VARIATIONS IN NUTRITIONAL POTENTIALS OF SOME VEGETABLES GROWN ON CRUDE-OIL CONTAMINATED AND REMEDIATED AGRICULTURAL SOIL.

Reginald Chibueze Ohiri[a]*, Augustine Amadikwa Uwakwe[a] and Eugene Nwaogwugwu Onyeike[a]

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Variations in nutritional potentials of some edible vegetables 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 variations in both the vitamin content and proximate composition of the vegetables. *Telfairia occidentalis* grown on bioaugmented site had the highest concentration of vitamins B_2, B_3 and E with an increase of 16.5425 % for vitamin B_2 and decrease of 7.8747 % and 6.6143 % for vitamins B_3 and E, while *Tallinium triangulare* grown on natural attenuated site had the highest concentration of vitamins A, B_1, B_2, B_3, B_6, C and E with decrease of 12.7558 %, 5.3239 %, 6.2900 %, 2.4000 %, 2.8834 %, 0.1508 %, and 21.4117 %. Moreover *Amarantus hybridus* grown on the natural attenuated site had the highest increase in vitamins A, B_1, $B_{6,}$ C and E with decrease of 5.4216 %, 16.2200 %, 4.7159 %, 1.7580 % and 3.5965 %. Proximate composition of the vegetables showed that *Telfairia occidentalis* grown on bioaugmented site had the highest concentration of lipid, carbohydrate, moisture, and crude fibre with increase of 125.1487 %, 3.0111 %, 3.0600 % and 6.2500 % respectively, while *Tallinium triangulare* grown on bioaugmented site had the highest concentrations of lipid, carbohydrate and ash with increase of 430.0000 %, 27.7592 % and 31.2846 % respectively. However, *Amarantus hybridus* also grown on bioaugmented site had the highest concentrations of lipid, carbohydrate and moisture with increase of 349.9631%, and 1.4593% for lipid and moisture, while carbohydrate had a decrease of 12.4762% when compared to their respective controls.

Corresponding Authors
Tel: *+234 8038808077.*
E-Mail: raycohiri@yahoo.com
[a] Department of Biochemistry, University of Port Harcourt, P. M. B. 5323 East-West Road, Choba, Rivers State, Nigeria.

Introduction

The soil is a biologically active porous medium that developed in the uppermost layer of the earth's crust. It is one of the principal substrata of life on earth, serving as a reservoir of water and nutrients, as a medium for the filtration and breakdown of injurious wastes and as a participant in the cycling of carbon and other elements through the global ecosystem. Since the rise of agriculture and forestry in the 8th millennium B.C., there has also arisen by necessity a practical awareness of soils and their management. In the 18th and 19th 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 the 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. The presence of toxic compounds in soil has increased dramatically by the accelerated rate of extraction of minerals and fossil fuels and by highly technological industrial processes[1]. Notable among these extractable toxic fossil fuels are the petroleum hydrocarbons.

Petroleum like all fossil fuels primarily consists of complex mixtures of hydrocarbons. Petroleum hydrocarbons are composed of various portions of alkanes (e.g. methane, ethane, etc), aromatics (e.g. benzene, toluene, ethylene and xylene, collectively known as BTEX) and polycyclic aromatics hydrocarbons (PAHs) (e.g. naphthalene, phenanthrene, anthracene and benzo(α)pyrene, etc). In high concentrations, the hydrocarbons molecules that make up petroleum are highly toxic to many organisms, including humans.[2] The dominance of petroleum products in the world economy creates the conditions for distributing large amounts of these toxicants into populated areas and ecosystem around the globe.[3] 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.[4] 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. The penetration of high doses of petroleum hydrocarbons into plant cells may lead to significant deviations from the norm and in some cases, even to the complete cell destruction and plant death.[5] Plant cells subjected to high doses of these toxicants will be faced with oxidative stress, which will subsequently lead to the protective usage of its antioxidant molecules. Notable among these antioxidant molecules in plants (mainly in the leaves) are Tocopherol (Vitamin E) and Ascorbic acid (Vitamin C) and Dehydroascorbic acid (the oxidized form of ascorbic acid).[6]

Materials

Bonny light crude oil was obtained from Shell Petroleum Development Company (SPDC) flow station at Egbema, Imo State, Nigeria. Chicken drops (40kg) 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 7^0 10" E and latitude 4^0 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 4.8600$\pm$0.1200. There was no record of oil spillage or pipeline vandalization in the study area.

Pollution and bioremediation of research site

Approximately 18 m^2 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^3 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^3 of bonny light crude oil and bioaugmented with 40 kg of chicken drops. However, site C (natural attenuated site) was polluted with 40 dm^3 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 vitamin content of vegetable leaves

Pulverized leaf samples were allowed to attain atmospheric conditions after removing from the storage chamber at 4 ^{0}C. The samples were pressed carefully in different mortars and 0.10 g of each sample was weighed into a 10ml beaker for extraction. The extracted was analysed by the method of the Association of Official Analytical Chemist.[7] The extract was concentrated to 1.0ml volume. The concentrate was injected into a Hewlett and Packard model 5890 Gas Chromatography (GC) powered with Hewlett and Packard Chemstation Rev. A09.01 (1206) software. Vitamin content was determined by a spilt injection method with spilt ratio of 20:1 and nitrogen was used as the carrier gas at an inlet temperature of 250 ^{0}C. A HPS column type of dimension (30 X 0.25)mm X 0.25μm was used. The initial temperature was 50^{0}C and first ramping was done at 10 ^{0}C per minute for 20 minutes and maintained for 4 minutes while second ramping was done at 15 ^{0}C per minute for 4 minutes and maintained for 2 minutes. The detector temperature was 32 ^{0}C while hydrogen pressure and compressed air are 20 psi and 30 psi respectively.

Proximate analysis of vegetable leaves

Proximate analyses of vegetable leaves was carried out by the methods of the Association of Official Analytical Chemist.[7]

RESULTS AND DISCUSSIONS

Vitamin E is presently the most important lipid-soluble antioxidant that protects the cell membranes from oxidation by reacting with free radicals produced by lipid peroxidation chain reaction.[8] Thus, the decrease in vitamin E concentration as observed in this research maybe due to the enhanced use of vitamin E in the prevention of oxidative stress induced by petroleum hydrocarbon (See tables 1-3). However, the oxidized form of vitamin E (alpha-tocopheroxyl radical) produced in this process may be recycled back to the active reduced from through reduction by other antioxidants such as ascorbate and ubiquinol.[9]

Plants are generally good sources of vitamin C (ascorbic acid), but this depends on the precise variety of the plant, the soil condition, the climate in which they grew and the length of time before analysis. However, this study showed a slight decrease in vitamin C content of the vegetables grown on the bioaugmented and natural attenuated site (See tables 1-3). This work agrees with that of,[10] which reported a decrease in vitamin C concentration in *Tallinium triangulare* planted on crude oil polluted site. However, the high concentration of vitamin C as compared to vitamin E may be due to the ability of these vegetables to convert glucose into ascorbic acid through a sequence of four enzyme catalysed steps[6]. The decrease in vitamin B complex of vegetables grown on the bioaugmented and natural attenuated sites as compared to their control may be attributed to the reduction in plant nutrients caused by the presence of recalcitrant hydrocarbons in the rhizosphere of vegetables grown on both the bioaugmented and natural attenuated sites. Vitamin B_1 (thiamine) is considered as an "anti stress" vitamin because it may strengthen the human immune system.[11]

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

	Control site	Bioaugmented site	Natural attenuated site
Vit. A	4.6816$\pm$0.0365^a	4.8698$\pm$0.0298^{bc}	4.9060$\pm$0.0066^{bc}
Vit. B_1	7.1452$\pm$0.0052^a	7.0132$\pm$0.0008^{bc}	7.0182$\pm$0.0020^{bc}
Vit. B_2	1.5034$\pm$0.0042^a	1.7521$\pm$0.0019^b	1.5675$\pm$0.0075^c
Vit. B_3	1.8515$\pm$0.0045^a	1.7057$\pm$0.0071^b	1.6444$\pm$0.0010^c
Vit. B_6	4.4349$\pm$0.0006^{ab}	4.4823$\pm$0.0178^{abc}	4.4968$\pm$0.0249^{bc}
Vit. C	64.0402$\pm$0.4357^a	62.0920$\pm$0.0358^{bc}	62.4789$\pm$0.0286^{bc}
Vit. E	7.4838$\pm$0.0324^a	6.9888$\pm$0.0823^b	6.7104$\pm$0.0022^c

Values are means $\pm$ standard deviations of three determinations. Mean values in each row with different superscripts (either a, b or c) differ significantly at $P = 0.05$.

Proximate analysis of vegetable samples grown on the bioaugmented and natural attenuated sites showed a pronounced decrease in protein content and a slight decrease in ash content when compared to their control (See tables 4-6). The decrease in protein content may be as a result of oxidative stress induced by the presence of petroleum hydrocarbons in these vegetables. This may have interfered with the protein synthesis or an increase in protein oxidation and destruction of plant cells which may possibly lead to a decrease in protein content of these vegetables. The results of this study corroborates that of,[12] where a progressive reduction in the mean concentrations of protein thiol and total thiol attributed to increased oxidative protein damage due to the reactive intermediates from spent engine oil was reported.

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

	Control site	Bioaugmented site	Natural attenuated site
Vit. A	3.4259±0.0064^{a}	2.9507±0.0043^{b}	2.9889±0.0118^{c}
Vit. B$_1$	5.0876±0.0189^{a}	4.6095±0.0024^{b}	4.7879±0.0315^{c}
Vit. B$_2$	4.7663±0.0107^{a}	4.3940±0.0236^{b}	4.4665±0.0376^{c}
Vit. B$_3$	1.3625±0.0027^{ac}	1.3071±0.0002^{bc}	1.3298±0.0341^{abc}
Vit. B$_6$	2.3028±0.0003^{a}	2.1626±0.0046^{b}	2.2364±0.0038^{c}
Vit. C	43.6426±0.0770^{abc}	43.5302±0.0029^{abc}	43.5768±0.0298^{abc}
Vit. E	1.6944±0.0074^{a}	1.2507±0.0041^{b}	1.3316±0.0043^{c}

Values are means ± standard deviations of three determinations. Mean values in each row with different superscripts (either a, b or c) differ significantly at P = 0.05.

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

	Control site	Bioaugmented site	Natural attenuated site
Vit. A	3.2813±0.0431^{a}	3.0947±0.0079^{bc}	3.1034±0.0490^{bc}
Vit. B$_1$	2.9630±0.0664^{a}	2.0620±0.0002^{b}	2.4824±0.0380^{c}
Vit. B$_2$	4.8750±0.1123^{ab}	4.7045±0.0270^{ab}	4.4312±0.0013^{c}
Vit. B$_3$	1.7239±0.0005^{a}	1.5343±0.0007^{b}	1.4506±0.0066^{c}
Vit. B$_6$	2.8075±0.0044^{a}	2.5303±0.0020^{b}	2.6751±0.0102^{c}
Vit. C	25.9443±0.0224^{a}	25.1470±0.1288^{b}	25.4882±0.0178^{c}
Vit. E	6.2228±0.0024^{a}	5.2987±0.0587^{b}	5.9990±0.0806^{c}

Values are means ± standard deviations of three determinations. Mean values in each row with different superscripts (either a, b or c) differ significantly at P = 0.05.

Table 4. Proximate composition of *Telfairia occidentalis* grown on crude oil bioremediated soil (%, m/m).

	Control site	Bioaugmented site	Natural attenuated site
Proteins	3.2000±0.0500^{a}	2.7000±0.0100^{b}	3.1100±0.0755^{c}
Lipids	1.1933±0.0902^{a}	2.6867±0.0833^{bc}	2.6000±0.0800^{bc}
Carbohydrates	6.3100±0.3000^{abc}	6.5000±0.0110^{abc}	6.3000±0.3300^{abc}
Moisture	81.7000±0.2000^{ac}	84.2000±0.5300^{b}	81.4000±0.4400^{ac}
Crude fibre	3.4100±0.2152^{abc}	3.3000±0.0600^{abc}	3.1000±0.0400^{abc}
Ash	3.2000±0.0240^{ac}	3.4000±0.0510^{b}	3.2000±0.0112^{ac}

Values are means ± standard deviations of three determinations. Mean values in each row with different superscripts (either a, b or c) differ significantly at P = 0.05.

Table 5. Proximate composition of *Tallinium triangulare* grown on crude oil bioremediated soil (%, m/m) .

	Control site	Bioaugmented site	Natural attenuated site
Proteins	2.2300±0.1044^{a}	1.8000±0.0420^{bc}	2.0100±0.0794^{bc}
Lipids	0.3000±0.0200^{a}	1.5900±0.3606^{b}	1.4367±0.0355^{c}
Carbohydrates	4.2267±0.1168^{a}	5.4000±0.0800^{b}	4.6033±0.0751^{c}
Moisture	89.8133±0.3691^{abc}	88.7000±0.7600^{abc}	89.2000±0.5300^{abc}
Crude fibre	2.1067±0.0208^{ac}	1.6300±0.2858^{b}	2.4000±0.0820^{ac}
Ash	1.3000±0.0320^{ac}	1.7067±0.0603^{b}	1.4033±0.0116^{ac}

Values are means ± standard deviations of three determinations. Mean values in each row with different superscripts (either a, b or c) differ significantly at P = 0.05.

Table 6. Proximate composition of *Amarantus hybridus* grown on crude oil bioremediated soil (%, m/m).

	Control site	Bioaugmented site	Natural attenuated site
Proteins	2.9000±0.0600^{a}	2.3033±0.0208^{b}	2.5933±0.0704^{c}
Lipids	0.4067±0.0710^{a}	1.8300±0.0794^{bc}	1.7033±0.0404^{bc}
Carbohydrates	10.5000±0.1418^{a}	9.1900±0.2066^{bc}	8.9900±0.2751^{bc}
Moisture	82.2033±0.5330^{abc}	83.4029±0.7550^{ab}	82.7744±0.6997^{abc}
Crude fibre	1.3967±0.0153^{a}	1.2100±0.0200^{b}	1.7133±0.0603^{c}
Ash	1.7967±0.0551^{a}	2.1067±0.0351^{b}	2.3033±0.0503^{c}

Values are means ± standard deviations of three determinations. Mean values in each row with different superscripts (either a, b or c) differ significantly at P = 0.05.

However, petroleum contaminants, apart from increasing the concentration of hydrocarbon in the soil can also lead to the predominance of organic carbon over the content of nitrogen in the humus horizon, which can consequently lead to large supply of energy and proportionately decrease in the availability of nitrogen.[13] Thus, strong competition for nitrogen occurs between microorganisms that are degrading the hydrocarbons and the plant root systems. This competition may result to acute shortage of nitrogen for plants, thereby minimizing or inhibiting the plant growth. This may also be responsible for the decrease in plant protein content and slight increase in carbohydrate content as observed in this research.

The increase in lipid content observed in these vegetables may be due to the ability of these vegetables to absorb and bioaccumulate petroleum hydrocarbon in their aerial parts (see tables 4-6). This also corroborates the work of, [13] which reported that contamination of soil with petroleum substances may not weaken the vegetative development of crops but can cause accumulation of hydrocarbon in plants.

Petroleum hydrocarbons can also have a direct effect by producing oily films on aerial parts of the plants, thus reducing plant transpiration and respiration thereby decreasing membrane permeability, thus causing disorders in the metabolic processes, which may result in some modifications in the chemical compositions of plants.[13] This may be responsible for the increase in lipid content of the vegetables grown on both the bioaugmented and the natural attenuated sites.

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