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Evaluating the use of spiny pigweed (*Amaranthus Spinosus)* and Water Leaf (*Talinum Triangulare)* for Bioremediation of Crude Oil polluted Soil in Ikarama Community in Bayelsa State Nigeria.

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ABSTRACT: The potential soil amending impact of various concentrations of macerated roots of *Amaranthus spinosus* and *Talinum triangulare* singly and in combination on crude oil polluted soil of Ikarama community of Yenagoa in Bayelsa State Nigeria was investigated using gas chromatography technique for twelve weeks. The polluted soil was bagged in seven groups with the addition of 250g of *Amaranthus spinosus* root, 500g of *Amaranthus spinosus* root, 250g of *Talinum triangulare* root, 500g of *Talinum triangulare* root, 250g of combined roots of *Amaranthus spinosus* and *Talinum Triangulare*, 500g of combined roots of *Amaranthus spinosus* and *Talinum Triangulare* and labelled as follows *G*a, Gb, Wa, Wb, GWa and GWb respectively; and a polluted and not amended bag which served as control. Each bag contained 1000g of polluted soil. The Total Petroleum Hydrocarbon (TPH), Polycyclic Aromatic Hydrocarbon (PAH), pH and enzyme concentration were analysed at intervals of four weeks for twelve weeks. The result showed that TPH reduction in the impacted soil varied between 29.5% for Ga and 1.79% for Wa after week 4.The results also showed that PAH reduction varied between 53% for Gb and 14.2% in GWa at week 12 ($p<0.05$). The results suggested that the roots of the plants *Talinum triangulare* and *Amaranthus spinosus* are best used singularly and not in combination in the bioremediation of TPH and PAHs.

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***Keywords*:** *Amaranthus spinosus*, Bioremediation, crude oil, polycyclic aromatic hydrocarbon, *Talinum triangulare*, Total petroleum hydrocarbon.

Crude oils are extremely intricate combination of compounds that vary in composition based on their sources. Non-Hydrocarbons and Hydrocarbons are the major sum of chemical constituents found in crude oil. Hydrocarbons include alkanes which are either cyclic, straight or branched. Benzenes and naphthalene are examples of aromatics found in hydrocarbons. Some compounds containing metals, sulphur, nitrogen and others are examples of Non-Hydrocarbons. (VSDE, 1999). Compounds in crude can majorly be classified into three major groups consisting of saturated hydrocarbons, aromatic hydrocarbons and polar organic compounds (Jain *et al*., 2011).

In high concentrations, compounds in crude oil tend to be very toxic and thus become harmful to the chemical, biological and physical properties of the soil such as benzene and its substituted cycloalkane rings. The presence of high molecular weight compounds with very low solubility in water prevents natural biodegradation process from working efficiently in hydrocarbon contaminated soils (Esin and Ayten, 2011). Oil usually causes anaerobic environment in soil by smothering soil particles and blocking air diffusion in the soil pores, and these affects soil microbial communities (Wang *et al.,* 2013*).* These compounds also penetrate macro-and micro-pores in soil limiting water and air transport that would be necessary for organic matter conversion. Generally, petroleum hydrocarbon compounds bind to soil components and are difficult to remove or degrade (Esin and Ayten, 2011). The daily maximum surface temperature of hydrocarbon-contaminated soils is often higher than that of adjacent control sites. Heavy crude oil pollution can cause complete mortality of marsh vegetation (Wang *et al.,* 2013*).*

Total petroleum hydrocarbon (TPH) is a term usually used to describe a large family of several hundreds of chemical compounds that originally come from crude oil (Alinnor and Nwachukwu, 2013). Mineral oil, hydrocarbon oil, extractable hydrocarbon and oil and grease are sometimes referred to as TPH. (TPHCWGS, 1998). They are called hydrocarbons because most of them are made entirely from hydrogen and carbon. Crude oils can vary in chemical content, and so affects the petroleum products that are made from them. TPH containing products will burn. Some of them easily evaporates and they are usually liquids which are either light coloured or clear while others may not evaporate easily and are usually semi solids, dark or thick liquids. Many of these products have characteristic gasoline, kerosene, or oily odours. Since modern society uses so many petroleum-based products such as gasoline, kerosene, fuel oil, mineral oil, and asphalt, contamination of the environment by them is potentially widespread (VSDE, 1999). The total petroleum hydrocarbons also include saturated alkanes, aromatic hydrocarbons, fuel oxygenated additives such as methyl t-butyl ether (MTBE), butanol and ethanol; and other compounds containing sulphur or nitrogen. These compounds are harmful or even toxic to the growth and development of plants and animals, being a source of long-term water and air pollution (Sudip *et al.*, 2002). Total Petroleum Hydrocarbon analysis is usually carried out wherever

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there is release such as gasoline, fuel or diesel leaks from oil tanks and spills of petroleum products (VSDE, 1999).

So many techniques exist for the measurement of TPH concentration in polluted environment. The concentrations of TPH determined are commonly utilized to confirm sites that need water or soil clean up and this is a common approach implemented by regulatory agencies in the United States. Approximately 75% of the states use TPH-based clean up criteria (TPHCWGS, 1998). These contaminants are derived from mixtures of fuels and crude oil by-products released into the environment through mining, industrial procedures, and accidental spills. (Wislocka, 2006). The fate of Total Petroleum Hydrocarbons in the environment relies on the pattern in which the main hydrocarbons partitions in the environment. Total Petroleum Hydrocarbon is discharged as emissions from industrial processes, or as waste released from private or commercial usage. When TPH is released directly into the water through spills or leaks, certain TPH fractions will float in water and form thin surface films. Other heavier fractions will accumulate as sediment at the bottom of the water, which may affect bottom- feeding fish and organisms. Some of the TPH fractions may be broken down by organisms found in the water (primarily bacteria and fungi).

When Total Petroleum Hydrocarbons are discharged onto the soil, they travel into the groundwater where they degrade into separate compounds, this depends on the chemical property of the compound. The more volatile compounds evaporate while others dissolve and enter the groundwater moving off from the discharge region. Some compounds fasten to soil particles and remain there for long periods; while some may break down to other products by soil organisms. The low molecular weight aliphatic and aromatic fractions which are the more soluble and volatile fractions are more likely to leach to groundwater, volatilize to the air, or biodegrade than the high molecular weight TPH compounds. These higher molecular weight compounds tend to adsorb to the soil and persist at the site of release (VSDE, 1999). Bioremediation is a process by which microorganisms and their products are used to remove or transform contaminants from the soil. Particularly, soil microorganisms of native origin play key roles during soil remediation as biogeochemical agents in the process of mineralization in which organic compounds that are complex are transformed to simpler inorganic compounds (Adams *et al,* 2015). Generally, soil particles have a negative charge, and microbes are usually absorbed into the particles of the soil by the action of polyvalent cations via the mechanism of ionic exchange. (Adams *et al,* 2015). Bioremediation technology uses microorganisms to reduce, eliminate, contain, or transform to benign contaminants present in soils, sediments, water, and air. Bioremediation is described as the use of microorganisms to destroy or immobilize waste materials. This process of detoxification targets the harmful chemicals by mineralization, transformation, or alteration. For centuries, civilizations have used natural bioremediation in wastewater treatment, but intentional use for the reduction of hazardous wastes is a more recent development.

Talinum triangulare commonly known as waterleaf can be described as an erect perennial herb with swollen roots and succulent stems, 30-100 cm tall. The branches have two lateral basal buds. The leaves are spirally arranged to nearly opposite, often crowded at the top of the stem. The waterleaf grows fast and once it restores efficiently it reseeds itself. Talinum starts to flower quickly year-round, and is mainly self-pollinating. The flowers are pink in color and open in the morning. *Amaranthus spinosus*, sometimes called spiny pigweed, is a troublesome weed of vegetables, row crops, and pasture in warm climates. Also called spiny amaranth, it is an erect, often bushy, much-branched summer annual, growing to heights of 2–5 feet. Stems and leaves are smooth and hairless, sometimes shiny in appearance. Like other pigweeds, spiny amaranth develops a strong taproot with a network of fibrous feeder roots. The taproot may or may not be distinctly reddish in colour. (Onwurah *et al.,* 2007).

Ikarama community is located in Yenegoa Local Government Area in Bayelsa state Nigeria.
The continual occurrence of oil spills in Ikarama community cannot be overemphasized. The oil spill used for this study took place on the night of October 4th, 2014 inside Okordia manifold which spread out of the gate into the community environment including those spewing into the air.

The impact of crude oil soil contamination in relation to TPH and PAH content in addition to increased land use due to urbanization, industrialization and agriculture brings to fore the need to use environmental friendly methods such as the use of macerated roots of *Talinum triangulare* and *Amaranthus spinosus* instead of planting crops that would lead to bioaccumulation and eventual long time health consequences on both plants and humans. Therefore, the aim of this research is to identify the effect of soil amendment on high molecular weight total petroleum hydrocarbon and polycyclic aromatic hydrocarbons of crude oil contaminated soil from Ikarama community of Yenagoa L.G.A in Bayelsa state using macerated roots of *Amaranthus spinosus* and *Talinum triangulare*.

MATERIALS AND METHOD

All reagents used in this research were of analytical grade and standards.
The soil samples were collected from a crude oil spill site in Okordia Manifold at Ikarama Community of Yenegoa Local Government Area in Bayelsa state, Nigeria.

Plant Materials: Amaranthus spinosus root sample were collected from plants samples of *Amaranthus spinosus* from various non-polluted bush sites in Rivers state and Imo state, while *Talinum triangulare* root samples were collected from *Talinum triangulare* farmers in Rivers state and Imo state.

*Methods***:** *Preparation Of Soil And Root Samples:* Collected soil samples which were already hardened were loosened and sieved to fine particles while the roots of the plants were washed and ground with manual blender.

Experinmental Design: A part of the sieved soil particles was taken and analysed for TPH and PAH concentrations before the commencement of the work. Then the soil was divided into seven (7) bags of 1000g each in which the following quantity of root samples were added and labeled as follows:
Ga –250g of *Amaranthus spinosus* root
Gb – 500g of *Amaranthus spinosus* root
Wa – 250g of *Talinum triangulare* root
Wb – 500g of *Talinum triangulare* root
GWa -250g of combined roots of *Amaranthus spinosus* and *Talinum Triangulare*.
GWb -500g of combined roots of *Amaranthus spinosus* and *Talinum Triangulare*.

Control -polluted and not amended
TPH, PAH, enzymatic activities and pH were analysed for twelve weeks at four weeks interval.

Determination of Ph of Soil Samples: The soil sample (20g) was placed in a beaker and 20ml of distilled water was added and stirred. This was allowed to stand for 30minutes to 1 hour. The pH meter was calibrated with standards and then dipped into the sample to take readings.

*Determination of Petroleum Activity***:** This was determined by the method of Cohen *et al* (1970).
About 2g of soil sample was weighed into a clean extraction container. 10ml of extraction solvent (dichloromethane, DCM) was added into the sample, mixed thoroughly and allowed to settle. The mixture was carefully filtered into a clean solvent extraction bottle. The extracts were then concentrated to 2ml and subsequently transferred for separation.

A moderate packed glass wool (1cm) was placed at the bottom of 10mm ID X 250mm loup chromatography column. Slurry of 2g of activated silica gel in 10ml methylene chloride was prepared and placed in the chromatographic column. To the top of the column was added 0.5cm of sodium sulphate. The column was rinsed with additional 10ml of methylene chloride.

The column was pre-eluted with 20ml of dichloromethane which flowed through the column at a rate of about 2 minutes until liquid in the column was just above the sulphate layer. Immediately 1ml of the extracted sample was transferred into the column. The extracted bottle was rinsed with 1ml of dichloromethane and added to the column as well.

The stop – cork of the column was opened and the eluent was collected with graduated cylinder. Just prior to exposure of the sodium sulphate layer to air dichloromethane was added to the column in 1-2ml increments. Accurately measured volume of 8-10ml of the eluent was collected and was labeled TPH/PAHs.

The concentrated petroleum fractions were transferred into labeled glass vials with teflon rubber crimp caps for G C analysis.

About 1µl of the concentrated sample was injected by means of hypodermic syringe through a rubber septum into the column. Separation occurs as the vapour constituent partition between the gas and liquid phases. The sample was automatically detected as it emerges from the column (at a constant flow rate) by the FID detector whose response is dependent upon the composition of the vapour.

*Determination of Peroxidase Activity***:** Peroxidase activity was analyzed using the method of titration described by Alef and Nannipren (1995).

About 5g of air dried sample was added 100ml of methanol and allowed to stand for 30 minutes after vigorous shaking. 20ml of the extract was pipetted into a conical flask. The extract was acidified by adding 5 drops of dilute sulphuric acid (H_2SO_4), 2 drops of methylene – blue indicator was added, and the content of the flask was titrated against $0.1ml^{-1}$ $KMnO_4$ until solution turns light purple. The sample hydrogen peroxidase was expressed as $0.1ml^{-1}KMnO_4$ dry soil.

Calculation

$C_KV_K = C_{HP}V_{HP}$

Where:
C_K = Concentration of $KMnO_4$
V_K = Volume of $KMnO_4$
C_{HP} = Concentration of hydrogen peroxidase
V_{HP} = Volume of hydrogen peroxidase

Determination of Catalase Activity: Catalase activity was analyzed using the method described by Cohen et al, 1970. It relies on the measurement of the rate of

decomposition of hydrogen peroxide (H_2SO_4) after the addition of material containing the enzyme by reacting it with excess potassium tetraoxomanganate (VII), $KMnO_4$and then measuring the residual spectrophotometrically at 480 nm.

Statistical Analysis: Data analysis was performed using the Statistical package for the Social Sciences software (SPSS, version 11.0). Data is displayed in mean + SD. The statistical method of one way analysis of variance (ANOVA) was used to compare the mean values obtained among different groups. Differences were considered significant whenever the p-value is P<0.05.

RESULTS AND DISCUSSION

Total Petroleum Hydrocarbon (Tph): The result for the TPH determination of the soil sample are summarized in the Table 1 shown below. The table indicated that the mean TPH concentration for soil of the control ranged from 6795.89±1.09 to 3767.52±3.62 from week 0 to week 12.

The Table 1 also showed that the mean TPH concentration of soil amended with Ga ranged from 4781.86±2.09 to 3002.92±2.97 Gb 5805±0.76 to 4313.06±3.04, Wa 6674.21±1.37 to 4890.17±3.28 and Wb 5832.61±5.35 to 4323.89±3.78, Gwa 5784.32±8.04 to 4586.73±3.78, Gwb 5543.98±4.15 to 3938.78±3.14, indicating that the TPH amended with Ga, Gb, Gwa, Gwb, Wa and Wb were significantly (p<0.05) reduced, relative to the contaminated soil sample

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Table 1: The Mean Tph Concentration Of Soil Amended With Plant Samples**.**

	Ga 250g	Gb 500g	GWa 250g	GWB 500g	Wa 250g	Wb 500g	Control
WEEK 4	4781.86±2.09[b]	5805±0.76[b]	5784.32±8.04[b]	5543.98±4.15[b]	6674.21±1.37[b]	5832.61±5.35[b]	5247.09±2.25[a]
WEEK 8	3929.15±2.81[b]	4838.04±2.65[b]	5614.62±2.42[b]	4683.79±2.58[b]	5082.62±1.94[b]	4559.49±2.12[b]	4366.29±2.34[a]
WEEK 12	3002.92±2.97[b]	4313.06±3.04[b]	4586.73±3.78[b]	3938.78±3.14[b]	4890.17±3.28[b]	4323.89±3.78[b]	3767.52±3.62[a]

Table 1: showing the mean (± S.D) TPH values of crude oil contaminated soil samples amended with Ga *Amaranthus spinosus* 250g, Gb *Amaranthus spinosus* 500g, Wa *Talinum triangulare* 250g, Wb *Talinum triangulare* 500g, Gwa both *Talinum triangulare* and *Amaranthus spinosus* 250g and Gwb *Talinum triangulare* and *Amaranthus spinosus* 500g; where Total TPH in polluted soil at week 0 was 6795.89±1.09.

Polycyclic Aromatic Hydrocarbon (Pah)**:** The result of the PAH determined for each of the sampled soil is shown in Table 2. The result indicated that the mean PAH concentration for soil of the control ranged from 12.05±0.36 to 7.37±1.54 from week 0 to week 12.

The Table 2 below also show that the mean PAH concentration of soil amended with Ga ranged from 9.3±1.08 to 6.1±0.47 Gb 8.7±0.49 to 5.66±0.5, Wa 10.24±0.87 to 7.52±0.43, Wb 7.03±1.0 to 6.68±1.27, Gwa 8.99±0.65 to 10.34±0.39 and Gwb 11.32±0.52 to 7.9±0.43 indicating that the PAH amended with Ga, Gb, Gwb, Wa and Wb were significantly (p<0.05) reduced, relative to the contaminated soil sample except GWa which showed an increase in PAH concentration.

Table 2: The Mean Pah Concentration of Soil Amended With Plant Samples

	Ga 250g	Gb 500g	GWa 250g	GWB 500g	Wa 250g	Wb 500g	CONTROL
WEEK 4	9.3±1.08[b]	8.7±0.49[b]	8.99±0.65[b]	11.32±0.52[a]	10.24±0.87[b]	7.03±1.0[b]	10.76±0.75a
WEEK 8	8.8±1.04b	8.15±0.52b	7.76±0.6b	8.5±0.46b	6.71±0.92b	7.06±0.96b	7.92±2.8a
WEEK 12	6.1±0.47[b]	5.66±0.5[b]	10.34±0.39[b]	7.9±0.43[b]	7.52±0.43[b]	6.68±1.27[b]	7.37±1.54a

Table 2: Table showing the mean (± S.D) PAH values of crude oil contaminated soil samples amended with Ga *Amaranthus spinosus* 250g, Gb *Amaranthus spinosus* 500g, Wa *Talinum triangulare* 250g, Wb *Talinum triangulare* 500g, Gwa both *Talinum triangulare* and *Amaranthus spinosus* 250g and Gwb *Talinum triangulare* and *Amaranthus spinosus* 500g; where Total PAH in polluted soil at week 0 was 12.05±0.36.

The result of the soil pH determined for each of the week are schematically shown in Table 3 below. The Table 3 below show that the mean pH of the soil sample from the control weeks were 6.47±0.03 for week 4 and 6.40±0.12 for week 8.

The soils amended with Ga had a mean pH range of 8.1±0.00 to 8.1±0.00 as against a mean pH range of 6.8±0.00 to 6.87±0.003 for same plant but with increased concentrations (250g to 500g) indicating that the pH of Ga amended soil samples were significantly (p<0.05) reducing as the concentration increases, relative to the contaminated soil samples.

The soils amended with Wa had a soil mean pH range of 8.27±0.03 to 8.33±0.03 and Wb 7.57±0.03 to 7.60±0.00, indicating that the pH of the Wa decrease as the concentration of the plant increase (250 to 500) and samples were significantly elevated with respect to the weeks.

Finally, the soil amended with the combination of both plants, had a soil mean pH of 7.27±0.03 to 7.20±0.00 and 7.30±0.00 to 7.20±0.00 indicating a reduction as the concentration of the plant increase (250g to 500g).

Table 3: The Soil Ph Concentration of Soil Amended With Plant Samples

	Ga 250g	Gb 500g	Wa 250g	Wb 500g	GWa 250g	GWb 500g	CONTROL
WEEK 4	8.1±0.00[b]	6.80±0.00[b]	8.27±0.03[b]	7.57±0.03[b]	7.27±0.03[b]	7.30±0.00[b]	6.47±0.03[a]
WEEK 8	8.0±0.00[b]	6.90±0.06[b]	8.40±0.06[b]	7.53±0.03[b]	7.30±0.00[b]	7.20±0.00[b]	6.40±0.12[a]
WEEK 12	8.1±0.00[b]	6.87±0.03[b]	8.33±0.03[b]	7.60±0.00[b]	7.20±0.00[b]	7.20±0.00[b]	6.37±0.03[a]

Table 3: Table showing the mean (± S.E) pH values of crude oil contaminated soil samples amended with Ga-*Amaranthus spinosus* (250g), Gb-*Amaranthus spinosus* (500g), Wa-*Talinum triangulare* (250g), Wb-*Talinum triangulare* (500g), Gwa-both *Talinum triangulare* and *Amaranthus spinosus* (250g) and Gwb-*Talinum triangulare* and *Amaranthus spinosus* (500g).

*Soil Catalase Activity***:** The result of soil catalase analyzed for each of the soil sample are shown in Table 4 below. The Table 4 below showed a mean soil catalase activity of 0.696±0.054 to 0.652±0.046 for the control.
The soil sample amended with Ga 250g has a mean catalase activity rang of 0.301±0.008 to 0.284± 0.002 and 0.172±0.004 to 0.164±0.0023 for Gb (500g) indicating a progressive decrease in activity as the concentration of the plant increases from week 4 to week 12.

Also soil sample amended with Wa 250g had a mean activity range of 0.594±0.02 to 0.582±0.0023 and 0.562±0.0023 to 0.540±0.0023 for Wb (500g) also indicating that catalase activity of soil amended with Wa and Wb was significantly ($p<0.05$) lower relative to the control soil sample.

The soil sample amended with the combination of both Ga and Wa (250g) which is the Gwa range from 0.300±0.0023 to 0.288±0.002 and Gb and Wb (500g) 0.296±0.002 to 0.276±0.002 indicating also a progressive decrease in catalase activity of Gwa and Gwb soil which was significantly ($p<0.05$) lower relative to the control soil sample from week 4 to week 12.

Table 4: The Soil Catalase Activity of Soil Amended With Plant Samples

	Ga 250g	Gb 500g	Wa 250g	Wb 500g	GWa 250g	GWb 500g	CONTROL
WEEK 4	0.301±0.008[b]	0.172±0.004[b]	0.594±0.002[b]	0.562±0.0023[b]	0.300±0.0023[b]	0.296±0.0020[b]	0.696±0.054[a]
WEEK 8	0.293±0.002[b]	0.166±0.005[b]	0.590±0.002[b]	0.544±0.002[b]	0.293±0.002[b]	0.284±0.002[b]	0.680±0.055[a]
WEEK 12	0.284±0.002[b]	0.164±0.002[b]	0.582±0.002[b]	0.540±0.002[b]	0.288±0.002[b]	0.276±0.002[b]	0.652±0.046[a]

Figure 4: Table showing the mean (± S.E) catalase values of crude oil contaminated soil samples amended with Ga: *Amaranthus spinosus* 250g, Gb: *Amaranthus spinosus* 500g, Wa: *Talinum triangulare* 250g, Wb: *Talinum triangulare* 500g, Gwa both *Talinum triangulare* and *Amaranthus spinosus* 250g and Gwb: *Talinum triangulare* and *Amaranthus spinosus* 500g.

*Soil Peroxidase Activity***:** The result of soil peroxidase analyzed for each of the soil sample are shown in Table 5 below. The Table 5 below showed a mean soil peroxidase activity of 0.230±0.004 to 0.187±0.002 for the control from week 4 to week 12.

The soil sample amended with Ga 250g has a mean peroxidase activity range of 0.146±0.0012 to 0.133± 0.0075 and 0.128±0.011 to 0.120±0.0011 for Gb (500g)

Also soil sample amended with Wa 250g had a mean activity range of 0.157±0.0031 to 0.143±0.0020 and 0.148±0.0020 to 0.143±0.0011 for Wb (500g).

The soil sample amended with GWa 250g had a mean peroxidase activity range from 0.156±0.0023 to 0.141±0.0011 and GWb 500g range from 0.130±0.0011 to 0.138±0.002 which also indicates a progressive decrease in peroxidase activity of Ga, Gb, Wa, Wb and Gwa as the percentage concentration of the plant increased in respect to the different weeks except for Gwb 500g which showed an increase in peroxidase activity. Also peroxidase activity of soil sample was significantly ($p<0.05$) lower relative to the control soil sample from week 4 to week 12.
Table 5: Table showing the mean (± S.D) peroxidase values of crude oil contaminated soil samples amended with Ga: *Amaranthus spinosus* 250g, Gb: *Amaranthus spinosus* 500g, Wa: *Talinum triangulare* 250g, Wb: *Talinum triangulare* 500g, Gwa both *Talinum triangulare* and *Amaranthus spinosus* 250g and Gwb: *Talinum triangulare* and *Amaranthus spinosus* 500g.

Table 5: The Soil Peroxidase Activity of Soil Amended With Plant Samples

	Ga 250g	Gb 500g	Wa 250g	Wb 500g	GWa 250g	GWb 500g	CONTROL
WEEK 4	0.146±0.0012[b]	0.128±0.0011[b]	0.157±0.0031[b]	0.148±0.0020[b]	0.156±0.0023[b]	0.130±0.0011[b]	0.230±0.004[a]
WEEK 8	0.136±0.006[b]	0.126±0.0046[b]	0.154±0.0023[b]	0.142±0.0011[b]	0.151±0.0011[b]	0.140±0.0011[b]	0.198±0.009[a]
WEEK 12	0.133±0.0075[b]	0.120±0.0011[b]	0.143±0.0020[b]	0.143±0.0011[b]	0.141±0.0011[b]	0.138±0.0011[b]	0.187±0.002[a]

Since the soil is the ultimate receptor of contaminants; the diffusion and dilution of contaminants especially the heavy molecular hydrocarbons tend to be very difficult. Also natural detoxification or remediation is very slow. Therefore, these contaminants often persist, producing potentially harmful effects on the environment (Park *et al.,* 2006) such as the soil becoming unavailable to grow food. If the contaminated soil is used to grow food, the land will produce lower yields, cause more harm since the lack of plants on the soil will cause increased erosion, change the makeup of the soil and the types of microorganisms that will live in it: thus it is possible for soil pollution to change whole ecosystems (Ashraf *et al.*, 2014). Many techniques can be employed to clean, eliminate, obliterate or sequester these hazardous pollutants from the soil. However, these techniques are usually costly, labor intensive, and often disquieting (Hakeen *et a*l., 2014). The most widely used procedure is the biostimulation of indigenous soil microorganisms by the addition of nutrients, as input of large quantities of carbon sources from hydrocarbon contamination tend to result into rapid depletion of the available pools of major inorganic nutrients such as Nitrogen and Phosphorous (Margesin and Schinner, 2001). Therefore, the application of macerated roots of legume plants known to have remediating properties such as *Amaranthus spinosus* in the bioremediation of heavy metals (Chinmayee et al., 2012) and *Talinum triangulare* for crude oil contaminated soil; (Uwagboe, 2008) on contaminated soil provides a more direct approach where the plant nutrient constituents are impacted directly to amend the soil for the remediation of contaminants.

The results of the soil pH investigation indicates that the pH of all the soil samples amended with Ga 250g, Gb 500g, Gwa 250g, Gwb 500g, Wa 250g and Wb 500g increased significantly ($p<0.05$) relative to the control, indicating that the contaminated soil were acidic in contrast to the amended soil which were alkaline.

The observed acidity in the contaminated soil and subsequent increase in pH in the amended soil was similar to the findings of Olusegun and Ramot (2013) who reported a reduction of pH in soil contaminated with hydrocarbon by artificial stimulation and subsequent increase in pH by the addition of exogenous nutrients.

The amended soil with Ga 250g, Wa 250g, were significantly elevated more than those amended with Gb 500g, Wb 500g in all the weeks, where Gb had a mean pH of 6.8±0.00 and Wb 7.57±0.03 as against the mean value of 8.1±0.00 and 8.27±0.00 observed for the respective soils amended with Ga and Wa. The ability of *Talinum triangulare* to effectively increase the pH of amended soil samples from week 4 to week 12 conformed to the study carried out by Ekpo *et al* (2013) who reported an increase in the pH of crude oil polluted soil in which *Talinum triangulare* was planted for six weeks.

TPH reduction in the impacted soil varied between 29% for Ga, 14% for Gb, 1.79% for GWa, 14% for GWb, 15% for Wa and 18% for Wb after week 4 respectively.

At week 8, the least reduction in TPH of 17% in GWa was recorded, and 28% GWa at week 12 was obtained. Also, the highest value of 42% of Ga at week 4 and 56% of Ga at week 12 were obtained showing that degradation ability of Ga is promising after 12 weeks of remediation period.

The ability of Ga, Gb, Wa, Wb GW and GWb in the containment of Polycyclic Aromatic Hydrocarbons (PAHs) in this study were as follows: The samples recorded the most steady reduction in PAH, 27%, 32% and 53% Gb 500g from week 4 to week 12 and the least reduction in PAH was observed in 14% in Gwa 250g and 34% GWb 500g at week 12 indicating that the degradation ability of Gb after week 12 is promising.

The ability of *Amaranthus spinosus* to amend contaminated soil samples have been shown in similar works. In a study carried out by Wislocka *et al* (2006). *Amaranthus spinosus* was found to extract almost all metals in large amounts when used in phytoremediation. Also, the study done by Chimayee *et al* (2012) showed that the bioaccumulation of copper, lead and cadmium was high in the roots of *Amaranthus spinosus* followed by the stem and leaves and that zinc and chromium remained high in the aerial parts. A steady increase was observed in the bioaccumulation of copper, zinc and cadmium on enhancing the concentration of the corresponding metal in the soil. This may account for the ability of mascerated roots of *Amaranthus spinosus* to amend contaminated soil samples used in this study.

The process of bioremediation mainly depends on microorganisms which enzymatically attack the pollutants and convert them to innocuous products. Bioremediation can be effective only where environmental conditions permit microbial growth and activity (Karigar and Rao, 2011; Zare-Maiven, 2011). The activities of catalase and peroxidase of soil sample amended with Ga, Gb, Gwa, Gwb, Wa and Wb were significantly different from those of the control ($p<0.05$).

The decrease in catalase and peroxidase observed mainly in all from week 4 to week 12 shows that the availability of some nutrients can induce a repressive effect on some catabolic genes responsible for synthesis of catabolic enzymes needed for the degradation of other nutrients. This may be responsible for the decrease in catalase and peroxidase activity observed in the soil of the amended soil as compared to the control.

These agree with similar works of Nwaugo et al, 2007, who observed that soil pollution reduces soil enzymatic activities.

*Conclusion***:** The results suggested that the roots of the plants *Talinum triangulare* and *Amaranthus spinosus* are best used singularly and not in combination in the degradation of TPH and PAHs. Enzymatic activity showed that all forms of the plant roots affected soil enzyme activity with the greatest impact by *Amaranthus spinosus.* Furthermore pH investigation showed increased alkalinity as the experiment progressed from week 4 to week 12. The ability of these roots to remediate could be as result of nitrogen fixation by soil bacteria on the nutritional components of the roots.

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