, Pb and

Fe) obtained using several techniques; it was observed that the least concentration of Fe was found in harvested maize seedlings when compared to their concentration in the total metals. Also, the highest concentrations of the metals were extracted by EDTA and the concentration of Pb accumulated in Ubeji soil by maize seedling was higher than the bioavailable concentration before germination.

TABLE I
PHYSICO-CHEMICAL PROPERTIES OF CRUDE OIL CONTAMINATED SOIL AND CONTROL SOILS (DRY WEIGHT)

Parameter/Sample	Control	CCS	UBEJI
pH (H ₂ O)	6.44±0.03 ^a	5.84±0.14 ^b	6.74±0.01 ^c
Soil Texture	Sandy clay loam	Sandy clay loam	Sandy loam
CEC (Cmol/kg)	7.08±0.01 ^a	9.05±0.07 ^b	9.98±0.02 ^c
THC (mg/kg)	0.00±0.01 ^a	17.34±0.01 ^b	74.88±0.01 ^c
TOC (%)	1.26±0.01 ^a	3.31±0.02 ^b	3.89±0.01 ^c
TOM (%)	2.17±0.02 ^a	5.71±0.05 ^b	5.41±0.02 ^{bc}
Clay (%)	26.68	28.0	16.28
Silt (%)	10.30	12.0	10.70
Sand (%)	63.02	60.0	73.02

Values are mean of three (n=3) replicates ± standard error of mean, CCS= crude oil contaminated soil, UBEJI = Spill site of crude oil, THC = Total Hydrocarbon Content. Means of the same row carrying different notations are statistically different at P<0.05 using Instat graphpad.

TABLE II
CONCENTRATION OF METALS IN CRUDE OIL CONTAMINATED SOIL AND CONTROL SOIL (MG/KG SOIL DRY WEIGHT).

Metal/Sample	Control	CCS	UBEJI
Cadmium	0.01±0.01 ^a	0.05±0.03 ^a	0.01±0.01 ^a
Lead	0.08±0.01 ^a	0.84±0.23 ^b	5.00±0.12 ^c
Zinc	10.00±0.03 ^a	46.12 ±0.03 ^b	50.00±0.04 ^c
Iron	119.75±0.07 ^a	165.18±0.04 ^b	300.95±0.14 ^c
Copper	1.05±0.02 ^a	4.60 ±0.02 ^b	6.00±0.02 ^c

Values are mean of three (n=3) replicates ± standard error of mean, CCS= crude oil contaminated soil, UBEJI = Spill site of crude oil, Means of the same row carrying different notations are statistically different at P<0.05 using Instat graphpad.

TABLE III (A)
FRACTIONATION TESTS OF CADMIUM IN THE CRUDE OIL CONTAMINATED SOIL BEFORE TREATMENT (MG/KG SOIL DRY WEIGHT)

Fraction	Control	CCS	UBEJI
Exchangeable	ND	ND	ND
Oxidizable	ND	ND	ND
Acid soluble	ND	0.05±0.02 ^a	0.01±0.01 ^b
Reducible	ND	ND	ND
Residual	0.01±0.01	ND	ND
Sum of BAF	000	100	100
Residual (%)	100	0.00	0.00

Values are mean of three (n=3) replicates ± standard error of mean, CCS= crude oil contaminated soil, UBEJI = Spill site of crude oil, BAF = Bioavailable Fraction, ND = Not Detectable., Means of the same row carrying different notations are statistically different at P<0.05 using Instat graphpad

TABLE III (B)
FRACTIONATION TESTS OF LEAD IN THE CRUDE OIL CONTAMINATED SOIL BEFORE TREATMENT (MG/KG SOIL DRY WEIGHT)

Fraction	Control	CCS	UBEJI
Exchangeable	ND	0.37 ±0.02 ^a	1.34 ±0.03 ^b
Oxidizable	ND	ND	ND
Acid soluble	ND	0.19±0.02 ^a	1.00±0.05 ^b
Reducible	ND	ND	0.24±0.02
Residual	ND	0.31 ±0.02 ^a	1.58±0.03 ^b
Sum of BAF	0.00	0.56	2.34
Residual (%)	0.00	36.79	37.98

Values are mean of three (n=3) replicates ± standard error of mean, CCS= crude oil contaminated soil, UBEJI = Spill site of crude oil, BAF = Bioavailable Fraction, ND = Not Detectable., Means of the same row carrying different notations are statistically different at P<0.05 using Instat graphpad

TABLE III (D)
FRACTIONATION TESTS OF IRON IN THE CRUDE OIL CONTAMINATED SOIL BEFORE TREATMENT (MG/KG SOIL DRY WEIGHT)

Fraction	Control	CCS	UBEJI
Exchangeable	19.18 ±0.01 ^a	43.14 ±0.02 ^b	20.80±0.07 ^c
Oxidizable	25.20 ±0.06 ^a	13.70±0.02 ^b	92.39±0.05 ^c
Acid soluble	16.68 ±0.06 ^a	0.10 ±0.02 ^b	46.90±0.03 ^c
Reducible	24.17 ±0.06 ^a	35.50 ±0.02 ^b	16.35±0.03 ^c
Residual	30.35 ±0.03 ^a	65.86 ±0.02 ^b	122.43±0.03 ^c
Sum of BA	35.86	43.24	67.70
Residual (%)	26.26	41.61	40.96

Values are mean of three (n=3) replicates ± standard error of mean, CCS= crude oil contaminated soil, UBEJI = Spill site of crude oil, BAF = Bioavailable Fraction, ND = Not Detectable., Means of the same row carrying different notations are statistically different at P<0.05 using Instat graphpad

TABLE III (C)
FRACTIONATION TESTS OF ZINC IN THE CRUDE OIL CONTAMINATED SOIL BEFORE TREATMENT (MG/KG SOIL DRY WEIGHT)

Fraction	Control	CCS	UBEJI
Exchangeable	0.28 ±0.01 ^a	3.14 ±0.02 ^b	8.85 ±0.03 ^c
Oxidizable	03.38 ±0.06 ^a	10.70 ±0.02 ^b	10.11±0.03 ^c
Acid soluble	0.68 ±0.06 ^a	8.92 ±0.02 ^b	7.75 ±0.03 ^c
Reducible	03.35 ±0.03 ^a	6.50 ±0.02 ^b	9.08 ±0.05 ^c
Residual	1.36 ±0.03 ^a	14.08±0.02 ^b	15.28±0.03 ^c
Sum of BAF	0.96	12.06	16.60
Residual (%)	15.03	32.48	29.92

Values are mean of three (n=3) replicates ± standard error of mean, CCS= crude oil contaminated soil, UBEJI = Spill site of crude oil, BAF = Bioavailable Fraction, ND = Not Detectable., Means of the same row carrying different notations are statistically different at P<0.05 using Instat graphpad

TABLE III (E)
FRACTIONATION TESTS OF COPPER IN THE CRUDE OIL CONTAMINATED SOIL BEFORE TREATMENT (MG/KG SOIL DRY WEIGHT)

Fraction	Control	CCS	UBEJI
Exchangeable	ND	0.50 ±0.02 ^a	1.89 ±0.03 ^b
Oxidizable	0.05±0.03	ND	ND
Acid soluble	ND	ND	ND
Reducible	0.04±0.03 ^a	0.93±0.05 ^b	ND
Residual	0.08 ±0.03 ^a	2.86 ±0.02 ^b	4.11±0.03 ^c
Sum of BAF	0	0.50	1.89
Residual (%)	47.06	67.14	68.50

Values are mean of three (n=3) replicates ± standard error of mean, CCS= crude oil contaminated soil, UBEJI = Spill site of crude oil, BAF = Bioavailable Fraction, ND = Not Detectable., Means of the same row carrying different notations are statistically different at P<0.05 using Instat graphpad

TABLE IV (A)
CONCENTRATION OF METALS IN MAIZE SEEDLINGS AFTER 21 DAYS POST GERMINATION AND EDTA EXTRACTABLE CONCENTRATIONS AFTER 6HRS EDTA EXTRACTION IN CCS (MG/KG SOIL DRY WEIGHT)

Samples/Metals	Cd (mg/kg)	Pb (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)
Maize seedlings	ND	0.09±0.03	11.47±0.05	24.95±0.03	0.87±0.03
EDTA extractable concs	ND	0.63±0.01	30.17±0.01	146.18±0.01	3.26±0.01
Soil after germination	ND	0.08±0.02	35.41±0.02	105.18±0.02	1.34±0.02
Soil after EDTA extraction	ND	0.21±0.01	15.95±0.01	19.00±0.01	1.34±0.01
% removal (by maize seedlings)	NA	11	25	15	19
% removal (by EDTA)	NA	75	65	88	71

Values are mean of three (n=3) replicates ± standard error of mean, CCS= crude oil contaminated soil, Concs.= concentrations, NA = Not applicable, ND = Not Detectable., Means of the same row carrying different notations are statistically different at P<0.05 using Instat graphpad.

TABLE IV (B)
CONCENTRATION OF METALS IN MAIZE SEEDLINGS AFTER 21 DAYS POST GERMINATION AND EDTA EXTRACTABLE CONCENTRATIONS AFTER 6HRS EDTA EXTRACTION IN UBEJI SOIL (MG/KG SOIL DRY WEIGHT).

Samples/Metals	Cd (mg/kg)	Pb (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)
Maize seedlings	ND	2.17±0.02	7.50±0.02	35.05±0.02	1.14±0.02
EDTA extractable Concs.	ND	3.74±0.03	39.48±0.03	274.05±0.07	4.39±0.02
Soil after germination	ND	0.15±0.01	37.44±0.02	257.40±0.05	2.74±0.02
Soil after EDTA extraction	ND	1.26 ±0.01	10.52±0.01	26.90±0.04	1.61±0.01
% removal (by maize seedlings)	NA	43	15	12	19
% removal (by EDTA)	NA	75	79	91	73

Values are mean of three (n=3) replicates ± standard error of mean, CCS= crude oil contaminated soil, Concs.= concentrations, NA = Not applicable, ND = Not Detectable., Means of the same row carrying different notations are statistically different at P<0.05 using Instat graphpad.

TABLE V (A)
COMPARISON OF THE CONCENTRATION OF METALS IN CCS USING SEVERAL TECHNIQUES

Metals	Concentration in maize seedlings	Total concentration	Bioavailable concentration	Concentration in EDTA
Cd	ND	0.05±0.03	0.05±0.02	0.03 ±0.01
Pb	0.09±0.03	0.84±0.23	0.56±0.01	0.63 ±0.02
Zn	11.47±0.05	46.12 ±0.03	12.06±0.03	30.17±0.02
Fe	24.95±0.03	165.18±0.04	43.24±0.02	146.18±0.05
Cu	0.87±0.03	4.60 ±0.02	0.50 ±0.02	3.26±0.02

Values are mean of three (n=3) replicates ± standard error of mean, CCS= crude oil contaminated soil, NA = Not applicable, ND = Not Detectable., Means of the same row carrying different notations are statistically different at P<0.05 using Instat graphpad

TABLE V (B)
COMPARISON OF THE CONCENTRATION OF METALS IN UBEJI USING SEVERAL TECHNIQUES

Metals	Concentration in maize seedlings	Total concentration	Bioavailable concentration	Concentration in EDTA
Cd	ND	0.01±0.01	0.01 ±0.01	ND
Pb	2.17±0.02	5.00±0.12	2.34±0.01	3.74±0.01
Zn	7.50±0.02	50.00±0.04	16.60±0.01	39.48±0.02
Fe	35.05±0.02	300.95±0.14	67.70±0.03	274.05±0.05
Cu	1.14±0.02	6.00±0.02	1.89±0.01	4.39±0.02

Values are mean of three (n=3) replicates ± standard error of mean, CCS= crude oil contaminated soil, NA = Not applicable, ND = Not Detectable., Means of the same row carrying different notations are statistically different at P<0.05 using Instat graphpad

V. CONCLUSION

The study shows that EDTA is more effective in the prediction of uptake of the metals in terms of their chemical forms and availability. However, when the bioavailable concentration of the uptake levels of the metals by the plant with the bioavailable and EDTA extractable concentrations were compared, the concentration of the metals taken up by maize was lower than the bioavailable concentration but the concentration extractable by EDTA was higher than the bioavailable concentrations.

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