

## Reginald Chibueze Ohiri<sup>[a]\*</sup>, Augustine Amadikwa Uwakwe<sup>[a]</sup> and Eugene Nwaogwugwu Onyeike<sup>[a]</sup>

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Variations in mineral element and heavy metal contents of some edible herbs grown on crude oil post remediated agricultural soil was carried out after four weeks of growth on the bioaugmented and natural attenuated soils. The result showed a slight decrease in all the mineral elements analyzed. However, Potassium had the highest concentration 5.5020±0.1056 % and 4.7007±0.6510 % for Tallinium triangulare and Amarantus hybridus planted on the bioaugmented site. Heavy metals concentration increased in all the vegetables after 4 weeks of growth with highest values of 0.0803+0.0006 %, 0.0813+0.0012 % and 0.8170+0.0015 % for Telfeiria occidentalis, Tallinium triangulare, and Amarantus hybridus planted on the natural attenuated site respectively.

Corresponding Authors Ťel: +234 8038808077.

E-Mail: <u>raycohiri@yahoo.com</u> Department of Biochemistry, University of Port Harcourt, P. [a] M. B. 5323 East-West Road, Choba, Rivers State, Nigeria.

## Introduction

Since the rise of agriculture and forestry in the 8<sup>th</sup> millennium B.C., there has also arisen by necessity a practical awareness of soils and their management. In the 18<sup>th</sup> and 19<sup>th</sup> centuries, the industrial revolution brought increasing pressure on soil to produce raw materials demanded by commerce, while the development of quantitative science offered new opportunities for improved soil management.1 This initial inquiry has expanded to an understanding of soil as a complex, dynamic, biogeochemical system that are vital to the life cycle of terrestrial vegetation and soil-inhabiting organisms and by extension to the human race as well. Industrial activities release substantial amount of crude oil and refined products into the environment, as a result of accidents such as storage tank leakage, oil spills during routine transportation and shipping operations or sabotage.<sup>2</sup> The contaminant load of soil and water is growing steadily each year in parallel with increasing industrialization and energy demand and therefore necessitate the need for remediation. Nonhydrocarbon compounds may also be found in crude oil and they include porphyrins and their derivatives.<sup>3</sup> Metals that could be found in crude oil via their association with porphyrins include Nickel, Vanadium, Iron, Zinc, Cobalt, Titanium and Copper.<sup>4</sup> Some priority contaminants of petroleum hydrocarbons and crude oil include Benzene, Heptane, Hexane, Isobutene, Isopentane and poly-aromatic hydrocarbons such as Benzo(a)anthracene, Benzo(a)pyrene, Benzo(b)pyrene etc.<sup>5</sup> Soils subjected to petroleum hydrocarbon bioremediation are used for crop growth without possible consideration to the concentration of the recalcitrant metals and hydrocarbons in the remediated soil.

However, not all contaminants are easily treated by bioremediation using microorganisms. For example, heavy metals such as Cadmium and Lead are not readily absorbed by microorganisms. The assimilation of metals such as Mercury into the food chain may pose a serious threat to human. Phytoremediation is useful in these circumstances, because natural plants and transgenic plants are able to bioaccumulate these toxins in their above ground parts, which can then be harvested for removal.<sup>6</sup> The heavy metals in the harvested biomass may be further concentrated by incineration or even recycled for industrial use.7 The aim of this research is to determine the variations in mineral elements and heavy metal concentrations of vegetables namely: fluted pumpkin (Telfairia occidentalis), water leaf (Tallinium triangulare) and African spinach (Amarantus hybridus) grown on crude oil post-remediated soil.

## **Materials**

Bonny light crude oil was obtained from Shell Petroleum Development Company (SPDC) flow station at Egbema, Imo State, Nigeria. Chicken drops (40 kg) was purchased from Godvine, Poultry Farm, Eliozu Obio Akpor, Rivers State, Nigeria. While, viable seeds of Telfairia occidentalis and healthy seedlings of Tallinium trangulare and Amarantus hybridus were purchased from Rumuokoro market, Obio Akpor Rivers State, Nigeria.

## **Study Area**

The study area was located along Eneka-Oyigbo new link road (longitude 7<sup>0</sup> 10" E and latitude 4<sup>0</sup> 40" N) in Obio Akpor Rivers State, Nigeria. The soil of this area belongs to the ultisols. Approximately the entire area consisted of deep uniform sand and clay sand, with slightly humus topsoil and a topsoil pH of approximately 4.86+0.12. There was no record of oil spillage or pipeline vandalization in the study area.

## Pollution and bioremediation of research site

Approximately 18 m<sup>2</sup> farmland was cleared and divided into three sites of 4  $m^2$  each with 2 m spaces in between them. These sites were polluted with 40 dm<sup>3</sup> of bonny light crude oil and bioremediated for 16 weeks as follows: Site A (Control site) was an unpolluted 4  $m^2$  farmland, while site B (Bioaugmented site) was a 4  $m^2$  farmland polluted with 40 dm<sup>3</sup> of bonny light crude oil and bioaugmented with 40 kg of chicken drops. However, site C (Natural attenuated site) was polluted with 40 dm<sup>3</sup> of bonny light crude oil.

### Planting and growing of vegetables

Viable seeds of *Telfairia occidentalis* (fluted pumpkin), and healthy seedlings *Tallinium Trianglerae* (water leaf) and *Amarantus hybridus* (African spinach) were planted on the three sites and allowed to grow for 4 weeks.

#### **Collection of plant samples**

Plant leaves were collected at fourth weeks after planting with an unused sterilized razor blade into sterilized plastic bags sealed with rubber bands. All samples were labelled with a permanent water-resistance marker and were taken to the laboratory within 1 hour of collection for analysis.

# Determination of mineral element and heavy metal contents of vegetable leaves

Mineral elements and heavy metal contents of vegetables leaves were determined by the emission spectroscopy method.<sup>8</sup>

**Principle:** Sample digests are burned in a carbon arc, causing each element to emit a unique wavelength of light. The density of light emitted by each element is directly proportional to the concentration of the element in the sample.

**Apparatus:** Arc-spark emission spectrography, Analytical weighing balance, Furnace, 10ml Porcelain crucibles, 50ml Burette, Drying oven, Wiley mill, Volumetric flasks and Pipettes.

**Reagents:** Lithium carbonate (LiCO<sub>3</sub>) use for the analysis was obtained from Sigma Aldrich, MO USA.

**Procedure:** A quantity of 1.0 g of dried leaf samples were ground and placed in 10 ml porcelain crucibles. The crucibles were placed in a cool muffle furnace and the samples were ashed at a temperature of 500 °C for 4 hours. The crucibles were removed and allowed to cool. Then 5.0 ml of lithium carbonate buffer was added and swirled gently to dissolve the ash. The digest was transferred to a teflon boat and analysed on a direct reading arc-spark emission spectrography.

## RESULTS

#### Mineral element content of vegetables samples

This study showed pronounced decrease in magnesium, calcium and potassium, while zinc and iron content increased (see Tables 1-3). The decrease in magnesium, calcium and potassium may be due to reduction in nutrient uptake by the vegetables caused by the presence of

petroleum hydrocarbons in the rhizosphere. Petroleum hydrocarbons may affect the uptake of many macro and micronutrients. However, the influence of petroleum hydrocarbons on the mineral element content of plants depends on the type and level of pollution and the species of plants involved.

Table 1. Mineral element content of *Telfairia occidentalis* grown on crude oil bioremediated soil (%, m/m).

	Control site	Bioaugmented	Natural
		site	attenuated site
Mg	$1.8010 \pm 0.1115^{a}$	$1.4027 \pm 0.0361^{b}$	1.4010 <u>+</u> 0.0705 <sup>c</sup>
Ca	2.0033 <u>+</u> 0.0551 <sup>a</sup>	$1.8080 \pm 0.0886^{b}$	1.1033 <u>+</u> 0.0512 <sup>c</sup>
Κ	2.3103 <u>+</u> 0.0823 <sup>a</sup>	$2.0010 \pm 0.0400^{b}$	1.6037 <u>+</u> 0.0210°
Zn	$0.0307 \pm 0.0021^{ac}$	$0.0813 \pm 0.0038^{b}$	$0.0417 \pm 0.0059^{ac}$
Fe	0.0810+0.0066abc	$0.0907 \pm 0.0021^{ab}$	0.0713 <u>+</u> 0.0025 <sup>ac</sup>

Values are means  $\pm$  standard deviations of three determinations. Mean values in each row with different superscripts differ significantly at P = 0.05.

**Table 2.** Mineral element content of *Tallinium triangulare* grown on crude oil bioremediated soil (%, m/m).

	Control site	Bioaugmented	Natural
		site	attenuated site
Mg	3.3353 <u>+</u> 0.2976 <sup>a</sup>	$2.9013 \pm 0.0305^{b}$	2.6347 <u>+</u> 0.5849 <sup>c</sup>
Ca	$1.2007 \pm 0.0400^{ac}$	$1.6010 \pm 0.0790^{b}$	1.3000 <u>+</u> 0.0174 <sup>ac</sup>
Κ	6.3027 <u>+</u> 0. 1910 <sup>a</sup>	$5.5020 \pm 0.1056^{b}$	5.9030 <u>+</u> 0.1305°
Zn	$0.0210 \pm 0.0020^{a}$	$0.0600 \pm 0.0046^{b}$	0.0317 <u>+</u> 0.0038 <sup>c</sup>
Fe	$0.0500 \pm 0.0030^{a}$	$0.0790 \pm 0.0028^{bc}$	$0.0803 \pm 0.0070^{bc}$

Values are means  $\pm$  standard deviations of three determinations. Mean values in each row with different superscripts differ significantly at P = 0.05.

**Table 3.** Mineral element content of *Amarantus hybridus* grown on crude oil bioremediated soil (%, m/m).

	Control site	Bioaugmented	Natural
		site	attenuated site
Mg	3.0057 <u>+</u> 0.0592 <sup>a</sup>	3.2023 <u>+</u> 0.0790 <sup>b</sup>	2.8027 <u>+</u> 0.0615 <sup>c</sup>
Ca	$1.7020 \pm 0.0770^{abc}$	1.9017+0.0595abc	1.6960+0.5513abc
Κ	5.1047+0.1991abc	4.7007+0.0510abc	4.9857 <u>+</u> 0.3095 <sup>abc</sup>
Zn	$0.0613 \pm 0.0049^{ab}$	$0.0813 \pm 0.0025^{abc}$	0.1013+0.015lbc
Fe	0.0907+0.0032ac	0.0683+0.0086 <sup>b</sup>	0.0900+0.0060ac

Values are means  $\pm$  standard deviations of three determinations. Mean values in each row with different superscripts differ significantly at P = 0.05.

Table 4. Heavy metal content of *Telfairia occidentalis* grown on crude oil bioremediated soil (%, m/m).

	Control site	Bioaugmented	Natural
		site	attenuated site
Cu	ND	$0.0217 \pm 0.0035^{b}$	0.0113 <u>+</u> 0.0032 <sup>c</sup>
Pb	ND	ND	0.0813 <u>+</u> 0.0012 <sup>c</sup>
Cr	ND	$0.0503 \pm 0.0070^{b}$	0.0413 <u>+</u> 0.0009 <sup>c</sup>
Cd	ND	ND	0.0113 <u>+</u> 0.0015°
As	ND	ND	ND

Values are means  $\pm$  standard deviations of three determinations. Mean values in each row with different superscripts differ significantly at P = 0.05. 

 Table 5. Heavy metal content of *Tallinium triangulare* grown on crude oil bioremediated soil (%, m/m).

	Control site	Bioaugmented	Natural
		site	attenuated site
Cu	$0.0107 \pm 0.0006^{ac}$	$0.0303 \pm 0.0031^{b}$	$0.0103 \pm 0.0029^{ac}$
Pb	ND	0.1017 <u>+</u> 0.0196 <sup>b</sup>	$0.0803 \pm 0.0006^{\circ}$
Cr	ND	$0.0600 \pm 0.0036^{b}$	ND
Cd	ND	$0.0307 \pm 0.0025^{b}$	0.0107 <u>+</u> 0.0006 <sup>c</sup>
As	ND	ND	ND

Values are means  $\pm$  standard deviations of three determinations. Mean values in each row with different superscripts differ significantly at P = 0.05.

Table 6. Heavy metal content of *Amarantus hybridus* grown on crude oil bioremediated soil (%, m/m).

	Control site	Bioaugmented	Natural
		site	attenuated site
Cu	$0.0200 \pm 0.0030^{ac}$	$0.0417 \pm 0.0076^{b}$	$0.0203 \pm 0.0012^{ac}$
Pb	ND	$0.1007 \pm 0.0075^{b}$	0.0817 <u>+</u> 0.0015 <sup>c</sup>
Cr	ND	$0.0613 \pm 0.0074^{b}$	$0.0407 \pm 0.0042^{\circ}$
Cd	ND	$0.0205 \pm 0.0032^{b}$	0.0110 <u>+</u> 0.0020 <sup>c</sup>
As	ND	$0.0263 \pm 0.0012^{b}$	ND

Values are means  $\pm$  standard deviations of three determinations. Mean values in each row with different superscripts differ significantly at P = 0.05.

This also agrees with the work of  ${}^{9}$ , which stated that small concentration of diesel oil raised the content of nitrogen, potassium and sodium in oat, whereas higher concentrations depressed these elements. This may be responsible for the decrease in magnesium, calcium and potassium contents of the vegetables as observed in this study. However, the increase in iron and zinc content as observed in this study may be attributed to the availability of these nutrients in the soil caused by their presence in the crude oil. This supports the work of,<sup>4</sup> which reported iron and zinc among the metals that are present in crude oil through their association with porphyrins.

The increase in heavy metal content observed in the bioaugmented and natural attenuated site may also be attributed to the presence of these metals in the crude oil (see Tables 4-6). This also corroborates the work of,<sup>4</sup> which also reported that heavy metal such as copper and nickel are found in crude via their association with porphyrins.

This work also agrees with the work of <sup>10</sup>, where an increase in cadmium concentration was observed in crude oil polluted arable soils in Isikwuato, Abia State, Nigeria. However, the concentration of cadmium observed in this research is below the Environmental Protection Agency (EPA) recommended level of 0.1mgkg<sup>-1</sup> as critical level form soil in Taiwan.<sup>11</sup>

## References

- <sup>1</sup>Sposito, G. *The chemistry of soils*, 2<sup>nd</sup> Edition, Oxford University Press, **2008**.
- <sup>2</sup>Gonza'Lez, J. L., Serrano, A., Gallego, M. and Tejada, M. J., J. *Environ. Pollut.* **2008**, *144*, 203-209.
- <sup>3</sup>Callot, H. J., Ocampo, R. Geochemistry of porphyrins. In Kadish, K. M., Smith, M., Guilard, B. (Editors.), The Porphyrin Handbook vol. 1. Academic Press San Diego, 2000.
- <sup>4</sup>Chicarelli, M. I, Eckardt, C. B., Owen, C. R., Maxwell, J. R., Eglington, G., Huston, R. C., Eaton, A. N., *J. Org. Geochem.* **1990**, *15*, 266-274.
- <sup>5</sup>Onwurah, I. N. E., Ogugua, V. N., Onyike, N. B., Ochonogor, A. E., Otitoju, O. F., *Int. J. Environ. Res.*, **2007**, *1*(4), 307-320.
- <sup>6</sup>Meagher, R. B., Curr. Opinion Plant Biol., 2000, 3(2), 153-162.
- <sup>7</sup>Hannik, N., Rosser, S. J., French, C. E., Basran, A., Muray, J. A., Nicklin, S., Bruce, N. C., *J. Nat. Biotechnol.*, **2001**, *19*(12), 1168-1172.
- <sup>8</sup>Jones, J. B. Jr., J. Assoc. Off. Anal. Chem., 1975, 58, 764-769.
- <sup>9</sup>Ziolkowskai, L., Wyszkowskii, H., J. Ecol. Chem. and Eng. **2010**, 17(1), 37-46.
- <sup>10</sup>Onweremadu, E. U., Duruigbo, C. I., *Int. J. Environ. Sci. Tech.*, **2007**, *4*(3), 363-366.
- <sup>11</sup>Environmental Protection Agency (EPA), Final reports of heavy metal contents in Taiwan agricultural soils, vol. 4. Tapei, Taiwan. 1989.

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