

Assessment of Plants at Petroleum Contaminated Site for Phytoremediation

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Abstract - The study is aimed at identifying native plants growing around petroleum hydrocarbon contaminated site in Umuahia, Nigeria that has the ability to phytoremediate the contaminant from the soil. A total of 28 native plants in the area were sampled. Most of the plants demonstrated the ability to grow in a high concentration of the total petroleum hydrocarbon which is an indication that they can be used for phytoremediation of a petroleum hydrocarbon contaminated soil. *Chromolaena odorata*, *Aspilla africana*, *Chloris babata*, *Paspalum vaginatum*, *Bryophyllum pinnatum*, *Paspalum scrobiculatum*, *Spondias mombim*, *Euragrostis atrovilens*, *Cyperus rotundus*, *Uvaria chamae* all demonstrated phytoremediation characteristics in the contaminated site. Among those characteristics was the ability to survive the toxic nature of the contamination and to uptake part of the contamination. Pearson correlation demonstrated a significant and positive relations ($r = 0.64$, $p = 0.003$) ($p < 0.05$) between the soil and root TPH. Using bioaccumulation factor as a module for phytoremediation resulted in Linear regression between TPH concentration in the soil and root tissue of $Y = 1.8696x + 608.55$. From this study, *chromolaena odorata*, *Aspilla Africana* and *Uvaria chamae* were selected as candidate plants for phytoremediation of PAHs.

Keywords: Native plants, contaminated sites, PAHs, TPHs, Phytoremediation, Organic compounds.

1. Introduction

Incessant human activities causing the release of large amounts of toxic compounds into the soil. These compounds that include heavy metals, metalloids as well as organic pollutants causes threats to the environment as they accumulate in soil and are readily passed unto man and animals through the food chain [1]. This lack of control in the contamination of the environment called for the need in the provision of appropriate measures to control or curtail the effects of those contaminations. The issue of environmental pollution is a global problem which has drawn a major concern since the recent past. Just as different contaminants predominates at different areas so will unique methods of management and control be employed based on what is obtainable in such areas [2].

Phytoremediation has been shown to be the most efficient and environmentally friendly means of restoring original soil conditions when contaminated by environmental organic contaminants [3]. The technology is generally accepted as the proven technology for the removal of environmental contaminants due to its minimal energy requirement and the beauty of the technique [1,3]. PAHs are group of highly toxic and recalcitrant organic compounds that are distributed widely in the environment. The problem of incomplete biodegradation of the higher molecular weight PAHs has been impacting in nature leading to various health effects to human and the animals [4]. However, plants have shown the ability to take up most of these PAHs and their toxic metabolites. This forms the major entry route of the compound into the food chain and the effects is been felt in almost every part of the ecosystem [5].

The biological action of plants has demonstrated an encouragement for organic contaminant reduction. It ultimately provides an enabling environment for microbial proliferation at the root area of the plant known as rhizodegradation. This phenomenon is possible as a result of the symbiotic relationship between plant and the microorganism, which is usually triggered by the unique properties of the plant [6-7]. Different plants have been used for the remediation of various environmental contaminants. And in all of those studies, the plants tested were able to present a successful phytoremediations effects towards the contaminants. Some plants have been used as a phytoaccumulator of metals [8], at

the same time various plants have identified as good phytoremediators of organic contaminated environment^{3,9-10}. Most specifically some have also been for the phytoremediation of PAHs [11-12].

However, there is little information with regards to what plant could be used at what area and why. Therefore the aim of the study encompasses identification of natives plants found to be growing in a PAHs contaminated soil at the Umuahia petroleum pipeline track, which has been previously vandalized allowing the flooding of the area with petroleum products. Total petroleum hydrocarbon (TPH) which has always been the primary criterion for environmental assessment of petroleum industries was measured in the area. And the level was found to be at a high threshold. Meanwhile the fact that some plants were still growing at the site means that such plants could have certain parameters that could of important for the management of PAH contaminated soil.

2. Materials and Methods

The study was conducted in Umuagu village located along the Portharcourt-Aba-Umuahia-Enugu Expressway a few kilometres from Umuahia in Abia state of Nigeria. The area lies within Latitude. 5°35'59.99" N and Longitude 7°31'59.99" E (Figure 1). It is an area that has faced multiple vandalization of petroleum pipeline with subsequent waste of refined petroleum products at the pipe line channel from the south eastern part of Nigeria to the rest of the country. Sampling of the soil at the contaminated site was carried out and the TPH concentration of the area was measured.

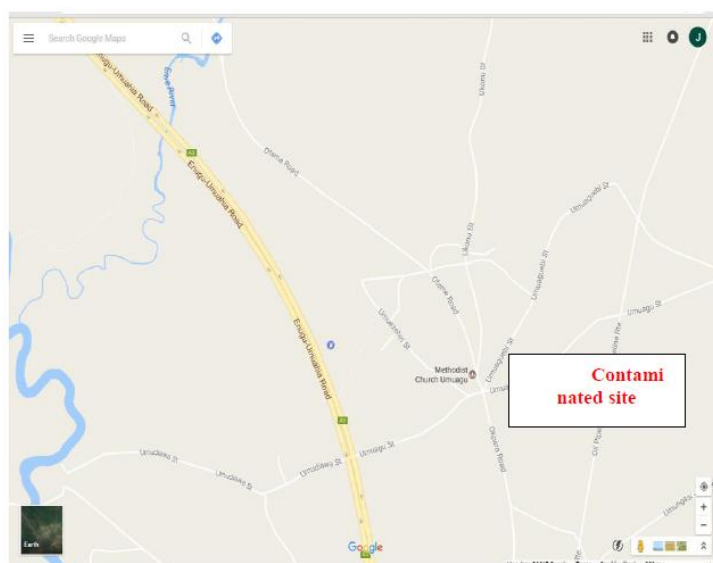


Fig. 1: Satellite map of Umuahia in Nigeria showing the study area and sample site 5°35'59.99" N 7°31'59.99" E (Google map).

3. How to Calculate the Biological Accumulation Coefficient (BAC) Values

BAC is a factor established so that we could measure how plant is able to uptake compounds from a substrate. This phenomenon is based on the equation bellow:

$$BAC = \frac{\text{Concentration in plant tissue } (\frac{\text{mg}}{\text{kg}})}{\text{Concentration in soil } (\frac{\text{mg}}{\text{kg}})} \quad (1)$$

Results derived from the determination of BAC are always being matched with plants categories so as to be able separate plants as hyperaccumulator or not (Table 2). This was determined using the metal uptake level of different plants. However, the same protocol can also be used to measure for the BAC of plants on organic contaminants.

Table 1: Category of BAC values in plants [13].

Plants category	Range
High accumulator plants	1.0 - 10
Moderate accumulator plants	0.1 – 1.0
Low accumulator plants	0.01 – 0.1
Non accumulator plants	< 0.01

4. Laboratory Analysis

Sludge preparation: Soil samples were air dried at room temperature for two weeks, homogenized with hand and pounded using a mortar, sieved through 1.00 mm mesh. This selection was done so as to encourage homogeneity of the mixture.

5. Extraction of the Soil for TPH Analysis

To carry out the extraction of soil for TPH analysis, USEPA [14] method of ultrasonic extraction method was used and amended. 10 g of the soil mixture sample was placed in a 250 mL volumetric flask and was mixed with approximately 4 g sodium sulfate (Na_2SO_4). The sodium sulphate acted as a dehydrating agent as the presence of water to alter the measure of the TPH. Final analysis of the TPH was done using Agilent 6890 Plus gas chromatograph equipped with a micro-63 Ni electron capture detector, an SPB TM-1 fused silica capillary column (30 m, 0.25 mm, ID x 0.25 μm film thickness) and HPChem station software. Data were collected in selective ion monitoring mode [15]. The injector and detection temperature was kept at 340 and 380°C respectively. And the oven temperature was programmed at 420°C held for 4 min and ramped at 10°C per min to 340 and held for 12 mins. The carrier gas used was helium at a flow rate of 1.00 mL per min. the total analysis was about 40 mins.

2 μL of the extract was injected into the GC to obtain the chromatogram of the TPH. The quantification was done with a five point's calibration plot (1, 5, 10, 20, 50 mgL^{-1}) of the aliphatic hydrocarbon ranges from C8 to C40. Quantitation for each carbon number was done by summarizing the area for each carbon number at the respective retention time and correlated with the area for individual carbon number of the calibration standards.

6. Elementary Analysis of the Soil

The EPA 3051 methods of extraction using Aqua Regia (AR) procedure [16], with a concentrated acid in a microwave oven, under controlled temperature and pressure was used. AR: 0.5 g of soil samples sieved through 100 mesh was transferred to Teflon tubes. Each tube received 9 ml of concentrated HCl (32 %) and 3 ml of concentrated HNO_3 (65 %) (3:1) of analytical-level purity, and the mixture was left undisturbed for 12 h of pre-digestion. The tubes were subsequently placed in a closed system in a microwave oven (Mars Xpress, CEM Corporation) for 20 min at 180 °C subsequently passing the temperature ramp. After cooling, samples were transferred to certified 25-ml flasks (NBR ISO/IEC), the flask volume was completed with ultrapure water, and the extracts were filtered. All analyses were carried out with three replicates [8.] Calibration curves to determine metal concentrations were prepared based on 1,000 mgL^{-1} standards, using ultrapure water for dilution, cleaning, and decontamination of the glassware, which was kept in 10 % nitric acid solution for 24 h and rinsed with distilled water and ultrapure water. Concentrations of Co, Cr, Cu, Ni, Pb, and Zn were determined via atomic absorption spectrophotometry (FAAS) and Cd concentrations in a graphite oven. However, methods in which a closed system is heated with a microwave oven are well established and widely employed [17].

Table 2: Average contents of heavy metals recovered for certified reference material.

Elements	Soil system	Recovery level	Certified value	Percentage recovery
Cd	Soil mixture	0.45	0.07±0.002	111
Co	“	11.3	12.80±0.2	76
Cr	“	55.0	130.0±9.0	43
Cu	“	27.2	33.0±0.05	75
Ni	“	32.4	85.0±2.0	73
Pb	“	11.0	17.3±0.1	50
Zn	“	74.2	103.0±4.0	72

7. Plants

Preparations: Before the commencement of the analysis plant samples were washed with tap water and rinsed with distilled water to remove adhering soil particles. Afterwards the samples were dried at room temperature for 48 hours and were then ground into powder using mortar and pestle after the weighing. The powdered sample was then used for analysis.

Extraction of metals from the soil: The methods used was wet digestion, the powdered plant samples was placed in a 100 mL cornical flask and added with 10 mL of conc HNO₃. The flask was covered with a glass cover and was allowed to stand overnight until the entire bubbles were dispersed. Afterwards, the sample was heated in a hot plate for 1 hr at 120°C. Distilled water was then added to enable complete digestion. When that is done, the flask was cooled and about 1 mL of H₂O₂ was added, and returned to a hot bath for continuous heating with intervally addition of H₂O₂ for every 4-8 mL of solution lost until digested sample becomes clear. Glass cover was then removed and temperature reduced to about 80°C. The digestion was continued until the samples become dried, and is then cooled. On cooling, 2 mL of acid mixture 1:3 HNO₃ – HCl was added, and the mixture filtered using a filter paper and afterwards analysed using ICP-OES (Perkin Elmer, USA).

8. Root Tissue Comparison Analysis

To establish the reason behind the high BAC of some of the plants growing in the contaminated site, root tissues from two species from each family of plants were selected analyzed for their carbon and lipid contents. Prior to analysis, the root tissues were air dried on at room temperature for one week. The air-dried root tissues were hand broken and then ground into powder using mortar and pestle. Carbon and lipid content was determined using Orita (2012) method.

9. Lipid Analysis

The lipid content of the root tissue was determined by extracting 2 g dry root tissue with ethyl ether for 24 hours in a Soxhlet apparatus. Lipid content was calculated by dividing the extracted lipid weight by the dry tissue weight added to the thimble. Fresh tissue lipid content was then calculated by multiplying the dry lipid content by the fractional water content.

To ensure quality control, all reagents used were of analytical grade and were used without further purification. All the glass wares were totally sterilized, and the analysis was one in triplicates.

10. Results

In the profiling of the plants, results showed that there were 5 families and 13 species that dominated the entire flooded area. *Astericeae* family which are shrubs was the most dominant. In the area outside the oil contaminated site, there were 9 species from 8 different families that were identified. The greater number of species in the non-contaminated site indicated the natural state of the area.

The *Astericeae* family which are shrubs were the dominant plants at 31%, followed by *Gramineae* (29%), *Cyperaceae* (which could also be called legumes) (17 %) and *Leguminoceae* (8 %) (Figure 2 and Table 4). The rest with lesser dominant approach were *Malvaceae*, *Euphobiaceae*, *Pteridophyte*, *Onagraceae*, *Sterculiaceae*, *Melastomaceae* each with (2 %), while *Vitaceae*, *Anacardiaceae* and *Annonaceae* were all had 1 % each. Although *Anacardiaceae* and *Annonaceae*

had lesser dominance, they however, seconded *Asteraceae* families in their ability to phytoaccumulate PAHs, hence were included in the induced phytoremediation study.

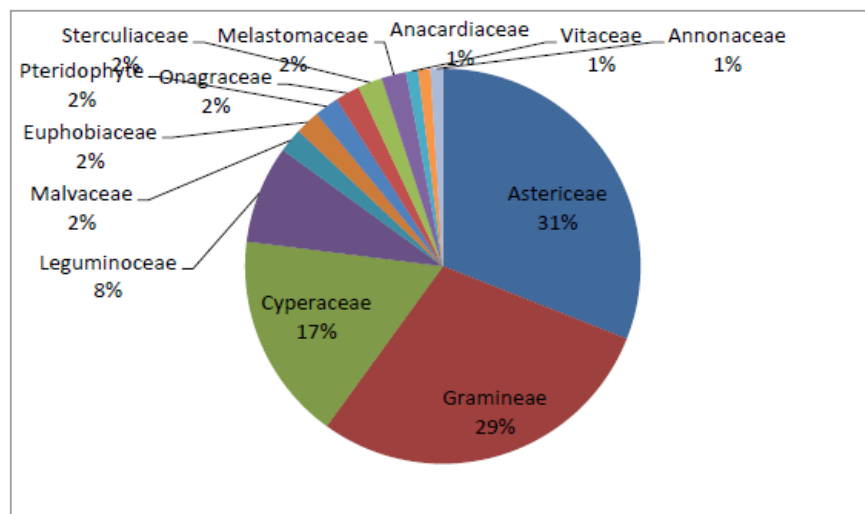


Fig. 2: Plant families sampled and their percentage dominance.

Table 3: Plant species samples at the petroleum contaminated site in Umuahia, Nigeria.

Family	Specie
<i>Asteraceae</i>	<i>Chromolaena odorata</i> , <i>Aspilla Africana</i> , <i>Chrysocoma ciliate</i> , <i>Dimorphotheca pisifera</i> , <i>Cosmos bupinnatus</i> , <i>Teraxacum sp.</i>
<i>Gramineae</i>	<i>Orinus longiglumis</i> , <i>Paspalum scrobiculatum</i> , <i>Paspalum vaginatum</i> , <i>Chloris babata</i>
<i>Vitaceae</i>	<i>Cayratia eurynemia</i>
<i>Malvaceae</i>	<i>Steculia Africana</i> , <i>Sterculia urceolata</i>
<i>Cyperaceae</i>	<i>Cyperus rotundus</i> , <i>Cyperus esculentus</i> , <i>Carex stricta</i> , <i>Scirpus cespitosus</i>
<i>Anacardiaceae</i>	<i>Buchanania acuminata</i>
<i>Euphobiaceae</i>	<i>Sabastiana chamaelea</i> , <i>Dysopsis Baill</i>
<i>Annonaceae</i>	<i>Uvaria chamae</i>
<i>Pteridophyte</i>	<i>Cyperus difformis</i> , <i>Salvinia natans</i>
<i>Onagraceae</i>	<i>Calylophus beriandieri</i> , <i>Calylophus serrulatus</i>
<i>Leguminoceae</i>	<i>Vigna marina</i> , <i>Vigna unbellata</i>
<i>Sterculiaceae</i>	<i>Melochia corcholifolia</i>
<i>Melastomaceae</i>	<i>Melastoma malabathricum</i>

11. Plant Variations and Screening of Potential Plants as Phytoremediator for PAHs.

For phytoremediation of organic contaminations site to be possible, plants must germinate, survive and grow in that atrocious condition. This is the only condition that could make such plant to be considered as a potential plant for phytoremediation [18]. The plant is resistant when it is able to survive and reproduce under contaminated soil condition. There are factors responsible for this phenomenon, and they include metabolic changes, hormone production and enzymes sequestration triggering substrate concentration and enzyme activity.

At a high concentration of TPH in the soil, *C. odorata*, *A. Africanus*, *C. ciliate*, *C. bipinatus*, *P. vaginatum*, *C. babata*, *E. atrovirens*, *B. acuminata*, *U. chamae*, were able to survive the harsh condition of the concentration (Table 5). *C. odorata*, *B. acuminata* and *U. chamae* demonstrated the ability to grow at the highest TPH contaminated condition which according to the site ranges from 329-214,241, 373-8011, 274-6318 mg/kg respectively.

Table 4: Concentration of TPH in soil taking from the rhizosphere area of the plants in the contaminated site.

Plant species	TPH concentration in soil (Mg/kg)	TPH in plant tissues (Mg/kg)	% absorbed
<i>C. odorata</i>	329-214,241	8.5-2235	Root
<i>A. africanus</i>	163-4264	0.5-18.9	Root
<i>C. ciliata</i>	105-1970	0.05-4.0	Root
<i>D. pisifera</i>	328-1714	0.5-2.4	Root
<i>C. bipinatus</i>	277-5928	0.5-30.0	Root
<i>O. lungighumis</i>	149-510	ND	Root
<i>P. scrobiculatum</i>	235-2993	..3-7.5	Root
<i>P. vaginatum</i>	210-3017	0.04-2.7	Root
<i>C. babata</i>	291-1168	ND	Root
<i>C. eurynemias</i>	59-1235	0.5	Root
<i>S. africana</i>	19-402	ND	Root
<i>S. urceolata</i>	121-3299	0.05-1.2	Root
<i>C. rotundus</i>	275-3664	1.3-4.2	Root
<i>C. esculentus</i>	232-2611	0.2-0.9	Root
<i>C. stricta</i>	672-5232	0.4-12.0	Root
<i>S. cespitosus</i>	659	ND	Root
<i>S. chamaelea</i>	67-538	ND	Root
<i>D. baill</i>	97-2009	0.3-0.5	Root
<i>C. difformis</i>	448	ND	Root
<i>S. natans</i>	79	ND	Root
<i>C. beviandieri</i>	107-276	ND	Root
<i>C. serrulatus</i>	140	ND	Root
<i>V. marina</i>	48-176	ND	Root
<i>V. umbellata</i>	32-94	ND	Root
<i>M. corcholifolia</i>	46-312	ND	Root
<i>M. malabatricum</i>	39-1556	0.28	Root
<i>C. acuminata</i>	373-8011	2.1-36.4	Root
<i>U. chamae</i>	274-6318	1.8-30.1	Root

ND = not detected

12. Assessment of the Root System of the Plants Growing in the Contaminated Site

The plants samples that demonstrated the ability to withstand the severity of high contamination of PAH in the petroleum contaminated site were found to possess fibrous rooting system able to spread across a large surface area. Most importantly, all the 3 selected plants that grew at high concentration of TPH all have fibrous root systems. Fibrous root system is known to support high colonization of soil microorganism, due to the large surface it generates. This is one of the qualities of plants that could be used for the cleaning of a petroleum hydrocarbon contaminated environment [18].

13. Distribution of TPH in Soil and Plants

The range of TPH of soil in the contaminated soil at the site varied between 0.5 to 3110 mg/kg, with a mean value of 1493.1. The concentration of TPH in the plant roots analysed ranges from 1100-8500 mg/kg with a mean value of 3400 mg/kg. The standard deviation of soil was 1013.2 and that of plant root was 2371.5, giving an indication of high variation in both the soil depth and the plant root.

14. Correlation Matrix

The relationship that existed between the concentration of TPH in soil and plants tissues was examined using correlation analysis (Table 7). According the Pearson correlation, a significant and positive relations ($r = 0.64$, $p = 0.003$) (significant level at $p < 0.05$) between the TPH concentration in depth of soil and root. The reason behind this might be that the increase of TPH in the soil also increases the uptake of PAH by the plants.

In the linear regression between TPH concentration in the soil depth and root tissue concentration indicated a linear model as described below:

$$Y = 1.8696x + 608.55 \quad (2)$$

15. Discussions

From the result received, the family of plant that dominated in the contaminated site were *Astericeae*, *Gramineae*, *Cyperaceae*, and *Leguminosae*. The rest with lesser dominant approach were *Malvaceae*, *Eupobiaceae*, *Pteridophyte*, *Onagraceae*, *Sterculiaceae*, *Melastomaceae*, while *Vitaceae*, *Anacardiaceae* and *Annonaceae* were scattered. Apart from the dominance presence of these plant, only *Asteraceae*, *Anacardiaceae*, and *Annonaceae* families that demonstrated the greater ability to phytoaccumulate PAHs. These sets of plants belong to the grass and legume family. It is reported in literature that grasses and legumes has high potential to remediate PAHs [19]. In the study done by Idris and his co-scholars to identify sets of plants at a petroleum contaminated site in Malaysia that could be suitable for phytoremediation, the dominated plants as was reported in that study to belong to the family of legumes and grasses⁷. The clear explanation to this could be the presence of extensive fibrous root system with diverse root surface area, hence can penetrate deep down into the soil.

Literature reports indicated that success of phytoremediation of environmental contaminants both organic and inorganic has been achieved with the following plants: Poplar tree (*Populus deltoides*), Willow (*Salix species*), Indian mustard (*Brassica juncea L.*), Indian grass (*Sorghastrum nutans*), and Sunflower (*Helianthus Annuus L.*) [3,20]. This success is as a result of their diverse root system that enabled even microbes an environment for successful growth, and also absorption of large quantities of water [21].

Optimal growth of plant in the contaminated soil is significant for phytoremediation to be possible. But for plant to grow in a contaminated environment it must have to severe the phytotoxic nature of the contamination, hence such plant should be able to germinate, survive and grow in such harsh condition [22]. There was high growth incidence of the selected plants at the contaminated site even at the high concentration of TPH. This means that such plant were able to resist the contamination of the petroleum products dispersed over the soil. For plants to resist the obnoxious condition of a PAH contaminated environment, it has to undergo metabolic changes resulting in hormone production and enzyme sequestration which brings about substrate modification and increased enzyme activity [23]. The toxicity of hydrocarbons is as a result of its volatility and hydrophobicity. Low molecular weight hydrocarbons which are mostly volatile have the tendency to move easily through cell membranes, resulting in their toxicities [7,24]. However, the presence of oil in the hydrocarbons causes less water infiltration and aeration required for optimal growth and development of plants, but most of the plants in this study were able to survive such toxicity.

There was high standard deviation in both the soil sample and the root of the plant showing a high deviation in their concentrations. High value of root TPH in plants explains the fact that there was high absorption of petroleum hydrocarbon by the roots. The proportion of the concentrations of TPH in both soil and plants shows that absorption and accumulation of petroleum hydrocarbon in plants depends on the concentration of the pollutant in the environment which in this case is the soil. The accumulation of high content of the contaminant resulted in the spillage of the petroleum products resulting from the vandalization of petroleum product pipeline in the area. The mean value of TPH in the soil exceeded the permissible limit of global TPH in soil (1000 mg/kg), indicating a high concentration of PAH in the site. Furthermore, the average value of the plant tissue TPH exceeded that global permissible limit in soil, but the value was within the phytotoxic level in plant (1000-12000 mg/l). This result is in agreement with that of Lotfinasabasl et al. [25], that documented a high TPH values in soil and plants sampled in that soil as a result of an oil spill event.

From the result of the BAC of the plants, it was indicated that some of the plants tolerated the high concentration of TPH in the contaminated site. The value of BAC in plants usually expresses the accumulation capability of toxicants by plant in relation to such contaminants bioavailability in soil [26]. The ability of the plants in the sampled site to tolerate the high concentration of TPH is an indication that the plants are potential hyperaccumulator plants for petroleum

hydrocarbons. This was demonstrated by the significant and positive relations between the TPH concentration in depth of soil and root during correlation and regression analysis. To assess which plants have the most potentials, further tests done presented plants from the family of *Asteraceae*, *Anacardiaceae*, and *Annonaceae* as those plants. These plant species belong to the shrubs and grass category of plants. Idris et al. [7] reported high BAC values in shrubs and grasses with fibrous root systems. Although plants TPH was assessed only in the root tissues of the plants in this study, authors further study involved the assessment of the value in the entire plant tissues (stem and leaf). Because this study only concentrated in the plants screening, translocation abilities of the plants were not recorded.

16. Conclusions

The result of this study involving the screening of plants at a petroleum hydrocarbon contaminated site in Umuahia-Nigeria, presented *C. odorata*, *B. acuminata*, and *U. chamae* as potential plants for phytoremediation of TPH. These plant species were able to grow in that contaminated site with high TPH values possessing other factors of a good phytoremediation plants as reported in literature. About 8 plant species could suffice as potential TPH phytoremediator as was recorded by their degree of growth; the plants included *C. odorata*, *A. Africanus*, *C. ciliate*, *C. bipinatus*, *P. vaginatum*, *C. babata*, *E. atrovirens*, *B. acuminata*, *U. chamae*. BAC factors were used to select the potential plant absorption of PAHs and to be able to determine which plant serves the most potential for phytoremediation of organic contaminated soil. The entire assessment of the screened plants in the contaminated site demonstrated good potentials for petroleum hydrocarbon remediation. Since phytoremediation has been reported as the most preferred method of contaminant remediation, the sampled plant could be used with amendments for ecosystem protection.

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