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Phytoextraction and Phytoaccumulation Potentials of *Chrysopogonzizanioides (L. Roberty)* and *Eucalyptus camaldulensis (Dehnh)* in the Bioattenuation of Petroleum Hydrocarbon Polluted Soil

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Abstract:
***Background**: The need of an efficient phytoaccumulator plant species for use in the phytoremediation procedure led to the study on the efficacy of two plant species for their ability to phytoextract and phytoaccumulate organic pollutants from soils.*
***Materials and Method:** The experiment was laid in the split-split plot design. This design was selected to ensure more precision to the selected plant species. The main plots were the Crude oil contamination (4 levels), Sub plot were the soil amendments (4 levels) while the sub-sub plot factor were the plant species (2 levels).*
***Results:**Results indicated that total plant biomass production was best at contaminant's concentrations of 0.5 L/kg soil while that of root elongation was at concentrations of 0.3 L/kg soil. Although phytoaccumulation of the soluble total petroleum hydrocarbon (TPH) was irrespective of contaminant's concentrations in soils, the insoluble asphaltene accumulation tends to increase with increased concentrations of contaminants in soils. In addition, while TPH and asphaltene accumulation was possible in E. camaldulensis species, that of C. zizanioides was twice the former and the rate at which C. zizanioides species uptake, translocate and accumulate organic contaminants was four times higher than that of E. camaldulensis species.*
***Conclusion:** It was concluded that the grass(C. zizanioides) was the best phytoaccumulator species for the phytoremediation of petroleum hydrocarbon from contaminated soils.*

***Key Word**: Phytoaccumulation; Phytoextraction; Phytoremediation; Bioaccumulation factor.*

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I. Introduction

Environmental pollution occurring mostly due to anthropogenic activities is usually xenobiotic in nature. Many of these xenobiotic substances are mostly toxic, carcinogenic and persistent within ecosystems. They are believed to be released into the environment through spills (crude oil, solvents), military activities (explosives such as trinutoulene – TNT), agricultural activities (pesticides, fungicides, herbicides) and industrial wastes (sludge from petrochemicals) [1, 2].

Organic pollutants such as crude spills are not only persistent within environment but leads to problems such as the inhibition of soil water infiltration capacity [3] and nutrient deficiency that is necessary for plant growth [4]. In addition, they are well known to accumulate through food chains in biological tissues in a process called bioaccumulation or phytoaccumulation and this leads to health and environmental issues to man and other organisms.

In an oil exploring country such as Nigeria, accidental spills from pipeline rupture and bunkering activities leads to both area source and point source pollution with its resultant adverse effects on the environment. For instance, more than 2 million barrels of crude oil leakage between 1976 and 1996 have been reported in the Niger delta where oil exploration is currently ongoing [5]. In addition, [6] reported groundwater contamination and its resultant mortality effects on aquatic life with associated difficulty in livelihood on inhabitants of the Niger delta aside conflict and biodiversity loss.

Several efforts have been made towards the ex-situ and in-situ remediation of organic contaminants by scientists [1, 7, 8] but such methods are associated with problems of cost, expertise and often leaves some contaminants in the environment [9, 10]. Although bioremediation as an in-situ biological method has been widely considered as good remediation strategy, phytoremediation that uses plants and its rhizosphere microbes is mostly considered [1, 7, 13].

In spite of the fact that phytoremediation is cheap and environmentally friendly, not all plants are efficient in the decontamination process on sites. The choice of plant species for the phytoremediation procedure is based on the occurrence of plants under specific climatic conditions, its resistance to pollutant toxicity, presence of phenolic compounds in root exudates and their capability to reduce pollutant concentrations in soil [12]. Additionally, plants are considered suitable in the phytoremediation procedure if they are tolerant to contaminants' toxicity and has the ability to extract and accumulate contaminants in its tissues. Some plants have been reported to uptake organic contaminants, translocate, accumulate and assist in its degradation in conjunction with soil microbes [1, 7, 13]. In the past, several researchers expressed concern over the disposal of plants used in the phytoremediation procedure but it is now known that phytomining of contaminants from the species used during phytoremediation process is possible [7].

In Nigeria, despite the promising nature of phytoremediation, not many research is carried out on common plants to ascertain their efficacies as good phytoaccumulator species. This stated fact could make application of indigenous plant species in the phytoremediation procedure worrisome. To this extent, identifying hydrocarbon resistant species that are efficient in extraction and accumulation into its tissues is necessary therefore, a comparative study on the efficacy of *Chrysopogonzizanioides* (a common grass) and *Eucalyptus camaldulensis* (woody species) for their ability to extract and accumulate organic contaminants from soils is considered in the conduct of this research.

II. Material And Methods

2.1 The Study Area

The study was carried out at the nursery of the Federal University Dutse, Jigawa State in the North West geopolitical zone of Nigeria. Jigawa state shares an international border with Republic of Niger to the north and the Nigerian states of Yobe to the northeast, Katsina to the northwest, Bauchi to the southeast and Kano to the southwest. It covers an area of about 24, 516 square kilometers and lies between latitude 11^o N to 13^o N and longitudes 8^oE to 10^o 15' E. It has a population of 4,361,002 (NPC 2006 Census figure). It is underlain by granites, schists and gneisses of the basement complex that emanates from the Pre Cambrian rocks of the Chad Formation. The soils are generally sandy at the top and compact at depth with often hard pans having aeolian deposits from the Sahara desert forming substantial part of the soils.

The mixing of the subsoil in these deposits has given rise to clayey subsoil, which dominates the northern parts of the state. The relief is generally undulating, but rock outcrops are common in areas of basement complex rocks. The climate is semi-arid characterized by a long dry season (June – September) and a short wet season (October – May). The climate varies considerably and is erratic. The mean annual temperature is about 25°C but the mean monthly values range between 21°C in the coolest month and 31°C in the hottest month. Total annual rainfall ranges from 600 mm in the north to 1000 mm in the south with variations leading to severe and prolonged droughts that causes crop failure, death of livestock and overall human sorrow. The vegetation falls within the Sudan Savannah vegetation belt, but traces of Guinea savannah vegetation are found in parts of the southern districts characterized by extensive open grasslands with few scattered stunted trees[14].

2.2 Materials

2.2.1 *Plant material*

Plants used as materials for the study were the seedlings of *Chrysopogonzizanioides* and *Eucalyptus cammaldule*nsis obtained from a farm in Kiyawa town about 70 km from Dutse, the Jigawa state capital. *E. camaldulensis*was selected because of its possible use in agroforestry systems in the Niger delta polluted sites and the Lake Chad Basin where oil exploration is targeted while vativer grass was selected for its possible hydrocarbon remedial capabilities.

2.2.2 *Crude oil*

Crude oil (Bonny light) was obtained from the Kaduna Refining and Petroleum Limited (A subsidiary company of the NNPC).

2.3 Experimental Technique

Seedlings of *E. camaldulensis*and *C.* zizanioides were cultivated in an uncontaminated pot soil at the nursery for two (2) months (November - December) to acclimatize prior totransplanting into crude oil contaminated soil.Experimental plots were plastic basins of known capacity (5 L). The pot soil was contaminated using four crude oil contamination levels (Control, 0.3 L/4.0 kg soil, 0.5 L/4.0 kg soil and 0.7

L/4.0 kg soil) as modified from the study by [15]. Seedlings of the two plant species were transplanted early morning into the contaminated plastic medium for three months (January - March) and replicated three times.

2.4 Experimental Design and Treatments

The design was the Split - Split Plot (4 x 4 x 2) experiment. This design was selected to ensure more precision to the selected plant species. The main plots were the Crude oil contamination (4 levels), Sub plot were the soil amendments (4 levels) while the sub-sub plot factor were the plant species (2 levels).

The following were the treatments (soil amendments):

T_1=Control
T_2 =NPK (g kg^{-1}soil)
T_3= Cow-dung (3:1 v/v)
T_4= NPK (g kg^{-1}soil) + Cow-dung (3:1 v/v)
Note: All experimental units were tilled and watered daily to ensure aeration.

2.5 Samples and Sampling Technique

2.5.1 *Plant samples*

Plant samples from each experimental unit were collected at the end of the three months; these were then divided into three groups: roots, leaves and stem cuttings (in case of the woody species) or root and turf (for the grass). All sampled plants were oven dried to constant weight using YC/JY series Analytical Precision Balance, China (0.001 precision) at ~ 60°C for at least 48 hours and weighed individually to determine plant biomass.

2.5.2 *Determination of plant biomass*

Plant biomass was then determined using the following mathematical relationships:

$Bm = W_r\,(g) + W_t(g)$ i (*C. zizanioides*)

$Bm\ =\ W_r(g) +\ W_s(g) +\ W_l(g)$ ii (*E. camaldulensis*)

where: W_r = Dry weight of root; W_s = Dry weight of stem; W_l = Dry weight of leaf;
W_t = Dry weight of turf; and B_m = Dry Biomass.

2.5.3 *Determination of phytoaccumulation of total petroleum hydrocarbon (TPH) and its associated asphaltenein plant tissues*

The accumulation of TPH and insolubleasphaltene in plant tissueswere determined using the method adoted by [16]. Ten grammes (10 g) of the air dried plant samples was mixed with 10 grams anhydrous sodium sulphate to remove moisture. The hydrocarbon was soxhlet extracted with chloroform for 8 hrs. The chloroform extract was then evaporated in a pre-weighed dish and the amount of total petroluem hydrocarbons (TPHs) was determined with the loss of TPH as shown in equation iii. The crude oil extracted was suspended in n-hexane and filtered through tared filter paper to remove and determine the insoluble fraction (Asphaltene) in the mathematical relationship in equation iv

$TPH_P = (W_E\,(g) + E_t\,(g)) – E_t\,(g)$ … … … … … … … iii

$ASP_P = (W_f(g) + F_t\,(g)) – F_t\,(g)$ … … … … … … … iv

where: TPH_P = Phytoaccumulated TPH, W_E= Weight of extract, E_t = Weight of tared evaporating dish, ASP_P = Phytoaccumulated asphaltene, W_f = Weight of filtrate, F_t = weight of tarred filter paper.

2.5.4 *Determination of bioaccumulation factor (BAF)*

The bioaccumulation factor (BAF) for each plant-soil pair was calculated to obtain useful information using the formula that was modified as obtained from [17] as follows:

$$BAF = \frac{Croot + Cturf}{Csoil} \qquad \text{v}$$

$$BAF = \frac{Croot + Cstem + Cleaf}{Csoil} \qquad \text{vi}$$

where:
C_{root} = Dry weight of accumulated petroleum hydrocarbon in root (g g^{-1})
C_{turf} = Dry weight of accumulated petroleum hydrocarbon in turf (g g^{-1})
C_{stem} = Dry weight of accumulated petroleum hydrocarbon in stem (g g^{-1})
C_{soil} = Dry weight of accumulated petroleum hydrocarbon in soil (g g^{-1})
Note: Equations v and vi were used to obtain BAF for *C. zizanioides* and *E. camaldulensisi*respectively.

BAF is the ratio of accumulated petroleum hydrocarbon in plant tissues to the residual petroleum hydrocarbon in soil. This model assumes that a certain mass of the contaminant had been taken up into the plant from the beginning of its growth in the contaminated soil to the time of harvest.

2.5.5 *Determination of uptake kinetics*

Time dependent uptake kinetics was based on the bioaccumulation factor (BAF) values and this was useful in estimating the effectiveness of the phytoextraction potentials of the tested species. Derivation of the uptake kinetics was based on uptake of contaminants from the contaminated soil through destructive sampling and not based on the kinetics of physiological uptake of contaminants into the plant. Note that the uptake rate is a continuous process though it may vary during the life of the plant therefore, the total mass of the contaminant removed (influx mass) was determined using equation vii below:

$$M_{influx} = C_{Plant} \ x \ BM \qquad \text{vii}$$

where:
C_{influx} = Mass of contaminant in plant species (g)
C_{plant} = Dry weight accumulated amount of contaminant in plant species (g)
BM = Dry biomass of plant species (g)

2.6 Data Analysis

Data collected were analyzed using Descriptive Statistics and Analysis of Variance (ANOVA), the split-split plot model, using GenStat Discovery Edition 4 software but due to limitation of ranking the Generalized Linear Model (GLM) procedure of SAS (Statistical Analysis System, 1999) was also used. The probability level of certainty in the research was at 95 % confidence limit or $\alpha = 0.05$ although, $\alpha = 0.01$ was also used. Statistical means were compared using the Fisher's Least Significant Difference (LSD) at $p \leq 0.05$ and $p \leq 0.01$. Means were also represented with bar charts and line graphs for easy comparison.

III. Result

3.1 Total Dry Biomass (DBM_T) Yield and Root Elongation of the Plant Species during Bioattenuation of Petroleum Hydrocarbon

The table of mean squares from ANOVA for total dry biomass (DBM_T) and root elongation produced during the bioattenuation of total petroleum hydrocarbon (TPH) were presented in Table 1. The analysis revealed that there were highly significant variability ($p < 0.05$ and $p < 0.01$) in the yield of total dry biomass (DBM_T) among the various levels of crude oil contamination, soil amendments and among the tested plant species at the end of 12 weeks after transplant (WAT) when destructive sampling of plants species took place.

On the other hand, results of Table 2 displayed the means obtained for DBM_T and the total root length (RL_T) during the bioattenuation of TPH. The result revealed that at soil contamination of C3 (0.5 L), both plant species attained the highest DBM_T with mean value of 34.82 g. That of C4 (0.7 L) was the next that encouraged production of DBM_T in the tested species with mean value of 30.86 g. It was obvious that the control C1 and C2 (0.3 L) that did not differ significantly yielded the least amount of DBM_T with mean values of 29.53 g and 29.42 g respectively. In terms of total root length (RL_T), C2 (0.3 L) produced the highest RL_T with mean value of 4.10 cm. This was closely followed by the root elongation produced in C1 with mean value of 3.83 cm. However, C3 (0.5 L) yielded the least root elongation with a mean value of 2.73 cm.

Table 1: Mean Squares from ANOVA for Root Length and Total Dry Biomass of tested Species at the end of experiment

		Growth Parameters	
Source of variation	Df	Rt Length (cm)	Dry Biomass (g)
Crude Contamination			
REP	2	0.2316	0.6139
Crude Oil Conc. (A)	3	9.2340**	153.0173**
Error	6	0.1496	0.3457
Soil Amendments			
Treatment (B)	3	12.6070**	194.0497**
A x B	9	1.0450**	113.6034**
Error	24	0.1226	0.2729
Plant Species			
Plant Species (C)	1	33.7251**	16242.4653**
A x C	3	1.7529**	245.3827**
B x C	3	0.4420*	220.6849**
A x B x C	9	1.8213**	107.3702**
Error	32	0.1689	0.1649
Total	95		

** = Highly Significant at $p < 0.01$
* = Significant at $p < 0.05$

Table 2: Means of Total Root Length (RL_T) and Total Dry Biomass (DBM_T) in the tested Species during Bioattenuation of Total Petroleum Hydrocarbon

Treatments	Root Length (cm)	Total Dry BM (g)
Crude Oil Contamination		
C1 (0 L)	3.83b	29.53c
C2 (0.3 L)	4.10a	29.42c
C3 (0.5 L)	2.73d	34.82a
C4 (0.7 L)	3.19c	30.86b
Mean	3.47	31.16
p of f	0.001	0.001
S.E.D	0.1117	0.1697
Soil Amendments		
T1	4.36a	29.86c
T2	2.99c	34.86a
T3	3.73b	31.68b
T4	2.78c	28.23d
Mean	3.47	31.16
p of f	0.001	0.001
S.E.D	0.1011	0.1508
Plant Species		
C. zizanioides	4.06a	44.16a
E. camaldulensis	2.87b	18.15b
Mean	3.47	31.16
p of f	0.001	0.001
S.E.D	0.0839	0.0829

T1 = Control; T2 = NPK (g kg^{-1}); T3 = Cow-dung (3:1 v/v); T4 = NPK (g kg^{-1}) + Cow-dung (3:1 v/v);
Figures with same alphabets within columns do not differ significantly for Crude contamination, Soil amendments and Plant species respectively p of f = Probability value of F. S.E.D = Standard Error Deviation.

Results in Table 2 further indicated that the soil amended with T2 (NPK g^{-1} Kg^{-1}) yielded the highest DBM_T with mean value of 34.86 g as compared to T4 (NPK g^{-1} $Kg^{-}1$ + Cow-dung 3:1 v/v) that yielded the least DBM_T with mean value of 28.23 g. In terms of the RL_T, the control soil amendment (T1) produced the highest root elongation with a mean value of 4.36 cm. The RL_T of T2 and T4 that did not differ significantly was the least with mean values of 2.99 cm and 2.78 cm respectively.

It was further revealed from Table 2 that *C. zizanioides* species yielded the highest DBM_T and RL_T with means of 44.16 g and 4.06 cm respectively as compared to that of *E. camaldulensis* that yielded the least DBM_T and RL_T with means of 18.15 g and 2.87 cm respectively.

3.2 Phytoaccumulation of Total Petroleum Hydrocarbon in Plant Tissues

The excerpt of mean squares from ANOVA for phytoaccumulation of TPH and the insoluble asphaltene were presented in Table 3. The analysis suggested that there were highly significant differences ($p < 0.05$ and $p < 0.01$) in the phytoaccumulation of both TPH and asphaltene among various levels of crude oil contamination, soil amendments and the tested species at the end of 12 WAT.

Table 3: Mean Squares from the Analysis of Variance for accumulated TPH and its associated Asphaltene content in tested Species

		Accumulation in Plants	
Source of variation	Df	TPH (g)	Asphaltene (g)
Crude Oil Contamination			
REP	2	0.005018	0.000837
Crude Oil Conc. (A)	2	0.052018**	0.123450**
Error	4	0.001624	0.001175
Soil Amendments			
Treatment (B)	3	0.074068**	0.195638**
A x B	6	0.181368**	0.052826**
Error	18	0.002061	0.001155
Plant Species			
Plant Species (C)	1	1.677501**	0.491701**
A x C	2	0.119935**	0.006689**
B x C	3	0.126898**	0.090638**
A x B x C	6	0.119014**	0.0023031**
Error	24	0.002387	0.001290
Total	71		

** = Highly Significant at $p < 0.01$

The mean values for TPH and asphaltene in the contamination levels, soil amendments and the tested plant species were presented in Table 4. From the result, phytoaccumulation of TPH in tissues of the tested plants was found to be higher in both soils contaminated at the lowest level (C2 with 0.3 L) and that of highest level (C4 with 0.7 L) that do not differ significantly with mean values for TPH as 0.58 g and 0.57 g respectively. Likewise, accumulated asphaltene was found to be higher in C4 (0.7 L) with a mean value of 0.47 g than in C2 (0.3 L) and C3 (0.5 L) that do not differ significantly with mean values of 0.35 g and 0. 34 g respectively. This entails that, the higher the contamination of soil by crude oil, the more the phytoaccumulation of same into tissues of plants.

The soil without amendment except tillage and watering at field capacity (T1) was observed to yield the highest mean phytoaccumulation value for both TPH and asphaltene in plant tissues with 0.64 g and 0.48 g respectively. On the other hand, while the soil amended with T4 (NPK g^{-1} $Kg^{-}1$ + Cow-dung 3:1 v/v) showed the least accumulation of asphaltene with 0.24 g, T2 (NPK g^{-1} Kg^{-1}) and T3 (Cow-dung 3:1 v/v) that do not differ significantly yielded the least for TPH accumulation in plant tissues with mean values of 0.50 g and 0.51 g respectively.

A closer glance at Table 4 further revealed that *C. zizanioides* was the best species for the phytoaccumulation of both TPH and the insoluble asphaltene during the course of this study with mean values of 0.70 g and 0.47 g respectively.

Table 4: Mean accumulated TPH and its associated Asphaltene content in tested Species during Bioattenuation

Treatments	Accumulated TPH (g)	Accumulated Asphaltene (g)
Crude Oil Contamination		
C2 (0.3 L)	0.58a	0.35b
C3 (0.5 L)	0.50b	0.34b
C4 (0.7 L)	0.57a	0.47a
Mean	0.55	0.39
p of f	0.001	0.001
S.E.D	0.01163	0.0099
Soil Amendments		
T1	0.64a	0.48a
T2	0.50c	0.43b
T3	0.51c	0.41b
T4	0.56b	0.24c
Mean	0.55	0.39
p of f	0.001	0.001
S.E.D	0.01513	0.01133
Plant Species		
C. zizanioides	0.70a	0.47a
E. camaldulensis	0.40b	0.30b
Mean	0.55	0.39
p of f	0.001	0.001
S.E.D	0.01152	0.0085

T1 = Control; T2 = NPK (g kg^{-1}); T3 = Cow-dung (3:1 v/v); T4 = NPK (g kg^{-1}) + Cow-dung (3:1 v/v);
Figures with same alphabets within columns do not differ significantly. S.E.D = Standard Error Deviation.

3.3 Phytoextraction Potentials of the tested Plant Species during Bioattenuation of Total Petroleum Hydrocarbon

Phytoextraction potentials for the tested species were found in using the bioaccumulation factor (BAF) and mass influx (M_{influx}) calculated from the results of accumulation of hydrocarbon contaminants in plant tissues. The mean square values from ANOVA for the Bioaccumulation Factor (BAF) and the Mass influx (M_{influx}) of petroleum hydrocarbon in the studied species were presented in Table 5. The result of the analysis showed that there were highly significant variability ($p < 0.05$ and $p < 0.01$) in the BAF as well as M_{influx} for both TPH and asphaltene among the various levels of crude oil contamination, soil amendments and the tested plant species.

3.3.1 *Bioaccumulation Factor (BAF) for petroleum hydrocarbon in plant species*

Result indicating mean values of BAF for both TPH and asphaltene in the tested species were presented in Table 6. From the result, it was revealed that BAF for TPH was highest in C2 (0.3 L) with a mean value of 2.28 g g^{-1} while the least BAF for TPH was recorded in C3 (0.5 L) with a mean value of 1.44 g g^{-1}. Similarly, the BAF for asphaltene in C2 (0.3 L) that did not differ significantly with that of C4 (0.7 L) was the best with mean values of 2.87 g g^{-1} and 2.64 g g^{-1} respectively.

The results further revealed that the soil amended with T4 (NPK g^{-1} Kg^{-}1 + Cow-dung 3:1 v/v) yielded the highest BAF for TPH with mean value of 2.13 (g g^{-1}); and the least was found in T3 (Cow-dung 3:1 v/v) with mean value of 1.71 g g^{-1}.

In terms of asphaltene, the soil amended with T2 (NPK g^{-1} Kg^{-1}) yielded the highest BAF value of 3.45 g g^{-1} while the least was observed in T4 (NPK g^{-1} Kg^{-}1 + Cow-dung 3:1 v/v) with mean value of 1.50 g g^{-1}. Looking at the tested plant species, it was observed that *C. zizanioides* species had the highest BAF for both TPH and asphaltene with mean values of 2.56 g g^{-1} and 3.67 g g^{-1} respectively. Contrarily, *E. camaldulensis* species yielded the least BAF for both TPH and asphaltene with mean values of 0.97 g g^{-1} and 1.73 g g^{-1} respectively.

Table 5: Mean Squares from Analysis of Variance for Bioaccumulation Factor (BAF) and Total Mass Influx (M_{influx}) for TPH and its associated Asphaltene content (g g^{-1}) in the tested Species

Source of variation	Df	BAF and M_{influx} in Plant Species BAF TPH (g g^{-1})	BAF Asphaltene (g g^{-1})	M_{influx} TPH (g)	M_{influx} Asphaltene (g g^{-1})
Crude Contamination					
REP	2	010548	0.5047	7.591	1.759
Crude Oil Conc. (A)	2	4.86133**	0.5344*	13.724**	116.605**
Error	4	0.01733	0.1188	1.648	0.954
Soil Amendments					
Treatment (B)	3	1.19099**	13.1125**	86.256**	439.510**
A x B	6	2.94849**	2.2593**	394.580**	142.160**
Error	18	0.02751	0.1592	4.607	2.504
Plant Species					
Plant Species (C)	1	45.58533**	67.2027**	10804.500	5213.907**
A x C	2	6.22002**	0.2121*	140.134**	37.777**
B x C	3	2.47988**	9.5446**	264.948**	406.782**
A x B x C	6	2.80777**	1.8653**	390.732**	102.426**
Error	24	0.02955	0.1710	4.374	1.928
Total	71				

** = Highly Significant at $P < 0.01$

* = Significant at $P < 0.05$

Table 6: Bioaccumulation Factor (BAF) and Mass Influx (M_{influx}) for TPH and associated Asphaltene (g g^{-1}) in the tested Species

Treatments	BAF TPH	BAF Asphaltene	M_{influx} TPH	M_{influx} Asphaltene
Crude Oil Contamination				
C2 (0.3 L)	2.28^{a}	2.87^{a}	19.33^{ab}	12.40^{c}
C3 (0.5 L)	1.44^{c}	2.59^{b}	20.32^{a}	13.52^{b}
C4 (0.7 L)	1.58^{b}	2.64^{ab}	18.83^{b}	16.65^{a}
Mean	1.77	2.70	19.49	14.19
p of f	0.001	0.095	0.037	0.001
S.E.D	0.0380	0.0995	0.371	0.282
Soil Amendments				
T1	1.72^{b}	3.12^{b}	20.04^{b}	14.62^{b}
T2	1.51^{c}	3.45^{a}	22.14^{a}	19.31^{a}
T3	1.71^{b}	2.73^{c}	16.90^{c}	15.38^{b}
T4	2.13^{a}	1.50^{d}	18.90^{b}	7.45^{c}
Mean	1.77	2.70	19.49	14.19
p of f	0.001	0.001	0.001	0.001
S.E.D	0.0553	0.1330	0.715	0.527
Plant Species				
C. zizanioides	2.56a	3.67a	31.74a	22.70a
E. camaldulensis	0.97b	1.73b	7.24b	5.68b
Mean	1.77	2.70	19.49	14.19
p of f	0.001	0.001	0.001	0.001
S.E.D	0.0405	0.0975	0.493	0.327

T1 = Control; T2 = NPK (g kg^{-1}); T3 = Cow-dung (3:1 v/v); T4 = NPK (g kg^{-1}) + Cow-dung (3:1 v/v);

Figures with same alphabets within columns do not differ significantly for Crude contamination, Soil amendments and Plant species respectively p of f = Probability value of F. S.E.D = Standard Error Deviation.

3.3.2 *Mass Influx (M_{influx}) of petroleum hydrocarbon in plant species*

The mean M_{influx} values for both TPH and asphaltene in the tested species were also presented in Table 6. It revealed that C3 (0.5 L) with a mean value of 20.32 g g^{-1} that did not differ significantly with that of C2 (0.3 L) with a mean value of 19.33 g g^{-1} yielded the highest M_{influx}result for TPH. Ironically, the M_{influx} found in C4 (0.7 L) that did differ significantly with that of C3 yielded the least with mean values of 18.83 g g^{-1} and 19.33 g g^{-1} respectively. Additionally, M_{influx} of asphaltene was found to be highest in C4 (0.7 L) with a mean value of 16.65 g g^{-1}. This was closely followed by that of C3 (0.5 L) with a mean value of 13.52 g g^{-1}. The least M_{influx} was observed in C2 (0.3 L) with mean value of 12.40 g g^{-1}.

The soil amended with T2 (NPK g^{-1} Kg^{-1}) yielded the highest M_{influx} result for both TPH and asphaltene with mean values of 22.14 g g^{-1} and 19.31 g g^{-1} respectively. Contrarily, T3 (Cow-dung 3:1 v/v) yielded the least M_{influx} value for TPH with mean of 16.90 g g^{-1} while that of asphaltene was T4 (NPK g^{-1} $Kg^{-}1$ + Cow-dung 3:1 v/v) with a mean value of 7.45 g g^{-1}.

The species *C. zizanioides*yielded the highest M_{influx} values for both TPH asphaltene with mean values of 31.74 g $g^{-}1$ and 22.70 g g^{-1} respectively. Contrarily, that of *E. camaldulensis* yielded the least M_{influx} value for both TPH and asphaltene with mean values of 7.24 g g^{-1} and 5.68 g g^{-1} respectively.

IV. Discussion

4.1 Plant Biomass Accumulation and Root Elongation during Bioattenuation of TPH and Asphaltene

The acquisition of total biomass tends to differ significantly with the levels of crude oil contamination, soil amendments and among plant species. It was observed that the tested plant species attained the highest DBM_T in the soil contaminated with 0.5 L/kg soil as compared to that of 0.7L/kg soil. This means that the tested species can only attain maximum biomass production in concentrations not beyond 0.5 L in soils; although the average biomass production was observed in higher contamination levels in soils. This is despite the fact that crude oil causes among other things low permeability and infiltration of water into the soil; a condition that may lead to accumulation of water on the surface of soil thereby creating artificial drought on the sub surface soil layer as supported by [15]. Additionally, soils amended with NPK in addition to tillage and watering at field capacity tends to encourage DBM_T production in the tested plants. This could be partly because tillage and watering as part of the daily general treatment helps in the increase of aeration and soil moisture respectively. It could also be due to the fact that deficiency of phosphorous and potassium in soils are augmented readily by the inorganic fertilizer as it is believed that it dissolve easily in soils than that of the organic fertilizer.

On the contrary, root elongation for the tested species was found be best at 0.3 L/kg soil contamination and tends to reduce with increasing level of contamination. This means that roots of the tested species are affected by crude oil contamination at higher concentration as attested to by [18]. Additionally, soils amended with only tillage and watering (T1) at field capacity tends to encourage RL_T production in the tested plants. The increase could be partly because tillage and watering as part of daily treatment helps ameliorate aeration and soil moisture respectively. In case of the tested species, *C. zizanioides* was the best plant species that tends to have higher biomass and root elongation which was twice that of *E. camaldulensis*. This result was much anticipated since the root of the plant *C. zizanioides* could reach up to 3 m within one year as supported by [19]. It is also known that some plants do synthesize some root-growth regulating hormones in contaminated sites as supported by [18].

4.2 Phytoaccumulation of Petroleum Hydrocarbon in Plant Tissues

Phytoaccumulation of the soluble total petroleum hydrocarbon (TPH) in plant tissues does not differ significantly among the highest and lowest levels of soil contamination C4 (0.7 L kg^{-1} soil) and C2 (0.3l kg^{-1} soil) respectively. This implies that accumulation of the soluble TPH in tissues of the tested species is irrespective of the level of hydrocarbon contamination in soils. On the contrary, the accumulation of the insoluble asphaltene increased with increasing level of hydrocarbon contamination in soils. This is indicative of the fact that the higher the contaminations due to hydrocarbon in soils, the higher the accumulation of asphaltene in the tissues of plants. This implies that the tested species are very good phytoaccumulator plants as supported by [12]. These plants are readily available and can grow fast hence, it will be effective in the clean-up of hydrocarbon polluted sites in Nigeria.

Aside the control treatment (T1) with only daily tillage and watering at field capacity, soils amending with the combined treatment T4 (NPK g^{-1} $Kg^{-}1$ + Cow-dung 3:1 v/v) did encouraged better accumulation of contaminants in tissues of the tested species. This could be because tillage and watering does improve permeability and availability of contaminants to plants as well as improve aeration in contaminated soil while both the organic and inorganic nitrogen and phosphorus improves the activities of microorganisms in soil leading to reduced toxicity of contaminants to plant and increased accumulation as supported by [20].

4.3 Phytoextraction Efficiency of *C. zizanioides*and *E. camaldulensis* for Petroleum Hydrocarbon using

Bioaccumulation Factor

The bioextraction efficiency of the tested species as measured using the BAF and mass influx kinetics revealed that while BAF for the removal of TPH by plant species was higher at lower crude oil contamination, BAF for asphaltene was higher at higher crude oil contamination. This implies that TPH was removed from the soil by the tested species much better at lower crude contamination than at higher contamination. On the other hand, asphaltene content was removed much more from the soil at higher concentration; the higher the contamination the more the phytoextraction capability of the plants for the insoluble asphaltene.

Additionally, the combined soil amendment T4 (NPK g^{-1} $Kg^{-}1$ + Cow-dung 3:1 v/v) in addition to daily tillage and watering at field capacity favored BAF for TPH but T2 (NPK g^{-1} Kg^{-1}) was best for the insoluble asphaltene accumulation. The grass *C. zizanioides* was best in BAF for both TPH and asphaltene with its BAF mean values twice that of *E. camaldulensis*. And as observed that the BAF values of the two tested species is greater than 1, this implies that the tested species can tolerate and accumulate hydrocarbon contaminants from soils more than the concentrations found in soils to the point of clean-up to pave way for other uses of the contaminated sites. It also implies that *C. zizanioides* can clean-up hydrocarbon contaminated soils twice as much as the woody species *E. camaldulensis* can ever achieve in soils.

Generally speaking, the higher the plant biomass the more the amount of contaminant to be removed from the soil by such plant. This phytoextraction of contaminants, especially by *C. zizanioides* could be due to its massive root system and high phytoaccumulation and biomass yield. [21] agreed that effective pollutant removal by plants from soil depends on an adequate yield of plants and/or the efficient transfer of contaminants from the roots of the plants into its aerial parts. [22] reported that phytoextraction is more effective with vigorously growing plants that are easily harvested and which accumulate large concentration of contaminants in harvestable form.

4.4 Phytoextraction Efficiency of *C. zizanioides*and *E. camaldulensis* for Petroleum Hydrocarbon using Mass Influx ratio in Plants

It was observed that the rate at which the tested plant species accumulate the soluble TPH does not differ among the different levels of crude oil contaminations in soils. This is not true for the insoluble asphaltene in that it was found that its rate of uptake and accumulation in plant tissues was higher in heavily polluted soils than a mild contaminated site. This means that the tested species can uptake contaminants irrespective of the amount of its concentrations in soils.

Furthermore, amending the soil with inorganic fertilizer (NPK) was found to encourage the uptake, translocation, accumulation and possibly assimilation of both the soluble TPH and the insoluble asphaltene in the tested species. Additionally, the rate at which *C. zizanioides* species did uptake, translocate, accumulate, and possibly assimilate both TPH and asphaltene were found to be four times higher than that of *E. camaldulensis* species. This could be due to the fact that *C. zizanioides* species did produce more biomass than *E. camaldulensis* as observed during this experiment. This result is supported by [21] and [22] that the higher the biomass the more the accumulation of contaminants in tissues of plants.

V. Conclusion

Based on the results of this study, it was concluded that there were combined effects of the level of crude oil contamination, soil amendments and influence of plant species in the phytoaccumulation and phtoextraction of organic contaminants from soils. While phytoaccumulation of TPH in *C. zizanioides* reduced with increased contaminants that of asphaltene increased with increased contamination in soils. On the other hand, TPH and asphaltene accumulation increased with increased contamination in *E. camaldulensis*. However, the perennial grass (*C. zizanioides*) was the best phytoaccumulator species. Additionally, root elongation and production of dry biomass were affected by the levels of crude oil contamination in soils. While the production of total plant biomass was better in concentrations not more than 0.5 L, root elongation is better when concentrations does not exceed 0.3 L/kg soil. Generally, *C. zizanioides* had more total dry biomass than *E.* camaldulensis species.

In terms of bioextraction potentials using the BAF and Mass influx kinetics, it was revealed that extraction of TPH and asphaltene from contaminated soil was twice in quantity and four times faster using the grass *C. zizanioides* species. To this end, the combined effects of using NPK (inorganic fertilizer) and cow-dung (organic fertilizer) as soil amendments proved to be effective for phytoextraction and phytoaccumulation of contaminants by plants.

VI. Recommendations

It is recommended that for better plant biomass and root elongation in addition to the remediation of both soluble TPH and insoluble asphaltene components of crude oil from soils, contamination levels of the site should be reduced through non biological remediation procedures to about 0.5 L Kg^{-1} soil before applying phytoremediation procedure as final clean-up of contaminants. In addition, it is recommended that the mixture of NPK (inorganic) and cow-dung (organic) fertilizers with daily tillage and watering at field capacity should be maintained during phytoremediation procedure on contaminated sites to encourage phytoextraction, phytoaccumulation and plant growth. Moreso, the grass *C. zizanioides* should be rigorously considered in the decontamination of organic contaminants in sites.

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