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COMPARISON OF PHYTO-ACCUMULATION OF METALS BY *VIGNA UNGUICULATA L* (BEAN) AND *ZEA MAYS L* (MAIZE) GROWN IN CRUDE OIL CONTAMINATED SOIL

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ABSTRACT

The study compared the phyto-accumulation of copper (Cu), iron (Fe), lead (Pb), cadmium (Cd) and zinc (Zn) by bean and maize seedlings grown in crude oil-contaminated soils. A total of 300 bags containing 500g of soil were used for this study. Three viable seeds of bean and maize were planted in a set of 60 bags of sandy loam soil with no history of crude oil contamination, which served as control, 180 bags of sandy loam soil were mixed with crude oil to give 2%, 5% and 10% contamination, while another set of 60 bags had 500g of soil from crude oil spill site in Ubeji community, Niger Delta. Both plants were harvested 7, 14 and 21 days post germination and the soils and seedlings analyzed for some metals before and after planting. Metal levels in the crude oil contaminated soils were not detected for Cd but showed high concentrations for the other metals when compared with control. The values of Cu, Pb, Zn and Fe decreased in the crude oil contaminated soil and increased in both seedlings as the number of days increased. The study indicates that bean seedlings accumulated more Fe and Cu than maize whereas maize seedlings accumulated more Zn and Pb than bean in the crude oil contaminated soil. The results suggest that the plant species for phyto-accumulation measures for decontamination of crude oil contaminated soils is an important factor.

Keywords: bean, copper, iron, lead, maize, phyto-accumulation, sandy loam soil, zinc.

INTRODUCTION

The urban and rural environment is increasingly being exposed to various contaminants due to increased human and vehicular activities. A major soil contaminant is metals. Metals can be found in materials from chemical production, production of batteries, mining, scrap yards, crude oil spill, paints, pesticides, acid or alkali plants and chemical engineering works (Mohanty and Mahindrakar, 2011). Metals are considered among the most hazardous contaminants because they are very soluble and persist in the environment (Evans and Li, 2003).

Metals contamination is a recurring problem at many contaminated sites as it contributes to environmental pollution which affects plants and animals in the ecosystem. Metal contamination of soil may also make it unfit for agricultural purposes since the produce, if any, may adversely affect crop growth, the safety and marketability of the crop, or environmental health which eventually link humans in the food web (Ling *et al.*, 2007). As chemical hazards, metals are notorious for their toxicity and carcinogenicity in biological systems as a result of their chemical reactivity, radioactivity and their cumulative tendency in such systems (D'amore *et al.*, 2005).

Many communities have experienced crude oil spill since the discovery and exploration of crude petroleum in Nigeria. The most frequent spills occurring in the Niger-Delta area from where most of the crude oil is derived. Continuous spill of crude oil may lead to

contamination of the soils with metals. Contaminated soils often requires soil remediation practices for decontamination in order to prevent the soils from being toxic to man and other organisms in the ecosystem. Conventional remediation methods are usually expensive and appropriate only for small areas where rapid, complete decontamination is required (Pulford and Watson, 2003).

Some plants have been shown to accumulate high concentrations of some of these heavy metals (Brandy and Well, 2005; Pulford and Watson, 2003). The concentration accumulated by some of these plants are dependent on soil pH, moisture, cation exchange capacity, organic matter content, rooting depth, plant species, etc (Okonronkwo and Odoemenam, 2005).

Bean (*Vigna unguiculata L*) and maize (*Zea mays L*) are different plant species which serves as major source of proteins and carbohydrates consumed in Nigeria. While bean are legumes, maize is a cereal crop of temperate and subtropical zones. Both plants are grown in most agro ecological areas especially in the Niger Delta region where oil industrial activities are predominant (Agbogidi *et al.*, 2007). Bean and maize have been displayed as variable stress tolerant plants under environmental extremities ranging from drought or heavy metals (Shanker *et al.*, 2005). The use of plants to remove pollutants from the environment or to render them harmless (phyto-remediation) was a result of the high cost of other soil remediation methods (Salt *et al.*, 1998; Pulford and Watson, 2003).

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It involves different technologies which help to reduce the levels of contaminants and the chances of off-site movement of the contaminants, protects groundwater and river system from the risks of contamination, reduce risk to humans, the environment and consequently enhance food/water quality and achieve a healthy population (Kumar *et al.*, 1995).

The aim of the study is to compare the phyto-accumulation ability of bean and maize seedlings grown on crude oil contaminated soil.

MATERIALS AND METHODS

Study Location

The study was carried out at the University of Benin, Benin City, Edo State, Nigeria. The soil from an uncultivated land with no history of crude oil contamination as well as soil where there was crude oil spill was collected from Ubeji, Delta State, Nigeria.

Soil Sampling and Treatment

Top soil samples were collected from an uncultivated land with no history of crude oil contamination by digging holes with a plastic spade at five different points within the land to a depth of about 15cm. Soil was also collected from a community (Ubeji) where there was crude oil spill from a burst pipe line and used for planting. The soil samples were collected into polythene bags and taken to the laboratory. A composite of all the samples was made by mixing thoroughly equal amounts of soil from each point. 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.

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. The soil in the bags contaminated with crude oil was mixed thoroughly in their respective polythene bags containing 500g top soil with the aid of a plastic spade and treated with distilled water, 10ml, 25ml and 50ml of crude oil to obtain 0, 2, 5 and 10% v/w crude oil contamination and used for planting.

The pH, Particle Size Analysis, Organic Carbon, Cation Exchange Capacity, Phosphorus, Nitrogen, metals was

assayed according to methods described by A.O.A.C, 1995.

Plant Materials and Crude oil

Bean (*Vigna unguiculata L*) and maize (*Zea mays L*) seeds were bought from a local market in Benin City, Edo State, Nigeria and identified as ITA 189 - 288 and Dmr-Esr-w cultivars respectively, in the Department of Crop Science, University of Benin, Nigeria and floatation method was used to test seed viability.

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

Planting of Seeds and Germination Studies

Three viable seeds were sown in 500g sandy loam soil with a depth of about 1-2 cm and watered daily with distilled water.

The time and number of seeds that germinated from each bag were noted and the percentage seedling germination in each treatment was calculated using the formula:

$$\text{Percentage Seedling Germination} = \frac{\text{number of seeds that germinated}}{\text{number of seeds planted}} \times 100$$

Plant Analysis

Equal amounts of seeds that germinated were harvested at day 7, day 14 and day 21 post germination and washed thoroughly with running tap water. The whole plant tissues were cut into pieces, dried for 2 days at 80°C and ashed in a muffle furnace at 500°C for 6 hrs. The ash was dissolved in 20% hydrogen trioxonitrate (IV) acid. The metal content was determined with Atomic Adsorption Spectrophotometer (AAS), (Bulk Scientific VGP210).

All experiments were done in triplicate and results expressed as mean \pm 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.

RESULTS AND DISCUSSION

The physicochemical properties and concentration of some metals in the composite soils are presented in Table 1.

Table 1: Physicochemical properties of soils (dry weight).

Parameter/Sample	Control	CCS	UBEJI
pH(H ₂ O)	6.44 \pm 0.03 ^a	5.84 \pm 0.14 ^b	6.74 \pm 0.01 ^c
Clay (%)	26.68	28.0	16.28
Silt (%)	10.30	12.0	10.70
Sand (%)	63.02	60.0	73.02
Soil texture	Sandy clay loam	Sandy clay loam	Sandy loam
Total Hydrocarbon Content (THC. mg/kg)	0.00 \pm 0.01 ^a	17.34 \pm 0.01 ^b	74.88 \pm 0.01 ^e
Total Organic Carbon (%)	1.26 \pm 0.01 ^a	3.31 \pm 0.02 ^b	3.89 \pm 0.01 ^e
Total Organic Matter (%)	2.17 \pm 0.02 ^a	5.71 \pm 0.05 ^b	5.41 \pm 0.02 ^{be}
Cation Exchange Capacity (Cmol/kg)	7.08 \pm 0.01 ^a	9.05 \pm 0.07 ^b	9.98 \pm 0.02 ^e
Cadmium (mg/kg)	0.01 \pm 0.01 ^a	0.05 \pm 0.03 ^a	0.01 \pm 0.01 ^a
Lead (mg/kg)	0.08 \pm 0.01 ^a	0.84 \pm 0.23 ^b	5.00 \pm 0.12 ^c
Zinc(mg/kg)	10.00 \pm 0.03 ^a	46.12 \pm 0.03 ^b	50.00 \pm 0.04 ^c
Iron (mg/kg)	119.75 \pm 0.07 ^a	165.18 \pm 0.04 ^b	300.95 \pm 0.14 ^c
Copper (mg/kg)	1.05 \pm 0.02 ^a	4.60 \pm 0.02 ^b	6.00 \pm 0.02 ^c

Values are mean of three (n=3) replicates \pm 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.

Cation exchange capacity, total organic carbon (TOC) and total organic matter (TOM) in crude oil contaminated soil were significantly higher ($P < 0.05$) than control soil but pH was significantly lower ($P < 0.05$) in the crude oil contaminated soil except Ubeji soil (crude oil spill site, 6.74 ± 0.01) which had higher pH value than control (6.44 ± 0.03). This indicates that the soils were acidic in nature. Acidic pH values have been reported for Niger Delta soils (Oviasogie and Ofomaja, 2007) and some other regions of Nigeria (Noma *et al.*, 2008). The acidic pH values may have implications on the availability and uptake of metals by both seedlings. The high CEC may give the soil a buffering capacity which may slow down the leaching of nutrient cations and positively charged pollutants because they affect both soluble and exchangeable metal concentrations (Yoo and James, 2002). Total organic carbon and TOM values obtained are comparable to those reported by Uba *et al.*, (2008), however, the values are high based

on the classification of soil organic matter given by Enwezor *et al.*, (1988). The high organic carbon is suggestive of the degradation or presence of degradable and compostable wastes in the crude oil contaminated soils (CCS). Particle size shows that the soil in the contaminated samples is mainly sandy clay loam. The values of Cd, Pb, Fe, Cu, and Zn for crude oil contaminated soils were above the value of control soil but below those of 4.7%, 190, 720, 36, and 140 intervention and target values for soils provided by Department of Petroleum Resources (DPR) guidelines and reported by other researchers in contaminated sites in Nigeria (WHO 1996; David *et al.*, 2009; Iwegbue *et al.*, 2013) and other parts of the world (Kashem *et al.*, 2007). The low concentration of the selected metals studied may infer that the CCS are relatively not polluted in terms of metal contamination. The effects of crude oil contaminated soil on the percentage germination of bean and maize are presented in Table 2 and 3.

Table 2: Effect of crude oil contamination on percentage germination of bean seeds

Parameter/Sample	Control	CCS	UBEJI
2% Contamination			
No of seeds planted	90	90	90
% germination	96	67	41
5% Contamination			
No of seeds planted	90	90	90
% germination	96	52	41
10% Contamination			
No of seeds planted	90	90	90
% germination	96	41	41

Values are mean of three ($n=3$) replicates \pm 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 3: Effect of crude oil contamination on percentage germination of maize seeds

Parameter/Sample	Control	CCS	UBEJI
2% Contamination			
No of seeds planted	90	90	90
% germination	96	78	51
5% Contamination			
No of seeds planted	90	90	90
% germination	96	60	51
10% Contamination			
No of seeds planted	90	90	90
% germination	96	50	51

Values are mean of three ($n=3$) replicates \pm 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.

It was observed during the study that the seeds in crude oil contaminated soil (CCS) (which had 10% oil contamination and Ubeji) emerged two days after the ones in 2% and 5% contaminant levels. The hydrophobic properties of crude oil may have prevented the seeds from absorbing adequate water and air which may have caused the delayed germination recorded (Omosun *et al.*, 2008; Olubodun and Eriyamremu,

2013; Minai-Tehrani and Mohammadi, 2014). The percentage germination of both seeds decreased as the percentage contamination increased. The seeds grown on 10% CCS exhibited the lowest germination rate of 45% in bean while the highest germination rate in the contaminated soil was observed in maize on the soil contaminated with 2% (Table 3).

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During their growth period, the leaves were yellow (chlorosis) after about 5 days for bean and 7 days for maize post germination. The intensity of discoloration was dose dependent and increased in the plants for some days but eventually leveled off about 16 days post germination. The chlorosis observed may be a result of the plants' adaptive mechanism to accumulate and volatilize the contaminants (Olubodun and

Eriyamremu, 2013; Minai-Tehrani and Mohammadi, 2014; Schwab *et. al.*, 1999) or the low level of magnesium, an important element in chlorophyll (Kafkafi, 2005).

The phyto-accumulation of Cd, Pb, Zn, Fe and Cu in bean seedlings 21 days post germination are presented in Table 4 while that of maize is presented in Table 5.

Table 4: Phyto-accumulation of lead, zinc, iron and copper in bean seedlings post germination (mg/kg)

Days/Sample	Control	CCS	UBEJI
LEAD (Pb)			
0	ND	ND	ND
7	ND	0.08 ±0.01 ^a	0.08±0.01 ^b
14	ND	0.08±0.03 ^a	0.10±0.01 ^b
21	ND	0.10±0.03 ^a	0.27±0.05 ^b
ZINC (Zn)			
0	0.91±0.03 ^a	0.90 ±0.02 ^a	0.91±0.02 ^a
7	1.00±0.05 ^a	5.18 ±0.03 ^b	3.41±0.04 ^c
14	1.25±0.06 ^a	8.06±0.07 ^b	5.17±0.10 ^c
21	1.96 ±0.07 ^a	10.29 ±0.06 ^b	7.27 ±0.05 ^c
IRON (Fe)			
0	9.12 ±0.12 ^a	9.12 ±0.0 ^a	9.12±0.12 ^a
7	9.92±0.20 ^a	26.80 ±0.10 ^b	14.17±0.02 ^c
14	10.32±0.20 ^a	43.56±0.07 ^b	30.68±0.10 ^c
21	11.47±0.09 ^a	60.00 ±0.03 ^b	43.60±0.02 ^c
COPPER (Cu)			
0	1.05±0.19	1.05±0.19	1.05±0.19
7	ND	0.19 ±0.02 ^a	0.48±0.03 ^b
14	ND	0.47±0.03 ^a	0.96±0.04 ^b
21	0.05 ±0.01 ^a	0.92±0.02 ^b	1.43±0.02 ^c

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

Table 5: Phyto-accumulation of lead, zinc, iron and copper in maize seedlings post germination (mg/kg)

Days/Sample	Control	CCS	UBEJI
LEAD (Pb)			
0	ND	ND	ND
7	ND	0.08 ±0.03 ^a	0.60±0.02 ^b
14	ND	0.09±0.02 ^a	1.06±0.01 ^b
21	ND	0.09±0.03 ^a	2.17±0.02 ^b
ZINC (Zn)			
0	3.89 ±0.03 ^a	3.90 ±0.20 ^b	3.89±0.03 ^a
7	1.20±0.05 ^a	5.17 ±0.03 ^b	2.17±0.05 ^c
14	1.95±0.07 ^a	7.27±0.05 ^b	4.36±0.10 ^c
21	2.28 ±0.07 ^a	11.47 ±0.05 ^b	7.50 ±0.02 ^d
IRON (Fe)			
0	3.10 ±0.02 ^a	3.10 ±0.02 ^a	3.09±0.05 ^b
7	4.80±0.05 ^a	12.15 ±0.10 ^b	15.00±0.05 ^c
14	7.20±0.03 ^a	20.34±0.07 ^b	26.25±0.10 ^c
21	9.40 ±0.09 ^a	24.95 ±0.03 ^b	35.05±0.02 ^c
COPPER (Cu)			
0	1.12±0.20	1.12±0.20	1.12±0.20
7	ND	0.28 ±0.01 ^a	0.60±0.02 ^b
14	ND	0.54±0.01 ^a	1.02±0.03 ^b
21	0.05 ±0.01 ^a	0.87±0.03 ^b	1.14±0.02 ^c

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

From the result, the concentration phyto-accumulated increased as the days for germination increased in both seedlings in both contaminated and control soil, however Cd was not detected (results not shown). 22% Zn, 36% Fe, 20% Cu and 12% Pb were accumulated by bean in the crude oil contaminated soil 21 days post germination while 15% Zn, 14% Fe, 24% Cu and 5% Pb were accumulated from Ubeji soil. The maize seedlings accumulated 25% Zn, 15% Fe, 19% Cu and 11% Pb in the crude oil contaminated soil 21 days post germination while 15% Zn, 12% Fe, 19% Cu and 43% Pb were accumulated from Ubeji soil. The percentage increase reduced after 21 days post germination in all the fractions of crude oil

contaminated soil except for Fe and Cu which showed continued increase as the number of days increased in the seedlings. This may be as a result of the high amount of organic matter and the acidic nature of the crude oil contaminated soil (Brandy and Well, 2005, Sparks, 2003). The study indicates that bean seedlings accumulated more Fe and Cu than maize whereas maize seedling accumulated more Zn and Pb than bean in crude oil contaminated soil. The result shows that Fe accumulation was the highest in crude oil contaminated soil while Cu accumulation was highest in Ubeji. The residual concentrations of cadmium, lead, zinc, iron and copper in the soil (mg/kg) with crude oil on which the seeds were planted is presented in Table 6 and 7.

Table 6: Residual concentration of lead, zinc, iron and copper in soil with CCS and control post-harvest of bean seedlings

Days/Sample	Control	CCS	UBEJI
LEAD (Pb)			
0	0.08±0.01 ^a	0.84±0.023 ^b	5.00±0.12 ^c
7	ND	0.14±0.02 ^a	0.31±0.02 ^b
14	ND	0.10±0.04 ^a	0.27±0.02 ^b
21	ND	0.08 ±0.03 ^a	0.15±0.20 ^b
ZINC (Zn)			
0	10.00±0.20 ^a	46.12±0.41 ^b	50.00±1.12 ^c
7	9.20±0.20 ^a	41.00±0.10 ^b	41.30±0.20 ^c
14	8.70±0.20 ^a	37.94±0.87 ^b	39.54±0.10 ^c
21	8.00±0.29 ^a	35.41±0.73 ^b	37.44±0.20 ^c
IRON (Fe)			
0	119.75±0.07 ^a	165.18±0.14 ^b	300.95±0.12 ^c
7	110.20±0.05 ^a	138.40 ±0.10 ^b	287.00±0.20 ^c
14	109.40±0.07 ^a	121.34±0.17 ^b	270.50±0.10 ^c
21	108.28±0.12 ^a	105.18±0.13 ^b	257.40±0.20 ^c
COPPER (Cu)			
0	0.05±0.20 ^a	4.60±0.41 ^b	6.00±0.12 ^c
7	ND	2.70±0.01 ^b	4.40±0.20 ^c
14	ND	1.67±0.07 ^b	3.02±0.10 ^c
21	ND	1.34±0.03 ^b	2.74±0.20 ^c

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

Table 7: Residual concentration of lead, zinc, iron and copper in soil with CCS and control post-harvest of maize seedlings

Days/Sample	Control	CCS	UBEJI
LEAD (Pb)			
0	0.08±0.01 ^a	0.84±0.23 ^b	5.00±0.12 ^c
7	ND	0.14±0.02 ^b	4.40±0.02 ^c
14	ND	0.10±0.04 ^b	3.94±0.02 ^c
21	ND	0.08±0.03 ^b	2.83±0.20 ^c
ZINC (Zn)			
0	10.00±0.20 ^a	46.12±0.05 ^b	50.00±1.12 ^c
7	8.25±0.20 ^a	38.41±0.03 ^b	37.15±0.20 ^c
14	7.36±0.20 ^a	34.20±0.07 ^b	32.10±0.10 ^c
21	6.27±0.29 ^a	32.05±0.03 ^b	27.01±0.05 ^c
IRON (Fe)			
0	119.75±0.20 ^a	165.18±0.41 ^b	300.95±0.12 ^c
7	113.10±0.07 ^b	145.00±0.10 ^b	273.00±0.05 ^c
14	109.14±0.03 ^b	142.34±0.17 ^b	258.67±0.10 ^c
21	104.60±0.05 ^a	130.00±0.03 ^b	220.00±0.05 ^c
COPPER (Cu)			
0	0.05±0.20 ^a	4.60±0.41 ^b	6.00±0.12 ^c
7	ND	4.50 ±0.05 ^b	4.40±0.20 ^c
14	ND	4.17±0.07 ^b	3.02±0.10 ^c
21	0.05±0.02 ^a	3.37 ±0.03 ^b	2.74±0.20 ^c

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

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The results showed that the concentrations of residual metals in the contaminated soil decreased as the number of days increased. This shows that the metals were available for the plant to accumulate. However, Cd was relatively non-detectable (results not shown).

CONCLUSION

The study indicates that *Vigna unguiculata* L (bean) and *Zea mays* L (maize) seeds grown in crude oil contaminated soil had the ability to accumulate some of the metals. The study also indicates that bean seedlings accumulated more Fe and Cu than maize whereas maize seedling accumulated more Zn and Pb than bean

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