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PHYTOREMEDIATION OF CRUDE OIL CONTAMINATED SOIL USING BITTER LEAF (*Vernonia amygdalina*) AND WATER LEAF (*Talinum fruiticosum*) IN EFFURUN, NIGERIA

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Abstract
This study was carried out to assess the phytoremediation potential of Vernonia amygdalina (bitter leaf) and Talinum fruiticosum (water leaf) planted in crude oil contaminated soil. Soil samples were collected randomly from a site at the beginning of the rainy season. The experiment was conducted with different concentrations (0.1 and 1%) of crude oil-contaminated soil for 70 days. Soil samples were analysed for total organic carbon (TOC) using Walkey-Black chromic acid wet oxidation method and for total hydrocarbon content using UV spectrophotometer at the beginning and at the end of the experiment. The initial TOC levels in the control soil, 0.1% and 1% crude oil contaminated soil was found to be 0.28, 0.92 and 2.15% respectively. The initial THC levels in the control soil, 0.1% and 1% crude oil contaminated soil was determined to be 3.69 mg/Kg, 35.06 mg/Kg and 238.21mg/Kg respectively. The result showed 55 % growth in V. amygdalina plant height in uncontaminated soil, whereas; V. amygdalina transplants in 1% contaminated soil wilted by the 6th day of the experiment, V. amygdalina transplants in 0.1% contaminated soil died by the 30th day of the experiment, the transplants died. This indicates that the growth of V. amygdalina transplants was affected by the contaminants in the soil. T. fruiticosum transplants indicated a high potential of adaptation in the contaminated soil as shown by the growth after 10, 30 and 70 days of the experiment. These observations indicate that T. fruiticosum is less affected by contaminants than V. amygdalina.

Key Words: *Phyoremediation, V. amygdalina, T. fruiticosum*

Introduction

Soil and ground water pollution as a result of contaminant accumulation is one of the major problems in the world. Contaminants can either be organic, inorganic or even radioactive which are gotten from various sources. Major component of inorganic contaminants are heavy metals (Adriano, 1986., Alloway, 1990) they present a different problem than organic contaminants. Soil microorganisms can degrade organic contaminants, while metals need immobilisation or physical removal (Shivendra and Hardik, 2014).

Soil contamination by crude oil can cause adverse effect on the ecosystem and other soil properties. Methods of oil

pollution remediation in the environment can be done in three ways i.e. physical, chemical and biological (Okohand Trejo-Hernandez*,* 2010).

Phytoremediation is a developing remediation technique that employs the use of plants to decontaminate already contaminated soil and water through various mechanisms. Phytoremediation utilizes physical, chemical, and biological processes to remove, degrade, transform, or stabilize contaminants within soil and groundwater. Phytoremediation can be classified based on the mechanisms involved. They differ in the way plants deal with contaminants (removal, immobilization, degradation), as well as in the type of contaminant that the plant species can target (organic or inorganic contaminant). These mechanisms include, phytoextraction, phytostabilization, phytodegradation, phytovolatilization, rhizodegradation, rhizofiltration (United States Environmental Protection Agency, USEPA, 2000). Phytoremediation is an eco-friendly approach for remediation of contaminated soil and water using plants comprised of two components, one by the root colonizing microbes and the other by plants themselves, which accumulates the toxic compounds to further non-toxic metabolites (Shivendra and Hardik, 2014). Interactions between these plants and microorganisms that live in the soil can also contribute to phytoremediation (Khyde, 2010).

Major advantages reported for phytoremediation as compared to traditional remediation technologies include the possibility of generating less secondary wastes, minimal associated environmental disturbance, and the ability to leave soils in place and in a usable condition following treatment. Cited disadvantages include the long lengths of time required (usually several growing seasons), depth limitations (3 feet for soil and 10 feet for groundwater), and the possibility of contaminant entrance into the food chain through animal consumption of plant material (Ralinda and Miller, 1996). Phytoremediation is also limited by the growth rate of the plants. More time may be required to phytoremediate a site as compared with other more traditional cleanup technologies (USEPA, 2000)

Plants should be selected according to the needs of the application, the contaminants of concern and their potential to thrive on contaminated soil (Kamath *et al*., 2004). Vegetation should be fast growing, hardy, easy to plant and maintain (Kamath *et al*., 2004). In general, TPH values of 15% or greater can result in significant reductions in plant growth and in some cases mortality. It was found that plants pre-grown in clean soil and subsequently transplanted to the contaminated soil grew nearly as well as the control, showing that toxicity was associated with germination and/or early plant growth (Mohebi *et al.,* 2011).

The plants used for this research (bitter leaf and water leaf) were chosen because they are known to be adaptable, easy to plant and fast growing. They are also common plants found in Nigeria.

Materials and Method

Study Area

The sampling site is located in Ugbomoro, a residential community in Uvwie Local Government Area of Delta State, Nigeria found roughly between 5.40’ and 5.50’E longitude, 5.30’ and 5.50’N latitude. The area has two weather seasons; rainy season that lasts from April to October and the dry season which begins in November and ends in March.

Activities in the area include farming, fishing, crude oil exploration, burning of fossil fuels, wood and solid wastes.

Soil Sampling and Preparation

Soils samples (0 – 30 cm depth) were taken randomly from the site and handpicked to remove stones, sticks and dead plant matter and mixed thoroughly to obtain a homogenous soil from which 5 kg was weighed (each) into 9 buckets.

Density of Crude Oil

The mass of a measuring cylinder of capacity 50mL was measured using a weighing balance and tarred to zero, then the crude oil was poured into the measuring cylinder till the 50mL mark and the mass of the crude oil was measured and recorded.

Soil Contamination and Sample Design

Based on the density of the crude oil calculated from the experiment above, different concentrations (1000 mg kg^{-1} (0.1%), 10,000 mg kg^{-1}(1%)) of crude oil was measured and then used to contaminate the soil. A set of three buckets were used for a particular crude oil treatment, this gave a total of six buckets for the crude oil treatment. The plants was planted in sets of three cuttings per bucket for each concentration of crude oil - (0.1% and 1%) - and uncontaminated soil. Additional set that will make the total to be three sets (i.e. 9 buckets) was treated with water only.

Table 1: Sample Design

Contamination/ Vegetation	0%	0.10%	1%
Unplanted	F1 (Control)	F2	F3
Bitter Leaf	M1	M2	M3
Water Leaf	S1	S2	S3

Physico-chemical Soil Properties

The physico-chemical parameters analysed includes; pH, moisture content. Prior to each analysis, the soil samples were sieved through a 2mm mesh sieve. The procedures for the determination of these properties are outlined below.

Determination of Soil pH

The soil sample was sieved through a 2mm mesh to remove coarse soil fraction and 10g of air-dried and sieved soil was accurately weighed into a beaker. Approximately 10 mL of deionized water was added and thoroughly mixed. The mixture was left to stand for an hour and the pH of the soil was measured and recorded. The electrode was rinsed with deionized water and wiped dry with a clean tissue after each reading.

Determination of Soil Moisture Content

The weight of empty crucible (Wc) was measured and recorded, 20 g of the dried soil sample was weighed into the crucible, the weight of the crucible and soil sample (Wcs) was recorded. The crucible containing the sample was placed into the oven, set at 105°C and left to dry for one hour (after temperature reaches 105°C).The crucibles were removed from the oven and allowed to cool in a dry atmosphere for 20 minutes. The weight of the crucible with the dried sample (Wos) was measured and recorded.

The moisture content of the soil was calculated as a percentage of the dry soil weight.

$$\text{Moisture } (\%) = \frac{\text{Wcs} - \text{Wos}}{\text{Wcs} - \text{Wc}} x\ 100$$

Where,

Wc = Weight of empty crucible

Wcs = Weight of crucible plus soil before drying at 105°C

Wos = Weight of crucible plus soil after drying at 105°C

Determination of % Organic Carbon and Organic Matter (Using Walkey-Black Method)

0.5g of sieved soil sample was treated with 10mL of 1N Potassium Dichromate

($K_2Cr_2O_7$) and 20mL of concentrated Tetraoxosulphate (VI) acid (H_2SO_4). The mixture was swirled gently until properly mixed and left to cool. After about 30 minutes, 20mL of distilled water (H_2O) and 5 drops of ortho-phenanthroline indicator were added to the mixture. The mixture was titrated to end point with 0.5N iron sulphate ($FeSO_4$) until the colour changed to red. A blank titration was made in the same manner without the soil to standardize the dichromate.

The results were calculated as follows:

$$\% \text{ Organic Carbon} = \frac{Meq\ FeSO4\ for\ blank - Meq\ FeSO4\ for\ sample \times 0.003\ F \times 100}{Weight\ of\ air-dried\ soil}$$

Correction Factor, F = 1.33
Meq = Normality of solution × volume of solution used
% Organic Matter in soil = % Organic Carbon × 1.729

Determination of Total Hydrocarbon (THC)

2g of air-dried soil was weighed into a beaker and 10mL of xylene was added as an extracting agent and the mixture was shaken with a mechanical shaker at moderate speed for 30 minutes. After shaking, the mixture was filtered and the filtrate was made to mark using xylene and left to stand for 20 minutes.

The filtrate was placed in a plastic cuvette and the sample was run in the UV-Vis (Ultraviolet) Spectrophotometer at a wavelength of 420nm after a blank sample and standard (1000ppm) was run.

Using the value for absorbance, the THC value was calculated using the following equation;

$$\text{THC (mg/kg)} = \frac{Absorbance\ of\ sample - Absorbance\ of\ blank}{Absorbance\ of\ standard - Absorbance\ of\ blank} \times Concentration\ of\ standard \times Dilution\ Factor$$

Concentration of Standard = 1000ppm
Dilution Factor = 10mL/2g = 5mL/g

Results

The initial levels of TOC and TPH/Oil and Grease of the uncontaminated and crude oil contaminated soil from were determined and are shown in table 2. The percentage changes in TOC and THC content in each treatment shown by tables 3 and 4 and represented by figures 1 and 2 respectively.

Table 2: Initial Levels of Total Organic Carbon (TOC) Content and Total Hydrocarbon Content (THC)

CONTAMINATION	TOTAL ORGANIC CARBON (%)	TOTAL HYDROCARBON CONTENT (mg/Kg)
0%	0.28	3.69
0.10%	0.92	35.06
1%	2.15	238.21

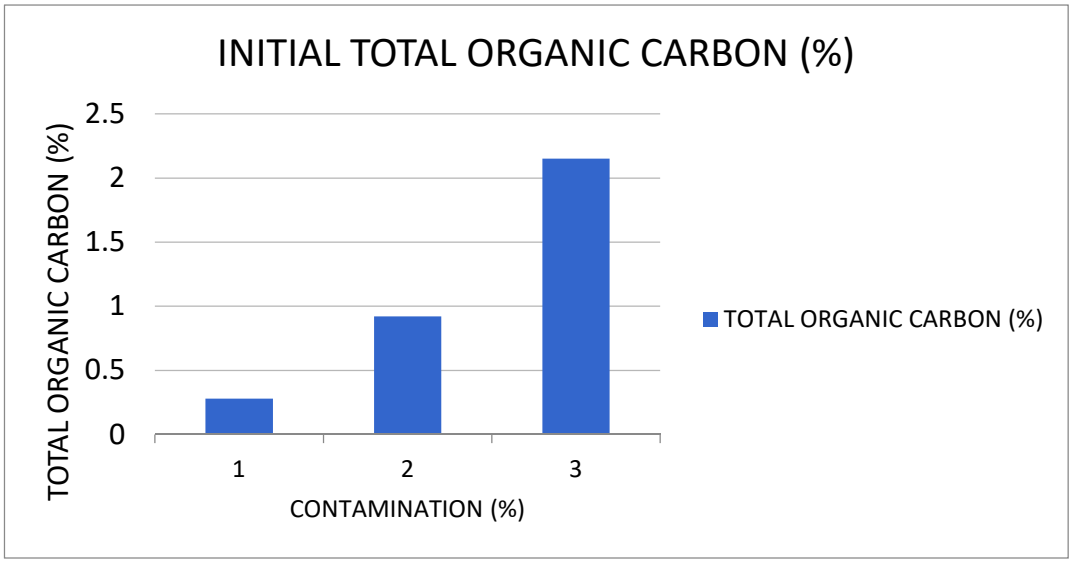

Fig. 1: Initial Total Organic Carbon

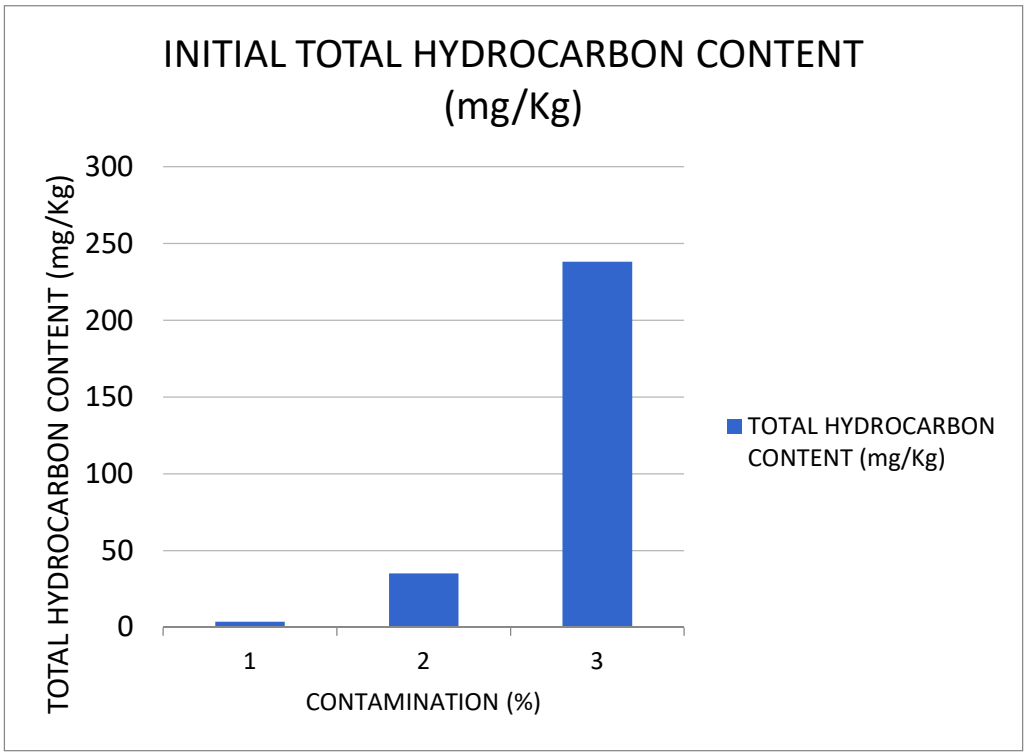

Fig. 2: Initial Total Hydrocarbon content

Table 3: Percentage Loss in Total Organic Carbon (TOC) Content

CONTAMINATION/ PLANT TREATMENT	0%	0.10%	1%	Mean	Standard deviation
Bitter Leaf	99.7	14.92	22.83	45.82	46.83
Water Leaf	76.15	17.63	32.61	42.13	30.40
Unplanted	54.2	12.89	7.16	24.75	25.66

Table 4: Percentage Loss in Total Hydrocarbon Content (THC)

CONTAMINATION/ PLANT TREATMENT	0%	0.10%	1%	Mean	Standard deviation
Bitter Leaf	42.86	48.91	27.44	39.74	11.07
Water Leaf	32.14	55.43	51.16	46.25	12.40
Unplanted	21.43	45.65	48.84	38.64	14.99

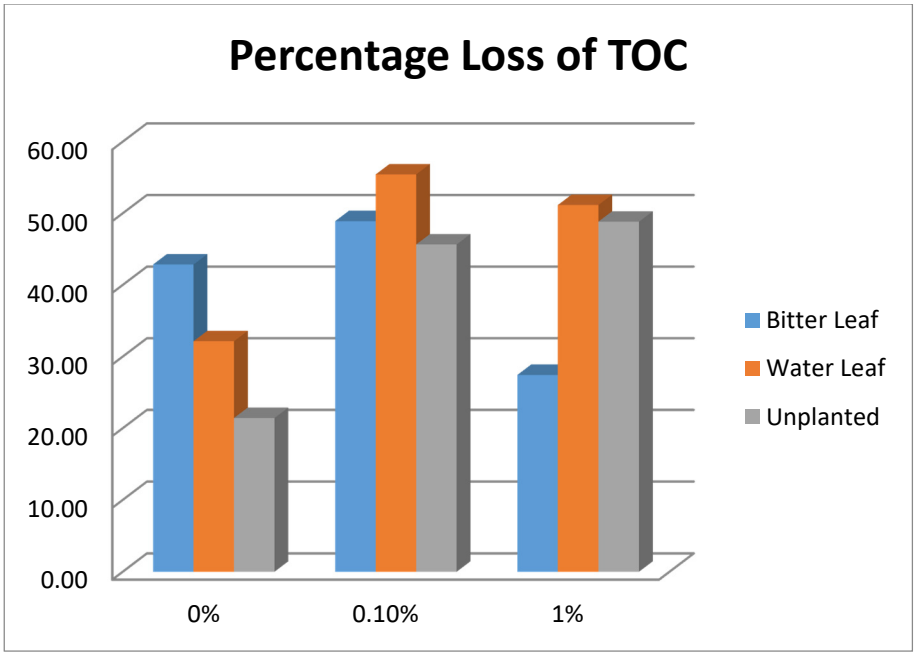

Fig. 3: Percentage Loss in Total Organic Carbon (TOC) Content

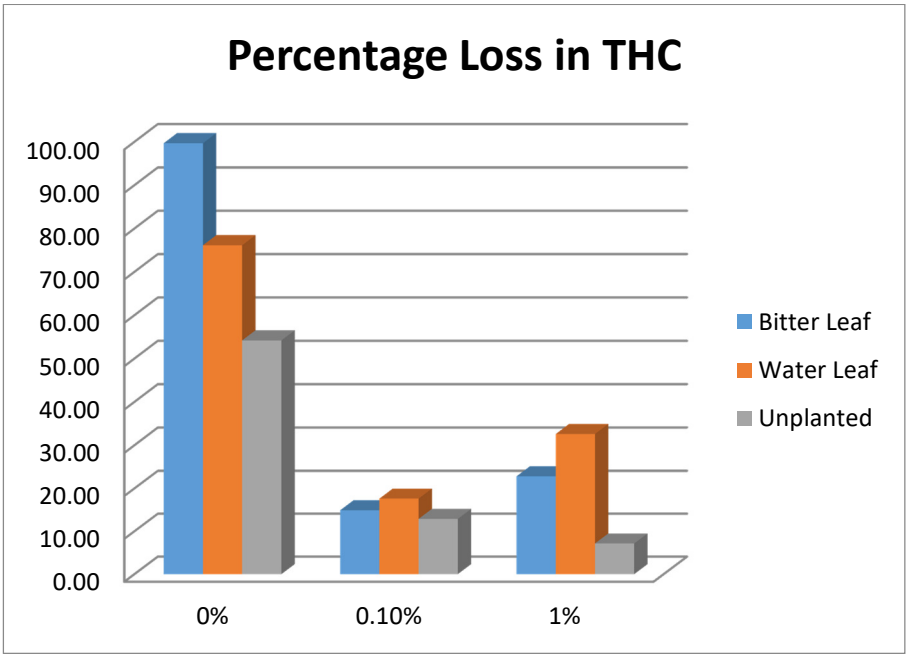

Fig. 4: Percentage Loss in Total Hydrocarbon Content

Discussion

Experimental bitter leaf transplants in uncontaminated soil had an average initial height of 20 cm. In 10 days, plant showed increased growth as the plant height increased by 15% and leaf count recorded 6 grown leaves. In 30 days, the plant height had increased by 37.5% and observed leaf count was 15 leaves. At the end of the experiment, there was 55 % growth in plant height, but there was an observed decline in leaf count because the leaves were fed on by insects, whereas; bitter leaf transplants in 1% contaminated soil wilted by the 6th day of the experiment, bitter leaf transplants in 0.1% contaminated soil had a recorded 6.5% growth in plant height with observed 6 leaves on the 10th day of the experiment. By the 30th day of the experiment, the transplants died. This indicates that the growth of the bitter leaf transplants was affected by the contaminants in the soil.

Water leaf transplants indicated a high potential of adaptation in the contaminated soil as shown by the growth after 10, 30 and 70 days of the experiment. The average growth of *T. fruiticosum* were 0.91%, 4.50% and0. 69% in 0.1% contaminated soil after 10, 30 and 70 days respectively in comparison to 2.47%, 1.75% and 3.20% in uncontaminated soil and 4.92%, 9.37% and 1.43% in 1% contaminated soil after these days. From the observations, *T. fruiticosum* seems to be less affected by contaminants than *V. amygdalina* probably due to the less broad and shallow roots water leaf plants have as compared to the broad, deep roots of the bitter leaf.

The differences in TOC loss in bitter leaf vegetated and water leaf vegetated treatments were not significant. There was no significant difference in loss of TOC for treatments with bitter leaf and treatments without plants, same with water leaf planted treatments. However, the presence of plants resulted in lower TOC concentration. The TOC content in the vegetated treatments decreased by 42.86% in M1, 48.91% in M2, 27.44% in M3, 32.14% in S1, 55.43% in S2 and

51.16% in S3 after 70 days. In comparison, the TOC decrease in unplanted treatments (F1, F2 and F3) was 21.43%, 45.65% and 48.84% respectively.

There was a recorded 99.70%, 14.92% and 22.83% loss in THC for bitter leaf transplants in M1, M2 and M3 respectively. The THC content reduced in water leaf vegetated treatment (S1, S2 and S3) by 76.15%, 17.63% and 32.61% respectively. The THC content in the unplanted treatment decreased by 54.20%, 12.89% and 7.16% in F1, F2 and F3 respectively. Similarly, (Egharevba *et al.,* 2017) observed 14.33% loss in THC for unplanted treatment, 45.89% THC loss in soybean planted treatment and 60.80% THC loss for guinea grass vegetated treatment. There no significant differences in THC decrease in bitter leaf vegetated and water leaf vegetated treatments. This was also observed for THC loss in water leaf vegetated treatments and unplanted treatments and in bitter leaf treatments and unplanted treatments. Also, Efe *et al*., 2014, reported that *Axonopus* sp. reduced the acidity of hydrocarbon concentration in soils (4.46 - 6.87 pH in Ubeji and 4.66 - 6.86 pH in Alesa Eleme). The decrease in TOC and THC levels of unplanted treatment of crude oil contaminated soil indicates that the organic contaminants underwent photo-volatilisation due to exposure to sunlight and microbial degradation caused by the microbial population in the soil.

Conclusion

This research project showed that *V. amygdalina* cannot thrive in hydrocarbon contaminated soils and *T. fruiticosum* can survive longer in hydrocarbon contaminated soil than *V. amgdalina*. The decrease in TOC content in unplanted treatment was lower than the TOC content decrease in vegetated treatment but is not significant. Although there was higher percentage decrease in TOC content in contaminated soil by *T. fruiticosum* than *V. amygdalina*, it is not significant. It was also observed that there was higher percentage loss in THC content by *T. fruiticosum* in contaminated soil than *V. amygdalina*, it is not significant. Therefore, while *T. fruiticosum* thrives more in contaminated soil than *V. amygdalina* and there is higher percentage loss in TOC and THC content by *T. fruiticosum* in contaminated soil, there is no significant degradation of crude oil contaminated soil by *V. amgdalina* and *T. fruiticosum* during rainy season. *T. fruiticosum* is not a viable plant for phytoremediation as already contaminated plants used for phytoremediation may be harvested and sold as food crops which would lead to bioaccumulation of hydrocarbon contaminants by the consumers.

Recommendation

- The plants should be pre-planted in a non-contaminated soil till it reaches a certain level of maturity before transplanting in the contaminated as the plant growth would not be greatly affected by the contaminant.
- Further research should be carried out in dry season and for a full year to evaluate the effect of season change on the phytoremediation ability of plants.

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