Jr. of Industrial Pollution Control 39(4) *1-10(2023)*
www.icontrolpollution.com
Research Article

TOLERANCE THRESHOLD OF GROWTH AND DEVELOPMENT OF FLUTED PUMPKIN (*TELFAIRIA OCCIDENTALIS*) ON DIESEL POLLUTED SOIL

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Citation: Ukaoma AA, Iwu IC, Ajuruchi VM, Nnodim NI, Nwamba IM, Orji-Kelechi Cl, Anyanwu CO. Tolerance Threshold of Growth and Development of Fluted Pumpkin (*Telfairia occidentalis*) On Diesel Polluted Soil. J Ind Pollut Control. 2023;39:003.

Key words: Diesel, Pollution, *Telfairia occidentalis*, Soil, Tolerance, Threshold

Received: 30-Oct-2023, Manuscript No. ICP-23-110132; Editor assigned: 03-Nov-2023, PreQC No. ICP-23-110132 (PQ); Reviewed: 17-Nov-2023, QC No. ICP-23-110132; Revised: 24-Nov-2023, Manuscript No. ICP-23-110132 (A); Published: 01-Dec-2023, DOI: 10.4172/0970-2083.39.4.003

ABSTRACT

Objective: The objective of this study was to evaluate the suitability of fluted pumpkin (*Telfairia occidentalis*) for use in the phytoremediation of Diesel oil contaminated soil.
Methodology: Planted seedlings of fluted pumpkin were subjected to varying concentrations of diesel oil (100 ml, 200 ml, 300 ml and 400 ml) and the control had no diesel oil. 5 kg of poultry manure was added to each of them to augment the process. The experiment incorporated a completely Randomized Design with three replicates. These treatments were applied to the soil before planting the *Telfairia occidentalis* seeds. The plants were allowed to grow for 10 weeks. The parameters studied include plant height leaf width leaf length and the number of leaves.
Results and discussion: Results showed that the 100 ml treatment gave the highest value of plant height (108 m) while the 400 ml treatment gave the lowest value of plant height (18.7 m), statistical analysis shows a significant difference (<0.05) in the plant height. The control maintained a lead over the polluted sample. It was observed that as the concentration of crude oil increased, there was a significant reduction in plant height (from 108.5 m to 18.7 m) and leaf width (from 13.33 m to 4.17 m). The total petroleum hydrocarbon content of baseline data (6786.54 mg/kg at week 0) was significantly greater than the total petroleum hydrocarbon content values in the soil during the six-week growth period. The result also shows a significant reduction of diesel oil in the soil during the period of growth from 6786.54 mg/kg to 4942.33 mg/kg for week 0 to week 6 respectively.
Conclusion: The reduction in petroleum hydrocarbons indicates that *Telfairia occidentalis* could be used in remedying soils polluted with diesel oil as the plant is found suitable for the uptake of heavy metals.

INTRODUCTION

Crude oil is a naturally occurring petroleum product consisting of hydrocarbon deposits and other organic materials (Aggarwal, et al., 2006). This is refined to produce usable products including petrol, gasoline, diesel, and various other forms of petrochemicals (Mnif, et al., 2017). Transportation of these refined products from one place to another resulted in spillage with adverse consequences (Hewelke, et al., 2018). The major methods employed in transportation include the use of oceanic tankers and pipelines overland. These methods eventually lead to pollution in an environment by accidental oil spills and operational discharge resulting in the loss of very large quantities of crude oil into land and sea bodies. (Huang, et al., 2019).

Petroleum hydrocarbon pollution could also form from anthropogenic sources as a result of pipeline vandalism which accounts for about 28% of the occurrence in Nigeria (Hewelke, et al., 2018). Oil production operations and inadequate or non-functional production equipment also account for 1% of most oil spillage occurrences in Nigeria (Baird, 2010).

Diesel contamination leads to significant impacts on soil properties that can lead to water and oxygen deficits as well as a shortage of available forms of nutrients such as nitrogen and phosphorus (Zarimkamar, et al., 2013). Hence, due to the high demand

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for diesel and the decline of fossil fuel reserves, less-polluting and renewable fuel sources such as biodiesel are currently being investigated (Ashnani, et al., 2014).

Diesel fuel pollution exerts tremendous effects on plants indirectly by adding toxic minerals to the soil which are absorbed by plants (Akoto, et al., 2008). Diesel fuel resulted in the deterioration of soil structure, loss of organic matter contents loss of soil minerals nutrients such as potassium, sodium, sulphate, phosphate, and nitrate (Akubugwo, et al., 2009). Pollution of soil by petroleum products is a universal problem as their effects on the soil ecosystem are still examining worldwide (Akoto, et al., 2008).

Phytoremediation technique utilizes plants to remediate the contaminated media including soil and water. Phytoremediation is an economically and environmentally favorable technique as it utilizes green plants to contain, sequester or detoxify contaminants from contaminated oil and water (Ashraf, et al., 2019).

Phytoremediation utilizes many mechanisms including degradation (Rhizome-degradation, Phytodegradation), accumulation (Phytoextraction, Hemofiltration), dissipation (Phytovolatilization), and immobilization (Hydraulic control and Phytostabilization) to degrade, remove, or immobilize the pollutants (Kafle, et al., 2020). Depending upon the contaminants, plants utilize one or more of these mechanisms to reduce their concentrations from soil and water. For example, plants uptake and accumulate heavy metals in their tissues and degrade the organic pollutants reducing their toxicity from soil and water resources (Saleem, et al., 2020).

Physiological processes in plants such as transpiration, respiration, photosynthesis, and translocation are affected adversely by oil contamination. Crude oil spillage on soil makes it unsatisfactory for plant growth. This is due to insufficient aeration of the soil because of the displacement of air from the spaces between the soil particles by crude oil. The oil blocks the soil pore sand and displaces soil water and soil air (Akubugwo, et al., 2009).

Phytoremediation of organic contaminated soils using endophytic bacteria, grasses (Poaceae), and legumes (Leguminosae) have been the subject of several studies (April, et al., 1990; Schwab, et al., 2006; Phillips, et al., 2008). There is a paucity of information about the use of *Telfairia occidentalis* (a mycorrhizal plant) on petroleum contaminated soils. Fluted pumpkin was used because it is a mycorrhizal plant with a taproot system having primary and extensive secondary roots. The plant can withstand stressed conditions, source of water and nutrients during drought, broad leaf that can cover the entire land within a short period giving hydrocarbon-degrading microorganisms favourable environment to act (Akpan, et al., 2014).

MATERIALS AND METHODS

Crude Oil

The Bonny light crude oil was obtained from Nigerian National Petroleum Corporation (NNPC) Port-Harcourt Refinery, Eleme, Rivers State Nigeria. It was collected in sterile containers in an unweather condition from the production plant. Determination of PH and Concentrations of SO_2, CO_2 and NO_2 in the Rainwater

Sample Collection

Viable seeds of the vegetable crop pumpkin (*Telfairia occidentalis*) used for this study were purchased at Ihiagwa Market in Owerri, Imo State. It is then stored at room temperature (25°c to 30°c) for 24 hours. The soil sample was collected from agricultural soil located in the Federal University of Technology Owerri farmland using sterile containers at the depth of 1 cm-15 cm the soil was mixed thoroughly and dried at room temperature for 7 days.

Pollution of Soil using Crude Oil

Before planting, 10 kg of filled polythene bags with different concentrations of crude oil (100 ml, 200 ml, 300 ml, and 400 ml) were poured into the soil samples and subsequently mixed thoroughly meanwhile the fifth pot, being the control was not polluted. Each treatment has three replicates making a total of 75 bags (i.e. 5 bags × 3 replicates × 5 samples). 5 kg of poultry manure was added to each of the treatment and control. The analysis of the sample was taken before surface pollution and after surface pollution to get baseline data.

Experimental Design

The experimental design used was a completely randomized design where sampling was done every two weeks for 10 weeks (Fig.1).

Fig. 1 Growth of fluted pumpkin (*Telfairia occidentalis*) plant at 8 weeks after pollution (right to left control, 100 ml, 200 ml, 300 ml, 400 ml) respectively.

Sterilization of Seeds for Planting

The seeds that were used for this study are the *Telfairia occidentalis*. It will be sterilized to eliminate contaminants as described by Yee (Yee, et al., 1998). This was achieved by washing the seeds initially with tap water, then with 95% ethanol for 45 minutes with continual swirling. After 45 minutes, ethanol will be decanted and 35% of the sodium hypochlorite solution will be used for further sterilization for 15 minutes accompanied by swirling. After which the seeds will be rinsed with sterile water, 3 times at 10 minutes each following sterilization, the seeds will be placed on Petri dishes containing sterile cotton wool soaked in sterile water. The moistened cotton wool provides the moisture needed for the seeds to germinate.

Planting of Seeds

After 72 hours -168 hours, two seeds of *Telfairia occidentalis* were sown in 10 kg of soil contained in each of the sterile perforated polythene bags. They were kept in a greenhouse and watered with 100 ml of sterile water using a sterile measuring cylinder at 48 hours interval as described by Yee.

Physico-Chemical Parameters

The Physicochemical properties of the soils were determined by using standard methods. The pH of the sample was determined using a Jewniary digital pH meter. Conductivity was determined using the CD303 conductivity meter. Total Organic Carbon (TOC) was determined by the Walkley-Black dichromate wet oxidation method and the content of available phosphorus was determined using the method reported by Awe (Awe, et al. 2018). Total Petroleum Hydrocarbon of Soil was determined by the method described by Eniola (Eniola, et al. 2014).

Analysis of Plant Growth by measuring agro morphology of Plant

Data was collected in terms of Plant height, Number of leaves, Leaf width, and Leaf length. The growth parameters of plants cultivated in contaminated soil were compared to reveal the effect of oil pollution on growth of plants.

RESULTS

Agronomic Characters

The hydrocarbon also significantly affected agronomic characteristics over the weeks of harvest. The effect of the concentrations (100 ml, 200 ml, 300 ml and 400 ml) on the agronomic character: leaf length, leaf width, number of leaves, and plant height are represented in Table 1 and the bar chat representation of this result is shown in Table 1.

Leaf Length

The crude oil affected the leaf width also in a significant way (P ≤ 0.05). The result confirms that the higher the pollution (increase in the concentration of crude) the lower the leaf width.

Number of Leaves

The number of leaves under different concentrations of crude oil pollution showed a significant difference (P ≤ 0.05) between treatments presented in Table 1. As the concentrations (level) of crude oil pollution increased, the number of leaves under evaluation decreased. The control had the highest no of leaves (18.00) which generally shows that crude oil affects the growth and development of a crop.

Plant Height

There was also a significant difference (P ≤ 0.05) in the plant height over the period of harvest. As the level of pollution increases the plant height was significantly reduced except for the control which maintained a steady increase throughout the growth period. The 400 ml concentration had the least values for plant height (18.7 m) (Table 2).

Physico-Chemical Parameters

The table for the result of the physico-chemical analysis is shown below in Table 3 and the effect of the analysis on the soil when it is unpolluted and polluted is presented as thus (Fig.2).

Table 1. Effect of concentration of crude oil pollution weeks after planting on the agronomic characteristics of *Telfairia occidentalis*.

Concentration	Leaf length	Leaf width	Number of leaves	Plant height
100	8.38^{a}	4.67^{a}	13.33^{a}	108.5^{a}
200	7.33^{a}	4.25^{a}	11.83^{a}	80.5^{b}
300	8.33^{a}	4.40^{a}	7.50^{b}	38.2^{c}
400	3.42^{b}	2.70^{b}	4.17^{c}	18.7^{d}
Control	9.92^{c}	4.67^{a}	18.00^{d}	111.5^{e}
LSD(0.05)	1.59	0.8	2.78	14.58
Note: Similar Alphabets are not significant while dissimilar alphabets are significant.				

Table 2. Effects of diesel weeks after planting on the agromorphological characteristics of *Telfairia occidentalis*.

Weeks after Planting	Leaf length	Leaf width	Number of leaves	Plant height
Week 3	4.07[a]	2.63[a]	6.40[a]	21.8[a]
Week 5	5.67[b]	3.60[b]	7.93[b]	34.8[b]
Week 7	10.70[c]	4.90[c]	11.20[c]	95.9[c]
Week 9	9.92[d]	5.42[d]	18.00[d]	133.3[d]
LSD(0.05)	1.42	0.8	2.49	13.04
Note: Similar Alphabets are not significant while dissimilar alphabets are significant				

Table 3. Effects on physicochemical parameters.

Treatment	N (%)	SO (ppm)	Ph (pp m/g)	K (cmol/kg)	Ca (cmol/kg)	C (%)	E.C (µS/cm)	Cd (mg/100g)	B (mg/100g)	pH	Organic matter (%)
Polluted soil	0.11	6.75	8.3	0.063	2.707	3.903	79	0.163	0.22	6.9	4.78
Unpollut-ed soil	0.28	3.72	6.3	0.063	1.367	1.747	76	0.11	0.1867	5.5	2.66
LSD(0.05)	0.24	2.32	2.26	0.057	0.539	0.407	2.267	0.081	0.054	0	0.292

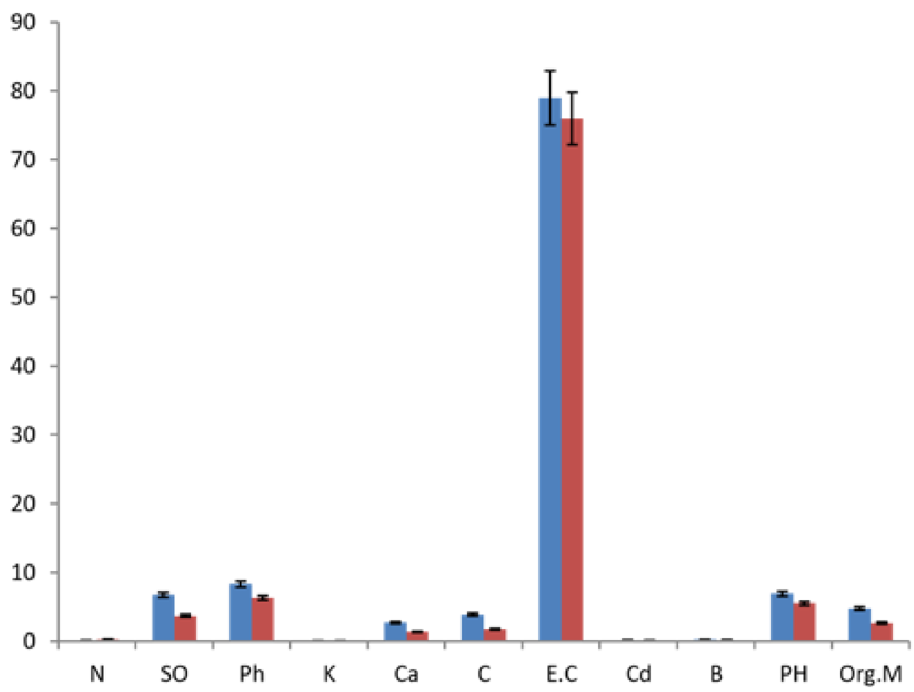

Fig. 2 Effect on physicochemical properties. **Note:** (■) Polluted soil; (■) Unpolluted soil.

Nitrogen

There was a significant difference (P ≤ 0.05) in the level of nitrogen between the polluted and the unpolluted soil sample. The unpolluted soil had a value of 0.277% while the polluted soil sample reduced to a value of 0.110%. it is observed that the level of nitrogen decreased with the increase in pollution.

Sulphate

There was also a significant difference (P ≤ 0.05) in the sulphate level compared with the unpolluted soil sample. The unpolluted soil sample has a value of 3.72 ppm while the polluted soil sample increased 6.75 ppm. The level of sulphur increases with the level of pollution.

Phosphorus

A significant difference (P ≤ 0.05) was observed between the level of phosphorus in the polluted and unpolluted soil sample. The unpolluted soil has a value of 6.30 ppm/g while the polluted soil sample increased to a value of 8.30 ppm/g.

Potassium

There was no significant difference (P ≤ 0.05) between the polluted and unpolluted soil sample. The polluted and unpolluted sample was observed to be having a value of 0.063 cmol/kg.

Calcium

There was significant difference (P ≤ 0.05) in the level of calcium between the polluted and unpolluted soil sample. The unpolluted soil sample has a value of 1.3667 cmol/kg while the polluted soil sample increased to 2.707 cmol/kg.

Carbon

There was significant difference (P ≤ 0.05) in the carbon content of polluted and unpolluted soil. The unpolluted soil sample has a value of 1.747% while the polluted sample increased to 3.903%. This is because of the carbon contained in the crude oil used to pollute the soil.

pH

There was a significant difference (P ≤ 0.05) in the level of this parameter when compared with the control which is the unpolluted soil. The control has a pH value of 5.487 while the polluted soil sample has a value of 6.897.

Electrical Conductivity

There was a significant difference (P ≤ 0.05) in the level of electrical conductivity when compared to the unpolluted sample (control). The electrical conductivity increased from 76 µS/cm to 79 µS/cm.

Cadmium

There was a significant difference (P ≤ 0.05) in the level of the cadmium content of the unpolluted and polluted soil. The unpolluted soil sample has a value of 0.110 mg/100 g while the polluted increased to 0.163 mg/100 g. This can be attributed to the crude oil introduced in the control sample

Boron

There was a significant difference (P ≤ 0.05) in the level of this parameter when compared to the unpolluted soil sample. The boron content of the soil polluted with crude oil has a value of 0.22 mg/100 g while the control has a lower value of 0.1867 mg/100 g.

Organic Matter

There was a significant difference (P ≤ 0.05) in the level of this parameter when compared with the control. The organic matter present in the soil increased from a value of 2.66% to 4.78% because of the crude oil content in the soil.

Total Petroleum Hydrocarbon Content of Crude Oil Polluted Soil

There was a highly significant difference in the total petroleum hydrocarbon content of the soil polluted with crude oil in different concentrations (Fig.3). The 100 ml had the least concentration of 5566.98 mg/kg while the highest concentration was recorded in 400 ml which had a value of 6155.88 mg/kg. The quantity of crude oil remnant in the soil is a factor of the concentration used in polluting the soil, thus the higher the concentration of crude oil used in polluting the soil the greater the quantity of hydrocarbon that will be present in the soil (Fig.4).

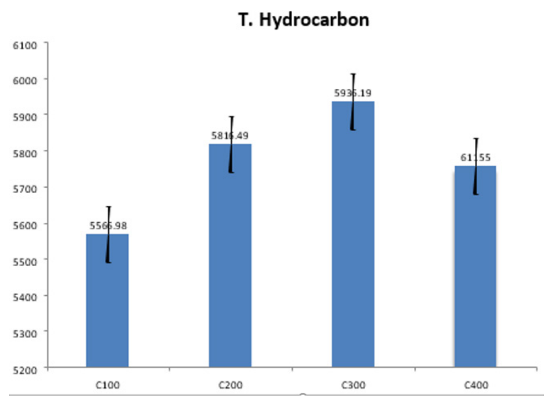

Fig. 3 Mean concentrations of hydrocarbon in the soil after pollution with different concentration of crude oil.

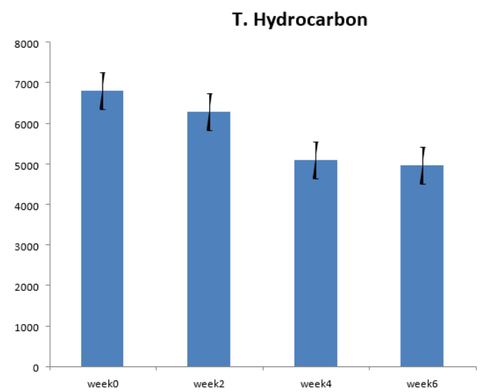

Fig. 4 Concentration of hydrocarbon in the soil weeks after pollution. **Note:**( )T. Hydrocarbon.

DISCUSSION

The result from this study shows the efficacy of *Telfairia occidentalis* to remove total hydrocarbon content from soil can be enhanced with poultry manure Murphy reported increased degradation of crude oil in the soil augmented with poultry manure while Davies reported that soil amendment of spent oil contaminated soil with organic waste led to the improved soil physical properties and increase agronomic parameters of such soil (Murphy, et al., 2011; Davies, et al., 2005). Davies also reported that soil amendment improved the physical properties of such soil, like water retention, water permeability, aeration, and structure of the soil; this will lead to more degradation by remediating polluted soil. The addition of poultry manure helped to improve plant growth by enhancing the uptake of the hydrocarbon by the plant. The result showed that as the plant grew over weeks, growth in the 400 ml parameters is attributed to the influence of metal especially in higher dosages. The TPH of the soil reduced from 6786.54 mg|kg recorded in the baseline data to 4942.33 mg/kg recorded after 6 weeks of remediation.

There was an observable discoloration on the leaves. This is a result of the loss of chlorophyll due to the imbalance of Manganese and Potassium Magnesium is known to be a major constituent of chlorophyll. The imbalance may have primarily affected leaf size and shape. *Telfairia occidentalis* germinated in all the treatments as well as the control. Seedling emergence was significantly delayed in the oil-treated soil compared to the control soil. The study agrees with the previous report of Kekere on cowpea reported the germination of seeds in oilpolluted soil varied with different plant species (Kekere, et al. 2011).

The increasing acidic condition of the soil following increased crude oil concentration increased the availability of heavy metal in the soil which in turn alters the growth parameters of the plant. Although growth was delayed in the polluted soil, the plant was able to compete and utilize the little nutrient available for survival. A similar study by Edem on six plant families in an oil-polluted soil produced an analogous result (Edem, et al., 2009). The ability of a plant to survive in crude oil-polluted soil depends on the level of toxicity and the degree of pollution received by the plant. Nwadinigwe recorded significant growth and yield reduction in soya beans planted on oil-polluted soil (Nwadinigwe, et al., 2003; Lasat MM,2000; Lee, et al., 2001). In conclusion, crude oil makes soil parameters unfavourable for plant growth and survival as evident in this study. The study demonstrated that although *Telfairia occidentalis* can grow in oil-polluted soil, it would depend on the nature and level of pollution.

CONCLUSION

TPhytoremediation has been recognized as one of the best methods of restoring contaminated sites. The result shows that *Telfairia occidentalis* pollution differed significantly when ($p \leq 0.05$) as when compared to control. This showed that plants were able to reduce the high level of hydrocarbon contaminant in the soil to minimal levels over time. The addition of soil with poultry manure also made the phytoremediation process a success. There was significant degradation of hydrocarbon-contaminated soil with the nutrient addition.

However, *Telfairia occidentalis* was able to tolerate the crude oil pollution at a concentration of 300 ml because at 400 ml pollution the uptake was minimal. This is an indication that the higher the level of crude oil pollution the more the activity of *Telfairia occidentalis* becomes minimal. Thus this plant is recommended as a pytoremediator of oil contaminated sites. In conclusion, crude makes soil conditions unfavourable for plant growth and survival as evident in the present study. Furthermore, the impact of oil on an already established plant was deleterious. This demonstrates the frustration of farmers in Niger Delta areas of Nigeria where there are incessant incidences of crude oil pollution in farmland. Thus the study demonstrated that although *Telfairia occidentalis* can grow in oil-polluted soil, it would depend on the nature and level of pollution.

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