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Tolerance and Bioaccumulation of TPH in *Caesalpinia Pulcherrima* L and *Imperata Cylindrica* L. of Crude Oil Contaminated Soils Amended with Cow Dung

Amadi Goodhope Onyemauche*, Tanee Franklin Banakuna Godson, Mubula Benson Diselph

Department of Plant Science and Biotechnology, University of Port Harcourt, Port Harcourt, Nigeria

Email address

goodhopeamadi@gmail.com (A. G. Onyemauche)

*Corresponding author

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Abstract: This study was carried out to provide information on the tolerance and bioaccumulation of TPH in *Caesalpinia pulcherrima* L. and *Imperata cylindrica* L. in crude oil polluted soils amended with cow-dung. 9kg soil was collected at a depth of 1-10cm and polluted with 250 ml of crude oil. A Latin Square Design comprising T1 (*Imperata cylindrica* + 300g cow dung + crude oil), T2 (*Caesalpinia pulcherrima* + 300gcow dung + crude oil), T3 (*Caesalpinia pulcherrima* + crude oil), T4 (*Imperata cylindrica* + crude oil), T5 (Polluted soil with 300gcow dung), T6 (Polluted soil without amendment (control) was used for this study. The plants vegetative parameters (plant height, leaf numbers, fresh and dry weight of root and shoot, root length) and shoot and root total hydrocarbon carbon content were analyzed. The results showed that addition of cow dung to the crude oil contaminated soil had much influence on the leaf number, fresh and dry matter content of *Imperata cylindrica* than *Caesalpinia pulcherrima*. The result likewise showed that there was a reduction in Total Petroleum Hydrocarbon (TPH) in the order T1>T4>T3>T2. The results gotten from this study have shown that augmenting crude oil contaminated soils with cow-dung will enhance remediation of petroleum polluted soil.

Keywords: Crude Oil, Cowdung, Soil, TPH, Contamination, Phytoremediation

1. Introduction

Environmental pollution has become a global problem affecting both developed and developing countries [1] and it has assumed global concern as it is a threat to the well-being of all life forms including humans. Oil exploration and exploitation in Nigeria has evolved through a long history [2]. However, the exploration and exploitation of this glossy façade of financial benefits have created serious environmental pollution for host communities. Nigeria has the largest natural gas reserve and the second largest oil reserve in Africa. The environmental impacts of exploration and exploitation have been of serious concern to Nigerian government regulatory agencies and other environmental outfits. Ghosh and Singh [3] reported oil exploration and exploitation, accidental and process spillage among others, as major factors responsible for the migration of pollutants into the soil and water ecosystem. Okoloko [4] reported the main causes of oil pollution in Nigeria to include discharge from sludge, production tests, drilling mud, spills from pipelines, well-blow outs, gas flaring and sabotage. The consequences of the aforementioned factors that lead to environmental hazards need to be remedied. Doelman [5] defined remediation as the management of a contaminant at a site so as to prevent, reduce or mitigate damage to human health, or the environment. Remediation techniques include: (i) ex-situ (excavation) or in-situ (on-site) soil washing/leaching/flushing with chemical agents, (ii) chemical immobilization/stabilization method to reduce the solubility of heavy metals by adding some non-toxic materials into the soils, (iii) electrokinetics (electromigration), (iv) covering the original polluted soil surface with clean soils, (v) dilution method (mixing polluted soils with surface and subsurface clean soils to reduce the concentration of heavy metals), (vi) phytoremediation by plants [6], [7]. These techniques, however, have their various merits and demerits. Detailed assessment of the advantages and disadvantages of various remediation techniques are reported by USEPA [8]. The harmful effects of oil in different environments, has led to the need to develop simple adoptable remediation techniques for petroleum polluted sites using different simple and affordable methods, which may include physical, chemical and biological processes [9]. Phytoremediation is the in-situ use of plants and their associated microorganisms to degrade, contain or render harmless contaminants in soil or groundwater [10]. It is a non-destructive and economical in-situ process that uses plants to remove, degrade or stabilize contaminants in soil. This remediation technique is especially promising due to favourable climatic conditions of the tropics. Karenlampi et al. [11] reported four characteristics that makes a plant suitable for phytoremediation, these include: (i) the plant's ability to accumulate extracted pollutant; (ii) plants should have enough tolerance to be able to not only survive in polluted soils, but to carry pollutants within their shoots; (iii) the species should be fast growing with an amplified ability to accumulate toxins; and (iv) the plant should be easily harvestable for simple disposal. Prasad &Freitas [12] reported that about 400 species of plants have been found with the potential of remedying crude oil polluted sites. Among the families documented by Hameed et al. [13] were Asteraceae, Brassiaceae, Caryophylaceae, Cyperaceae, Cunouniaceae, Fabaceae, Poaceae, Violaceae, Lamiaceae, Euphorbiaceae and Flacourtiaceae. In their study, the Poaceae family was reported to be the most abundant, frequent and most tolerant to crude oil contamination. Hameed et al. [13] reported that *Imperata cylindrica* Linn., a member of the family Poaceae showed specific root and stem anatomical adaptation for its better survival under harsh environments. Studies on the phytoremediation of soils contaminated with petroleum hydrocarbon using different plants in Nigeria and other parts of the world are well documented. However, there is little or no work on the remediation of soils contaminated with crude oil using *Imperata cylindrica and Caesalpinia pulcherrima.* This study therefore investigated the growth of *Imperata cylindrica and Caesalpinia pulcherrima* under different concentrations of crude oil and also determined the residual hydrocarbon content in the soil at plant maturity with a view to assessing its phytoremediating potential.

2. Methods

2.1. Description of Experimental Site

The study was carried out at the Centre for Ecological Studies of the Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria at Latitude 4° 00N and 5° 00N and Longitude 6° E and 7° E in southern Nigeria.

2.1.1. Soil Collection and Pollution

Soil samples were collected randomly from pristine land located close to the Faculty of Agriculture Demonstration farm, and then taken to the Ecological Centre. Weighing balance calibrated in kilogram was used to weigh 9kg of soil into 36 polythene bags. 250 ml of crude oil (pollutant) obtained from Nigerian National Petroleum Corporation (NNPC), Eleme, Port-Harcourt was added into the 36 polythene bags (Exprimental bags). The Soil was thoroughly mixed in the experimental bag. The bags were allowed to stand for 14 days after which amendment was added to it except in the bag which serves as the control. The amendment used, (cow dung manure) collected from an abattoir near Choba Market. The polythene bags were arranged in four (4) columns and four (4) rows each, designated as T1 (crude oil + *Imperata cylindrica* + cow dung), T2 (*Caesalpinia pulcherrima* + crude oil + cow dung), T3 (crude oil + *Caesalpinia pulcherrima)*, and T4 (crude oil + *Imperata cylindrica).* During this period, the soil samples were watered on weekly basis depending on the dryness of the soil using 0.05litres of water. Weeding was done when the need arose**.**

2.1.2. Sample Collection and Analysis

Seeds of *Caesalpina pulcherrima* were gotten from Obinna plant nursery, along Mgbuoba/Choba road near AIT station, Port Harcourt and were brought up in the nursery for two weeks before it was transplanted while *Imperata cylindrica* was gotten from University of Port Harcourt close to the Con Oil Filling station and was moved directly into the contaminated soil. Growth parameters such as plant height, leaf number were measured while fresh weight, dry weight, shoot and root total hydrocarbon content were determined at the termination of the experiment. Fresh weights of the sampled plants were obtained by weighing immediately after harvest using an electronic weighing balance (LP 502A model). The plants were oven dried in B & T hot air oven drier for 3 days (72 hrs) at 75°C. Dry weight of the whole plant was determined by weighing on an electronic weighing balance (LP 502A model) and the results recorded. Plant Height (cm) was done by measuring the heights of the seedlings from the ground level or base of the plant to the tip of the plant or plant's apex with a meter rule. The number of leaves was obtained by counting the leaves on both plants. The Total Petroleum Hydrocarbon (TPH) Total petroleum hydrocarbon content was determined by the Method of American Petroleum Institute (API) (1994). Determination of shoot and root total hydrocarbon content was done using APHA 5520D (American Public Health Association 1990) method.

2.2. Bioconcentration Factor (BCF)

The phytoremediation potentials of *Imperata cylindrica* and *Caesalpinia pulcherrima*was examined by the use of bioconcentration factor. This was evaluated using the formula [14].

$$\mathrm{BCF} = \frac{\text{Concentration of TPH in root}}{\text{Concentration of TPH in soil}} \quad (1)$$

Accumulation Factor (AF): this was computed using the following formula [15].

$$AF = \frac{\text{Concentration of TPH in plants}}{\text{Concentration of TPH in soil}} \quad (2)$$

Translocation Factor (TF): this was calculated in other to understand the transfer or mobility potential of petroleum hydrocarbon from the roots to the shoots of the plant. It was calculated using the formula below (Cui, 2007).

$$TF = \frac{\text{Concentration of TPH in shoot}}{\text{Concentration of TPH in root}} \quad (3)$$

2.3. Statistical Evaluation

The data collected were statistically analyzed using Analysis of Variance (ANOVA) at 5% level of probability and the Least Significant Difference (LSD) was computed. (SPSS, version 21).

3. Results

The results for the height of the plants used is as shown in Figure 1. The results showed that treatment 1 (crude oil + *Imperatacylindrica* + cow dung) had the highest plant height, followed by treatment 4 (crude oil + *Imperata cylindrica*). Treatments 2 (*Caesalpinia pulcherrima* + crude oil + cow dung) while treatment 3 (*Caesalpinia pulcherrima* + crude oil) had the least heights. At 3MAP, addition of cowdung to treatments significantly improved the height of *Imperata cylindrica* but no difference in the height of *Caesalpinia pulcherrima* between the treatments (p=0.05).

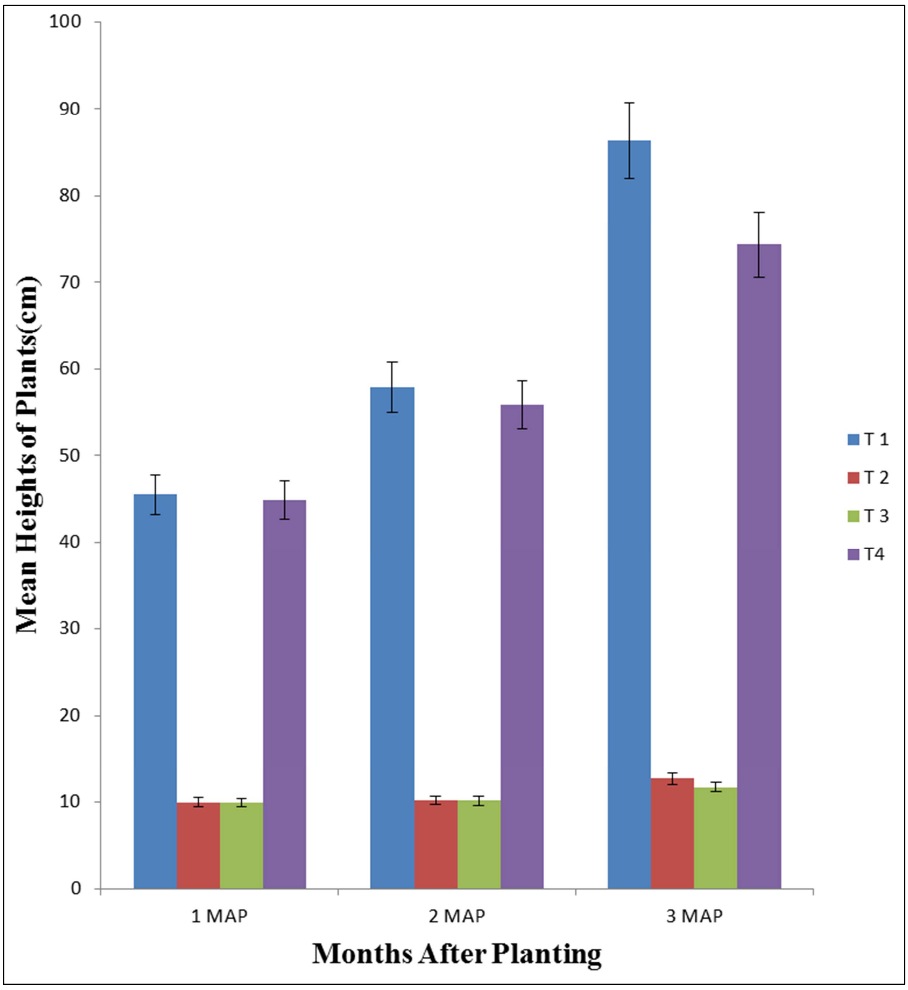

Figure 1. *Effects of treatment on the mean heights of Imperata cylindrical and Caesalpinia pulcherrima.*

Number of Leaves

The results for the number of leaves is as presented in Figure 2. The results showed that the highest number of leaves was observed in treatment 1 (crude oil + *Imperata cylindrica* + cow dung) at 3MAP (months after planting) while the least was seen in treatment 3 (crude oil + *Caesalpinia pulcherrima)* at 3MAP. The results showed that there were significant differences between all the treatments at 3MAP, while there was no significant difference between treatments 2, 3 and 4 at 1 MAP except treatment 1, where there was a significant difference (p=0.05) between treatment 1 and all other treatments.

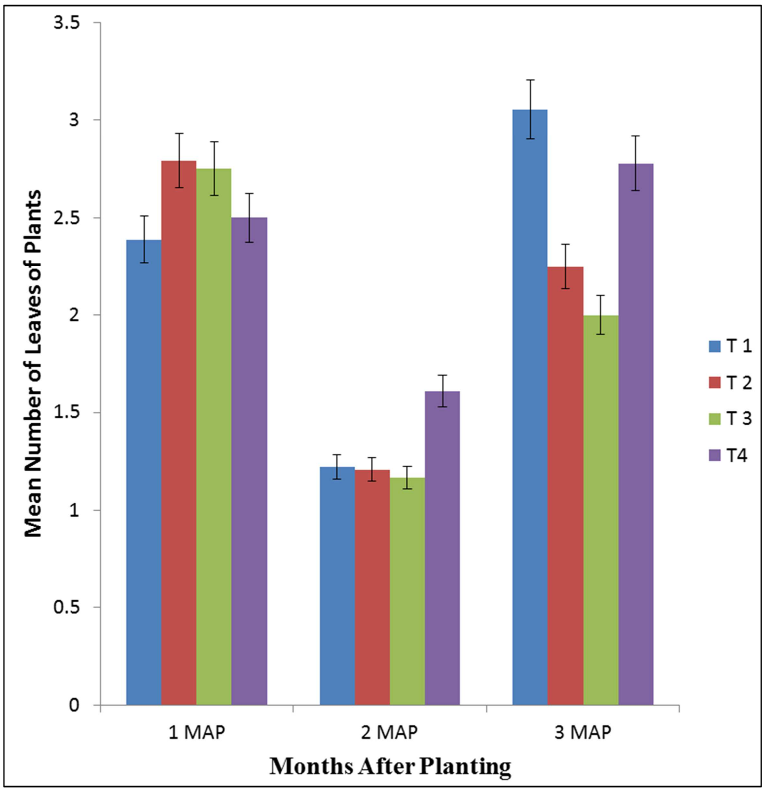

Figure 2. *Effects of treatment on the mean number of Imperata cylindrica and Caesalpinia pulcherrima.*

Root Length

The results for the root length i*s* as shown in Figure 3. The result showed that treatment 1 (crude oil + *Imperata cylindrica* + cow dung) had the highest root length followed by treatment 4 (crude oil + *Imperata cylindrica).* There was no significant difference between treatment 2 and 3, showing that cow dung addition did not have any significant effect on the root length of *Caesalpinia pulcherrima.*

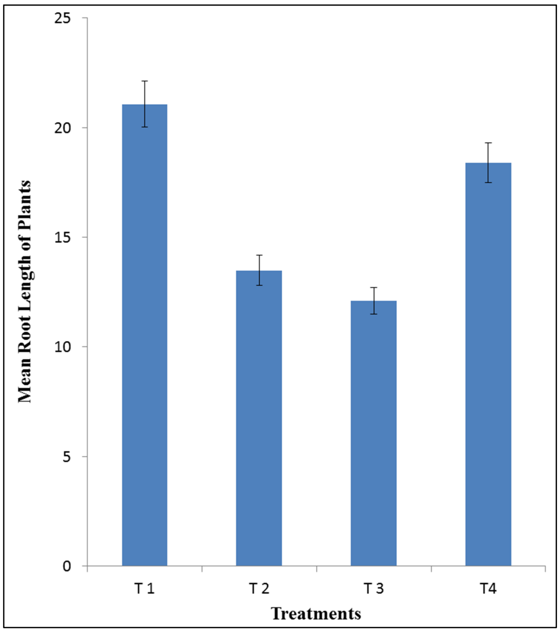

Figure 3. *Effects of treatment on the mean root length of Imperata cylindrica and Caesalpinia pulcherrima.*

Shoot Fresh Weight

The results for the shoot fresh weight is as shown in Figure 4. The result shows that addition of cowdung did not have any stimulating effect on shoot fresh weight of the Phytoremediation plants. Treatment 4 (crude oil +*Imperata cylindrica)* and treatment 1 (crude oil +*Imperata cylindrica*) had the highest shoot fresh weight while treatments 2 and 3 had the lowest. Also the results showed no significant differences in root length between all the treatments at p=0.05).

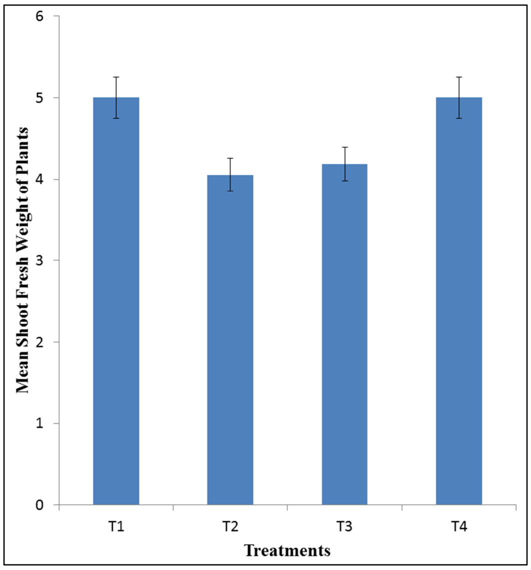

Figure 4. *Effects of treatment on the shoot fresh weight of Imperata cylindrica and Caesalpinia pulcherrima.*

Shoot Dry Weight

The results for the shoot dry weight is as shown in Figure 5. The results showed significant differences in root length between all the treatments at p=0.05). Treatment 4 (crude oil +*Imperata cylindrica)* was observed to have had a high shoot dry weight followed by treatment 1 (crude oil +cowdung +*Imperata cylindrica*) while treatments 2 and 3 had the lowest.

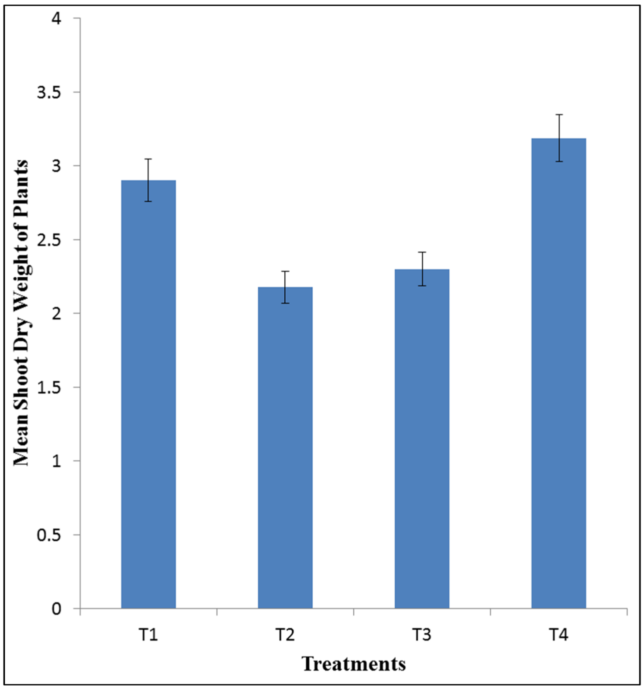

Figure 5. *Effects of treatment on the shoot dry weight of Imperata cylindrical and Caesalpinia pulcherrima.*

Shoot Total Hydrocarbon Content (THC)

The results for the shoot total hydrocarbon content (STHC) is as shown represented in Figure 6. Treatment 2 had the least THC value, while treatment 4 had the highest. The results for the shoot total hydrocarbon content (THC) showed that cow dung addition did not enhanced the accumulation of THC in the shoots of the 2 plants.

Root Total Hydrocarbon Content (THC)

The results for the root total hydrocarbon content (RTHC) of *Imperata cylindrica and Caesalpinia pulcherrima* is presented in Figure 7. Treatment 1 had the highest root THC followed by treatment 2. The result showed no significant difference between all the treatments.

Root Fresh Weight

The root fresh weight of *Imperata cylindrica and Caesalpinia pulcherrima* are as shown in Figure 8. Treatments 1 (crude oil + *Imperata cylindrica* + cow dung) and 4 (Crude oil soil + *Imperata cylindrica)* had high root fresh weights, followed by treatments 2 and 3. The result showed no significant difference in root fresh weight between all the treatments at p=0.05.

Root Dry Weight

The results of the root dry weight of *Imperata cylindrica and Caesalpinia pulcherrima* is as shown in Figure 9. Treatment 1 and 3 were observed to have the highest root dry weight while treatments 2 and 3 had the least root dry weight. There was no significant difference between treatments 2, 3, and 4, but significantly lower than treatment 1.

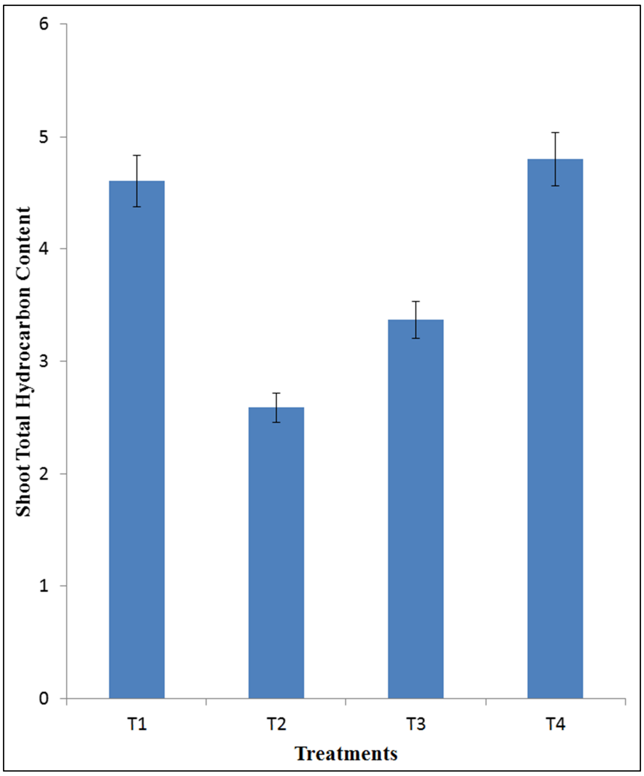

Figure 6. *Effects of treatment on the shoot total hydrocarbon content (STHC) Imperata cylindrica and Caesalpinia pulcherrima.*

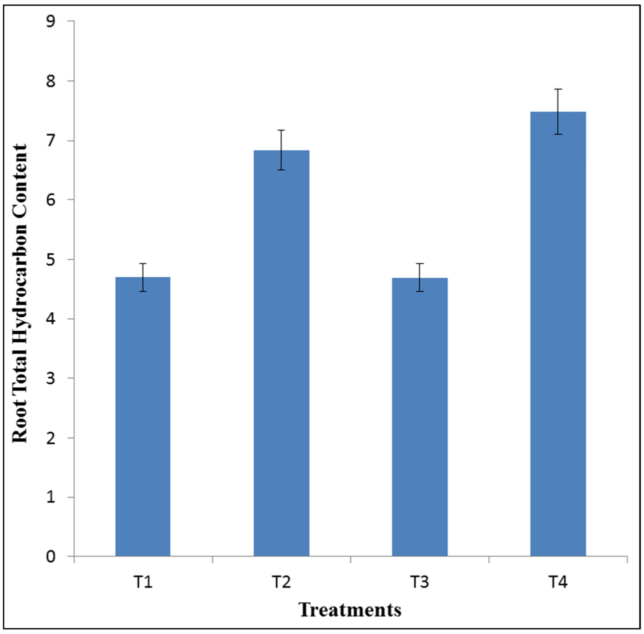

Figure 7. *Effects of treatment on the root total hydrocarbon content (RTHC) of Imperata cylindrica and Caesalpinia pulcherrima.*

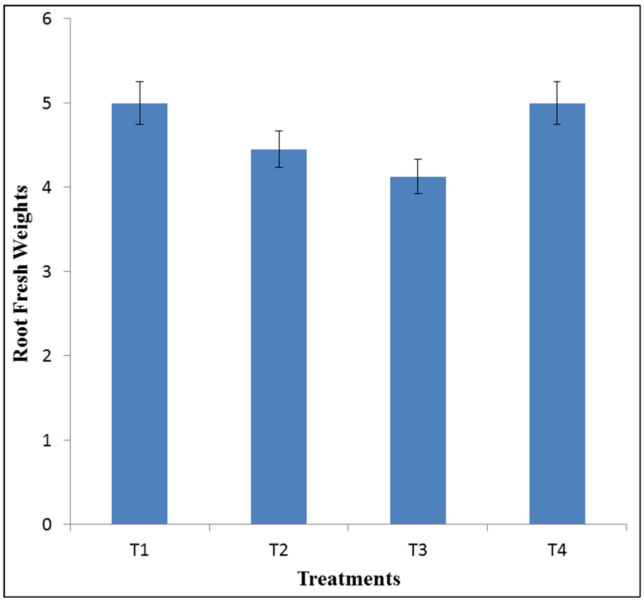

Figure 8. *Effects of treatment on the root fresh weights of Imperata cylindrica and Caesalpinia pulcherrima.*

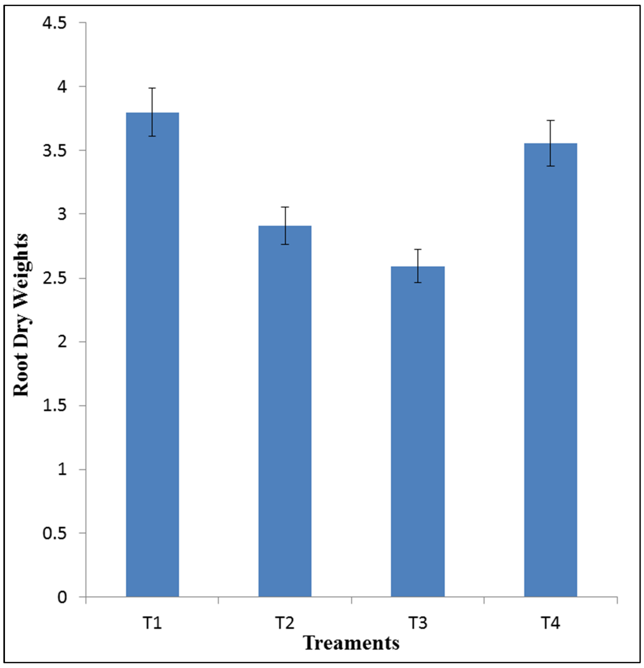

Figure 9. *Effects of treatment on the root dry weights of Imperata cylindrica and Caesalpinia pulcherrima.*

Table 1. *Bioconcentration factor (BCF), Accumulation Factor (AF) and Translocation Factor (TF) values of TPH in the root, shoot and soil samples.*

Treatments	BCF	AF	TF
T1	0.01	4.6	0.98
T2	0.01	2.6	0.38
T3	0.01	4.7	0.77
T4	0.01	4.8	0.64

4. Discussion

The continued growth of *Imperata cylindrica* and *C. pulcherrima* in a contaminant shows that the plants are potential phytoremediant. The effectiveness of *Imperata cylindrica* and *C. pulcherrima* in to accumulate hydrocarbon in its tissues as observed in this experiment was due to its ability to survive in a harsh environment. Similar results were obtained by Hameed *et al*., [13] where it was reported that *Imperata cylindrical* showed specific root and stem anatomical adaptations for its better survival under a harsh saline environment. The heights of *Imperata cylindrica* in soils contaminated and augmented with cow dung were greater than those of *Imperata cylindrica* in contaminated soils that had no cow dung. The height increased with increase in months. *C. pulcherrima* experienced stunted growth. It therefore means that *Imperata cylindrica* was able to tolerate and withstand the high levels of crude oil contamination. This agrees with Kirk *et al.* [17] who select plants for phytoremediation in petroleum contaminated sites on the basis of its high resistance to phytotoxic petroleum compounds. The addition of cow dung to the crude oil contaminated soil had much influence on the leaf number, fresh and dry matter content of *Imperata cylindrica* than *Caesalpinia pulcherrima*.

The shoot total hydrocarbon content of *Imperata cylindrica* was higher both in the soil amended with cow dung and in the soil without cow dung while it was low in the *Caesalpinia pulcherrima* soils with crude oil and cow dung and without cow dung. The root THC was highest in the polluted soil phytoremediated with *Imperata cylindrica* without cow dung than the one with cow dung. The high amounts of THC found in the shoots and roots of the plants could be inferred that these plants have the ability to absorb crude oil in their roots and shoots. This is also similar to what other researchers have noticed. For instance, Okolo *et al*. [18] reported increased degradation of crude oil in soil augmented with poultry manure. The high hydrocarbon loss in the organic manures amended soils is in line with Lee *et al*., [19] who reported that organic manures have effect in stimulating crude oil degradation by increasing the total heterotrophic microbial growth and activity. It could also be that the plants released root exudates which helped to break down the crude oil. The effect of *I. cylindrica and C. pulcherrima* on the removal of crude oil from the polluted soil with cow dung and without cow dung is similar to the findings of Aprill and Sims [20], and Merkl*et al.* [21] who reported higher degradation of petroleum hydrocarbon in vegetated soils than in non-vegetated soil. The higher removal of crude oil observed in this study conforms with the reports of Sampanpanish*et al*., [22], [23]; and Oti, [24] who listed *I. cylindrical* as one of the plants that can remediate petroleum hydrocarbon (anthracene) polluted soil. The removal of crude oil by *I. cylindrica and C. pulcherrima* possibly occurred through one of the several mechanisms of phytoremediation. Such mechanisms include polymerization of the contaminants, interaction of the plant with fungi and bacteria [25] and production of root exudates and plant materials which serve as source of carbon, nitrogen and phosphorus for petroleum degrading microbes [26], [27]. It is possible that the rhizospheres of these plants are rich in hydrocarbon utilizing microorganisms which could affect oil attenuation in soil [28]. Translocation Factor (TF) was calculated in other to understand the transfer or mobility potential of petroleum hydrocarbon from the roots to the shoots of the plant, while the accumulation and bioconcentration factors were calculated to determine whether there was a transfer of petroleum from the soil to the plants Osu and Ogoko [29]. The results revealed a variation in translocation factors in all the treatment used in this study. Low translocation factors as observed in all the treatment 2 (T2) reflects the strong sorption of pollutants to the colloids while higher translocation factors as seen in T1 and T3 reflects relatively poor retention in the soil or greater efficiency of plants to absorb the pollutants [29].

According to [30], when BCF < 1 as seen in this study for all the treatments it denotes that the plant only absorbs the pollutant but does not accumulate, but when BCF > 1, and this indicates that plant accumulates the pollutant. Translocation factors greater than one (BCF and TF>1) have the potential to be used in phytoextraction. Besides, plants with Bioconcentration factor (BCF) greater than one and translocation factor less than one (BCF>1 and TF <1) have the potential for phytostabilization [31]. In general, plant's ability to translocate pollutants from the roots to the shoots is measured using the translocation factor. Findings indicated that TF was less than 1 in all the treatments for. This means the quantities of pollutant accumulated in the roots tissues exceeded those in the shoots tissues, moreover the subsequent increasing order of the average TF values in all the treatments were as follows; T1>T3>T4>T2.

5. Conclusion

The results obtained from this study, have shown that the growth of *I. cylindrica* and *C. pulcherrima* in crude oil contaminated soils reduces the toxicity of crude oil in the soil. This is going by the improved vegetative vegetation of the plants. Since phytoremediation has been identified as aesthetically pleasing, cost effective and eco-friendly, *I. cylindrical* and*C. Pulcherrima* are viable plants for the remediation of crude oil contaminated soil. More so, the use of cow dung as the source of manure in this study is significant because it reduces the cost of using inorganic fertilizers which further reduces the cost of phytoremediation.

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