Phytoaccumulation and Bioextraction Potentials of Chrysopogon zizanioides L. Roberty in the Bioattenuation of Hydrocarbon Polluted Soils

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Abstract: Phytoaccumulation and bioextraction potentials of Chrysopogon zizanioides during bioattenuation of hydrocarbon contaminated soils was studied to assess accumulation and partitioning of Total Petroleum Hydrocarbon (TPH) and insoluble asphaltene in the plant tissues. Experimental design was Split-Split Plot (3 x 4 x 2). Main plot factors were crude oil contamination (3 levels), sub-Plot factors were soil amendments (4 levels), sub-sub-Plot factors were plant tissues (2 levels). Results indicated highly significant differences (P ≤ 0.01) in phytoaccumulation and bioextraction between plant tissues, crude oil contamination and soil amendments. Roots and turf of the grass were found to accumulate both TPH (0.52 g g⁻¹ and 0.18 g g⁻¹ respectively) and asphaltene (0.40 g g⁻¹ and 0.11 g g⁻¹ respectively). Bioaccumulation factor (BAF) for TPH was highest in C2 under treatment T4 (2.28 g g⁻¹ and 2.13 g g⁻¹ respectively) while asphaltene was highest in C2 not differing with C4 (2.87 g g⁻¹ and 2.64 g g⁻¹ respectively) under treatment T4 with 3.45 g g⁻¹. The plant was observed to phytoextract and accumulated 20.32 g g⁻¹ TPH under C4 and 16.65 g g⁻¹ asphaltene under C4 in three months. The best soil amendment for bioextraction and phytoaccumulation was T4 but T3 encouraged growth. Therefore, C. zizanioides was recommended as efficient hyper accumulator species for crude oil remediation. Phytonmining from the plant could be considered.

Keywords: Phytoaccumulation, Bioextraction, Bioaccumulation Factor (BAF), Phytoremediation, Bioattenuation

I. Introduction

The problem of environmental pollution, especially due to hydrocarbon, has been globally a challenge requiring multifaceted solution. Several authors [12,1], observed that anthropogenic activities such as oil spills from exploration sites and pipeline ruptures, mostly due to vandals, are the most common named culprits of this devastating environmental scourge. Most importantly, total petroleum hydrocarbon (TPH), as a major source of environmental pollution, is not only identified as the most widespread among hazardous wastes that is carcinogenic and mutagenic in nature but it is persistent in the environment. Its persistence constitutes threats to humans, animals and plants; including agricultural productivity [7].

In Nigeria, it has been reported that environmental pollution due to hydrocarbon have reached a frightening scale in the Niger Delta, the largest delta in Africa [13,2]. Notwithstanding, the Lake Chad Basin located in the North Eastern part of Nigeria have been recently targeted for possible oil exploration; this will further aggravate the negative environmental impact and other social and economic problems in Nigeria. Thus, it is necessary to draw urgent attention on feasible clean-up procedures that will combat this devastating environmental, social and economic impacts on the affected communities. In recent times, the observed militancy and fuel scarcity in Nigeria due to the activities of aggrieved vandals in affected communities has led to erratic power outages that in turn affects businesses; this is an obvious stern warning sign to the crude oil pollution crisis.

According to several authors [5,7,14], some strategies employed in the remedy of polluted terrestrial sites includes excavating contaminated soil and dumping it to landfills followed by subsequent processes such as chemical soil washing (through solvent extraction and UV oxidation), and biological treatments (e.g. bioventing and Biosparging). These methods did provide rapid solution to the immediate problem, but was observed to be very costly and leave unseen blemish on the landscape, and often have limited success.

It is obvious that a comparatively cheaper and feasible sustainable method of pollutants removal from the environment is necessary. One of such method is phytoremediation, which is the use of green plants and their associated rhizosphere microorganisms for the in-situ treatment of contaminated soil and groundwater [29,18,13,23]. In more recent times, phytoremediation has been the focus of researchers because it utilizes natural tendencies of plants and associated microorganisms to achieve decontamination.

In general, several researchers have established that there are various phyto-technologies utilized by plants to remediate environmental contamination. For instance, plants can be used as rhizofilters [17,28], as...
adsorption pumps in the root zone [24] and as a direct means of degradation of organic pollutants either by microbes in the rhizosphere (phytostimulation or rhizodegradation) [22,8] or enzymatic activities of plants (phytodegradation) [22,31]. Certain pollutants can escape the plant through phytovolatilization [32,15].

In addition, some plants can phyto-stabilize pollutants in soil either by simply preventing erosion, leaching, runoff or by converting pollutants to less bioavailable forms [4,8,10]. Other plants can also phytoextract or phytoaccumulate pollutants in their tissues [6,19] and can subsequently be used for non-food purposes, disposal in landfills or for recycling valuable elements (phytomining) [9]. Phytoextraction and phytoaccumulation are both important processes involved in phytostabilization of contaminants in soil. It is vital to note that these various phyto-technologies are not mutually exclusive [16,8].

Evidence abounds in literature that substantiate the fact that the adoptive avoidance mechanisms of some specialized hyperaccumulator plant species do reduced toxicity and therefore enable their growth on contaminated habitats. And according to [10], the avoidance mechanisms could be reduced uptake, chelation of sulphur polypeptide or sulphur-hydrogen proteins in the cytoplasm, immobilization of toxic ion in cell walls, impeded permeation across boundary layers of the protoplasm, induced stress proteins (phytochemicals), compartmentalization and formation of complexes (with either organic and inorganic acids, phenol derivatives or glycosides) in the vacuole, and lastly re-translocation.

Despite the various advantages of phytoremediation method that is cost effective and feasible, especially in combination with other in-situ bioremediation methods such as tilling and the addition of fertilizers (both organic and inorganic) in land farming, not much concerted effort is made to especially identify most plants species that could be potential phytoextractors and or phytoaccumulators in Nigeria.

To this end, this research proffers some feasible solutions to the environmental contamination problems using the phytoremediation technology believing that it will impact positively on the environment thereby improving the socioeconomic conditions of not only the Lake Chad Basin pruned hydrocarbon contamination but also the Niger delta and perhaps, wherever oil exploration is contemplated in Nigeria.

The aim of the study was to assess the efficacy of *C. zizanioides* species in the phytoextraction and phytoaccumulation of Total Petroleum Hydrocarbon (TPH) and associated asphaltene content in contaminated soils. The objectives were to:

i. estimate accumulation and partitioning of Total Petroleum Hydrocarbon (TPH) and associated asphaltene in plant tissues,
ii. assess the phytoextraction potentials of *C. zizanioides* during bioattenuation of crude oil contaminated soil,
iii. compare the relationship between level of crude oil contamination and TPH/asphaltene accumulation in plant tissues,
iv. assess the best soil amendments encouraging both phytoaccumulation and phytoextraction during bioattenuation of the contaminated soils under the influence of *C. zizanioides* species.

This study is limited to contaminated soils of the Lake Chad Basin, targeted for oil exploration, using mixtures of in-situ bioremediation methods to achieve decontamination of TPH but carried out in Dutse, Jigawa State, Nigeria. The phytoremediation procedure, in addition to some soil amendments such as tillage, watering and fertilizer (organic and inorganic fertilizers such as: Cow-dung and NPK) application, was considered to enhance remediation of the soil in question. The study did not account for the lost of TPH through volatilization process.

### II. Research Methodology

#### 2.1 Study Area

The study was carried out at a site near the Federal University Dutse, Jigawa State, Nigeria. Dutse is the state capital. Dutse lies between Latitude 11° N to 13° N and Longitude 8°E to 10° 15’ E. Its semi-arid climate is characterized by long erratic dry season (October - May) and a short wet season (June - September) heralded by violent dust storms followed by tornado and lightening. Its total annual rainfall ranges from 600 mm to 1000 mm. The mean annual temperature is about 25°C. Evapotranspiration is very high and relative humidity is highest in August (up to 80 per cent) and low in January through March (20 - 30). Most of the state falls within the Sudan Savannah vegetation belt, but traces of Guinea savannah vegetation are found in parts of the southern districts.

The ancient Pre Cambrian rocks of the basement complex comprising granites, schists and gneisses are separated from the younger sediment of the Chad Formation by a hydrological divide, which runs through Kiyawa, Dutse and Yankwash. The Chad formation occupies the north-eastern parts of the state. However, the basement complex rocks have undergone weathering to give rise to fairly deep soils which are often covered by a sheet of laterite which has been exposed by denudation in some places. The Chad sediments are concealed by sand dunes and its sandy beds, formed over the impervious clays of the Chad formation, form the main source of water supply in the dry season. The soils are generally sandy at the top and compact at depth with often hard
pans. Aeolian deposits from the Sahara Desert form substantial part of soils in the state especially towards the northern parts. The mixing of the subsoil in these deposits has given rise to clayey subsoil, which dominates the northern parts of the state [21].

2.2 Materials
2.2.1 Plant material
Seedlings of *Chrysopogon zizanioides* obtained from a farm in Kiyawa town about 70 km from Dutse, Jigawa state capital formed the plant used for the study. The plant 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 *C. zizanioides* were cultivated in an uncontaminated soil at the nursery for two (2) months (November - December 2014) for acclimatization prior to their being transplanted into crude oil contaminated potted soil. Experimental plots were plastic basins of known capacity (5 L). The pot soil was contaminated using three crude oil contamination levels: C2 (0.3 L/4.0 kg soil), C3 (0.5 L/4.0 kg soil) and C4 (0.7 L/4 kg soil) as modified from the study of [25]. Seedlings of the plant species was then transplanted early morning into the contaminated plastic medium and left for three months (January - March, 2015). The basic experiment described was then replicated three times.

2.4 Experimental Design and Treatments
The design was the Split - Split Plot (3 x 4 x 2). This design was selected to ensure more precision to the selected plant species. The main plots were the Crude oil contamination (3 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 soil amendments that served as treatments: T1=Control (tilled and watered daily), T2=NPK (g kg⁻¹soil), T3= Cow-dung (3:1 v/v), T4= NPK (g kg⁻¹soil) + Cow-dung (3:1 v/v). Note that all experimental units were watered and tilled daily to ensure aeration.

2.5 Samples and Sampling Technique
2.5.1 Soil samples
Soil was sampled randomly at depth of 0 - 10 cm (the surface and at middle) using soil auger from each sampling unit. The sampled soil was then homogenized and composite soil sample obtained from the experimental pots for each species every 21 days for a period of three months. All sampled soil for % MC, residual TPH quantification and microbiological analysis were collected as quickly as possible to prevent exposure to the environment and subsequent error in measurements. In addition, except for the tests mentioned, all soil was oven dried and passed through a 2 mm sieve prior to analysis.

2.5.2 Plant samples
Plant samples from each experimental unit were collected at the end of the three months; these were then divided into roots and turf. All sampled plants were oven dried and weighed 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. Plant biomass was then determined using the following mathematical relationship:

\[ B_m = W_r (g) + W_t (g) \]  

(1)

where: \( W_r \) = Dry weight of root; \( W_t \) = Dry weight of turf; and \( B_m \) = Dry Biomass. In addition, root length was measured for each experimental plant species using a metal measuring tape with 50 cm calibration.

2.6 Determination of Accumulated and Residual TPH and Asphaltene in Samples
The Residual TPH and its fraction were determined using the method adoted by [11]. Ten grammes (10 g) of the oven dried plant samples and air dried soil 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 petroleum hydrocarbons (TPHs) was determined with the loss of TPH as shown in equation ii. The extracted residual oil 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 iii.
where: \( TPH_R \) = Residual TPH, \( W_e \) = Weight of extract, \( E_e \) = Weight of tared evaporating dish, \( ASP_R \) = Residual asphaltene, \( W_f \) = Weight of filtrate, \( F_e \) = weight of tared filter paper.

2.7 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 [3] as follows:

\[
BAF = \frac{C_{\text{root}} + C_{\text{turf}}}{C_{\text{soil}}} \tag{4}
\]

where: \( C_{\text{root}} \) = Dry weight of accumulated petroleum hydrocarbon in root (g g\(^{-1}\)); \( C_{\text{turf}} \) = Dry weight of accumulated petroleum hydrocarbon in turf (g g\(^{-1}\)); \( C_{\text{soil}} \) = Dry weight of accumulated petroleum hydrocarbon in soil (g g\(^{-1}\))

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.

2.8 Determination of Uptake Kinetics

Time dependent uptake kinetics was based on the BAF values, as adopted from [3], 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. It is assumed that the uptake rate is a continuous process though, it may vary during the life of the plant. Thus, the total mass of the contaminant removed (influx mass) was determined using equation v below:

\[
M_{\text{influx}} = C_{\text{plant}} \times BM \tag{5}
\]

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

2.9 Data Analysis

Data collected were analyzed using Analysis of Variance (ANOVA) in the split-split plot model. The GenStat Discovery Edition 4 software was used in the analysis but due to its limitation in ranking, the Generalized Linear Model (GLM) procedure of SAS (Statistical Analysis System, 1999) was also used for the same analysis. The probability level of certainty was set 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 using some Descriptive Statistics such as bar charts and line graphs for ease in comparison.

III. Results and Discussion

3.1 Results

3.1.1 Bioaccumulation and Partitioning of Petroleum Hydrocarbon in C. zizanioides

The excerpt of mean squares from ANOVA table for both TPH and asphaltene contents in \( C. zizanioides \) were presented in Table 1. It revealed that there were highly significant differences (\( p < 0.05 \) and \( p < 0.01 \)) in the amount of both TPH and asphaltene accumulated in tissues of \( C. zizanioides \) among the levels of crude oil contamination, different soil amendments and the plant parts of turf and root. Also, there were highly significant interactions (\( p < 0.05 \) and \( p < 0.01 \)) for the accumulated TPH and associated asphaltene between the levels of crude oil contamination, soil amendments and the two plant parts.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>TPH (g)</th>
<th>Asphaltene (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Oil Contamination</td>
<td>2</td>
<td>0.0049014</td>
<td>0.00037917</td>
</tr>
<tr>
<td>REP</td>
<td>4</td>
<td>0.016056</td>
<td>0.00045208</td>
</tr>
<tr>
<td>Crude Contamination (A)</td>
<td>2</td>
<td>0.04102917**</td>
<td>0.04102917**</td>
</tr>
<tr>
<td>Error</td>
<td>4</td>
<td>0.0016056</td>
<td>0.00045208</td>
</tr>
</tbody>
</table>

Table 1: Mean Squares from Analysis of Variance showing partitioning of accumulated TPH and Asphaltene in \( C. zizanioides \).
Phytoaccumulation and Bioextraction Potentials of Chrysopogon zizanioides L. Roberty in the...

<table>
<thead>
<tr>
<th>Treatment (B)</th>
<th>3</th>
<th>0.0252378**</th>
<th>0.10495324**</th>
</tr>
</thead>
<tbody>
<tr>
<td>A x B</td>
<td>6</td>
<td>0.1362069**</td>
<td>0.03707546**</td>
</tr>
<tr>
<td>Error</td>
<td>18</td>
<td>0.0026042</td>
<td>0.00068333</td>
</tr>
<tr>
<td>Plant Parts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant Parts (C)</td>
<td>1</td>
<td>2.1012500**</td>
<td>1.2001250**</td>
</tr>
<tr>
<td>A x C</td>
<td>2</td>
<td>0.0055792**</td>
<td>0.00440417**</td>
</tr>
<tr>
<td>B x C</td>
<td>3</td>
<td>0.0288241**</td>
<td>0.03041250**</td>
</tr>
<tr>
<td>A x B x C</td>
<td>6</td>
<td>0.0117810**</td>
<td>0.03775417**</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.0007514</td>
<td>0.00007778</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = Highly Significant at p < 0.01

Table 2: TPH and Asphaltene content partitioned in C. zizanioides

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TPH (g)</th>
<th>Asphaltene (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Oil Contamination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2 (0.3 L)</td>
<td>0.40</td>
<td>0.23</td>
</tr>
<tr>
<td>C3 (0.5 L)</td>
<td>0.32</td>
<td>0.20</td>
</tr>
<tr>
<td>C4 (0.7 L)</td>
<td>0.33</td>
<td>0.28</td>
</tr>
<tr>
<td>Mean</td>
<td>0.35</td>
<td>0.24</td>
</tr>
<tr>
<td>S.E.D</td>
<td>0.01157</td>
<td>0.00614</td>
</tr>
<tr>
<td>Soil Amendments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>0.34</td>
<td>0.28</td>
</tr>
<tr>
<td>T2</td>
<td>0.34</td>
<td>0.31</td>
</tr>
<tr>
<td>T3</td>
<td>0.31</td>
<td>0.27</td>
</tr>
<tr>
<td>T4</td>
<td>0.40a</td>
<td>0.13d</td>
</tr>
<tr>
<td>Mean</td>
<td>0.35</td>
<td>0.24</td>
</tr>
<tr>
<td>S.E.D</td>
<td>0.01701</td>
<td>0.00871</td>
</tr>
<tr>
<td>Plant Tissues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turf</td>
<td>0.18</td>
<td>0.11</td>
</tr>
<tr>
<td>Root</td>
<td>0.52</td>
<td>0.40</td>
</tr>
<tr>
<td>Mean</td>
<td>0.35</td>
<td>0.24</td>
</tr>
<tr>
<td>S.E.D</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>
| 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 presented in Table 2 indicated the accumulated and partitioned TPH and asphaltene in different tissues of C. zizanioides. It revealed that TPH accumulation was higher in the lowest contamination level (C2) with mean value of 0.40 g and the least was observed in C3 (0.32 g) that did not differ significantly from C4 (0.33 g). Inversely, asphaltene accumulation was observed to be higher in the highest contamination level (C4) with mean value of 0.28 g while the least was observed in C3 with mean value of 0.20 g.

The best soil amendment that encouraged accumulation of TPH was T4 with mean value of 0.40 g while that of asphaltene accumulation was T2 with mean value of 0.31 g. In addition, C. zizanioides species accumulated more of both TPH and asphaltene in its roots with 0.52 g and 0.40 g respectively than in its turf with 0.18 g and 0.11 g respectively.

3.1.2 Bioextraction Potentials of C. zizanioides during Bioattenuation of Petroleum Hydrocarbon

The bioextraction potentials of C. zizanioides species was observed using the Bioaccumulation Factor (BAF) and Mass Influx (M_influx) as calculated from the results of TPH and asphaltene bioaccumulation in the plant tissues as in 4.1 above.

A. Bioaccumulation Factor (BAF) for petroleum hydrocarbon in plant species

Results of the mean values of BAF for both TPH and Asphaltenes of the tested species was presented in Figure 1, 2 and 3. From the result of Figure 1, BAF for TPH was highest in C2 (2.28 g g⁻¹) but the least BAF value for TPH was recorded in C3 with mean value of 1.44 g g⁻¹. Similarly, BAF for Asphaltene content in C2 that did not differ significantly with that of C4 gave the highest value of 2.87 g g⁻¹ and 2.64 g g⁻¹ respectively.

Figure 2 further revealed that T4 was the best soil amendment that yielded the highest BAF for TPH with mean value of 2.13 (g g⁻¹) while the least for TPH was recorded in T3 with mean value of 1.71 (g g⁻¹). In terms of Asphaltenes, T2 yielded the highest BAF value of 3.45 (g g⁻¹) while the least was observed in T4 with mean value of 1.50 (g g⁻¹).
B. Mass Influx ($M_{\text{influx}}$) for Petroleum Hydrocarbon in plant species

The results of the mean $M_{\text{influx}}$ values for both TPH and its Asphaltene of the tested species was also presented in the Figure 1, 2 and 3. The results in Figure 1 revealed that C3 (20.32 g g$^{-1}$) yielded the highest $M_{\text{influx}}$ result for TPH and that this is followed by that of C2 (19.33 g g$^{-1}$) which did not differ significantly with the $M_{\text{influx}}$ yield of C4 (18.83 g g$^{-1}$). However, $M_{\text{influx}}$ for Asphaltene content had the highest value in C4 which was closely followed by C3 with 16.65 g g$^{-1}$ and 13.52 g g$^{-1}$ respectively. The least value of $M_{\text{influx}}$ was seen in C2 with mean value of 12.40 g g$^{-1}$.

In respect to the soil amendments in Fig. 2, T2 yielded the highest $M_{\text{influx}}$ values for both TPH and associated Asphaltene content with 22.14 g g$^{-1}$ and 19.31 g g$^{-1}$ respectively. However, T3 yielded the least $M_{\text{influx}}$ value for TPH with mean value of 16.90 g g$^{-1}$ while that of Asphaltene was T4 with mean value of 7.45 g g$^{-1}$.

IV. Discussion

The results presented for both the accumulation of TPH and associated insoluble asphaltene with that of the BAF (for both TPH and Asphaltene) agreed to the fact that $C. zizanioides$ species can grow and tolerate contamination in sites due to hydrocarbon and can accumulate same, especially the insoluble asphaltene content at higher concentration levels of up to 0.7 L Kg$^{-1}$ soil. This is not withstanding the fact that it is more effective in the accumulation of the soluble fraction of TPH at concentrations levels not above 0.3 L Kg$^{-1}$ soil. This agrees with the results of authors like [6] and [19] that certain species of plants can phytoextract or phytoaccumulate pollutants into their tissues; and also contradicting the general believe that the uptake of hydrocarbons by plants is primarily from atmosphere through gas and particle bound deposition [30].

The grass been a perennial species with rugged roots that grows downward to about 2-4 meters with tall rigid turfs [35] had proved to be adaptive to wide range of environmental and edaphic conditions in that it
grows well in crude oil contaminated environment with up to 0.7 L Kg\(^{-1}\) soil. Additionally, the fact that it does not only accumulate TPH and its insoluble asphaltene in its roots but also in its turf has proved that the plant is an excellent hyper accumulator of crude oil and not only limited to that of heavy metals as suggested by \[33\].

The fact that \textit{C. zizanioides} species shows some signs of leaf burns during its growth in the contaminated pot soils does not in any way dispute the hyper accumulative potentials of the species but it indicated that there is reduction in contaminant toxicity in the plant as suggested by \[34\] that plants can reduce the amount of hydrocarbon toxicity by gaseous diffusion and intercepting particles on their leaf and bark surface.

The \textit{M\textsubscript{loth}} as an indicator of the uptake kinetics indicated that \textit{C. zizanioides} species can uptake, translocate and phytoaccumulation TPH at 0.5 L Kg\(^{-1}\) soil contamination of up to 20.32 g g\(^{-1}\) in three months. On the other hand, the insoluble asphaltene could be translocated and phytoaccumulated at a higher soil contamination level of 0.7 L Kg\(^{-1}\) soil to about 16.65 g g\(^{-1}\) within three months. The more the contamination level, the more the adsorption of asphaltene in this species. This indicates that \textit{C. zizanioides} species can, under favourable condition, remediate crude oil contamination on site as long as the plant grows; because the grass is a perennial species. \[27\] also reported translocation of long chain heavy alkanes slowly into the stem and roots of plants. This is contrary to the report of \[36\] that plants are only effective in phytoremediation at the first 90 days of growth.

In addition, it was observed that while the mixture of both cow dung and NPK fertilizer \(T_4\) favours phytoaccumulation of TPH in the plant, the use of NPK fertilizer \(T_3\) was the soil amendment that favours phytoaccumulation of asphaltene fractions as well as the general uptake of the crude oil contaminants from the soil (Uptake kinetics). This agrees with the results of \[26\] that the use of cow dung can increase the growth of plants in crude oil contaminated environments.

Organic matter content in soils tends to improve the soil condition by increasing its water retentive capacity and making nutrient more available to plant roots. \[25\] NPK fertilizer on the other hand do supplement for deficiency within crude oil contaminated environments thereby encouraging plant growth. Plant roots that penetrates the soil gives room for aeration in the soil thereby encouraging the activities of aerobic microorganisms in degrading crude contaminants therefore making it more bioavailable and less toxic to plants.

V. Conclusion

This study found that \textit{C. zizanioides} species is an excellent hyper accumulator species of crude oil contaminants. The perennial grass can phytoaccumulate TPH and insoluble asphaltene in both its roots and turf in reasonable quantities but limited to specific soil amendments. It can withstand harsh environmental and edaphic conditions and can phytoaccumulate crude contaminants of up to 20.32 g g\(^{-1}\) TPH and 16.65 g g\(^{-1}\) insoluble Asphaltene using soil amendments of mixtures of cow dung and NPK fertilizer \(T_4\) within a span of three months. Thus, it is not only recommended that \textit{C. zizanioides} species should be considered in phytoremediation of crude oil contaminated sites but that phytomining (extraction of crude accumulated in its tissues) should be considered and the product extracted should be tested and put to appropriate usage.

Acknowledgements

The Authors wishes to acknowledge the efforts of Prof. Ivyola O. Tella (Departments of Forestry and Wildlife Management) and Prof. Jeffrey Barminas (Department of Chemistry), Modibbo Adama University of Technology, Yola, Adamawa State - Nigeria for their guidance during the research.

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