Original Research Article

Evaluation of Total Hydrocarbon Contents in the Leaves of Selected Crops Grown on Crude Oil Polluted Agricultural Soil

ABSTRACT

Aims: The Total Hydrocarbon Content (THC) levels in the leaves of three edible plants (*Allium cepa, Telfairia occidentalis and Zea mays*) grown on soil polluted with 100ml of crude oil were examined.
Study design: This experiment was conducted in two groups where the soil samples were polluted before planting (PB) and polluted 2 weeks after planting (PA).
Place and Duration of Study: The research was carried out at the Federal University of Technology, Owerri (FUTO), Imo State, Nigeria, within a period of 4 weeks based on each group's pollution time.
Methodology: The tests for total hydrocarbon content (THC) were analyzed at different wavelengths for polluted soil and leaf samples using UV-Vis spectrophotometric method. Soil THC tests were determined on days 0, 14 and 28 respectively while THC tests on leaves of study plants were assessed on day 14 and 28.
Results: The total hydrocarbon content values of the unpolluted soil (UPS) and the non-planted polluted soil (PS) subjected to this analysis on day zero were (UPS = 96.38mg/kg; PS = 1082.80mg/kg). However, results obtained on day 14 and 28 showed significant difference ($P < 0.05$) between the unpolluted soil sample (UPS) and all the planted polluted soil for *Telfairia occidentalis* (TOPB, TOPA), *Allium cepa* (ACPB, ACPA), *Zea mays* (ZMPB, ZMPA) as well as, the non-planted polluted soil (PS). For the leaf samples, TOPB had the highest THC value of 14.47mg/kg and 36.73mg/kg for day 14 and 28 respectively while ZMPB had the lowest value of 5.38mg/kg at day 14 and ZMPA, the least THC value of 7.76mg/kg at day 28.
Conclusion: Based on the varying THC levels observed in the leaves of crops used for this study; it was connoted that, bioaccumulation of hydrocarbons depends on the plants phytoremediation capability and the mode of pollution as observed in TOPB.

Keywords: hydrocarbon, foliar, petroleum, assessment, monocots, dicots, clean-up

1. INTRODUCTION

Several thousands of aliphatic and aromatic hydrocarbons, as well as different trace metals and non-hydrocarbons, can be found in crude oil [1]. The origin of the oil, the amount of hydrocarbons present, and the proportions in which they are blended all play a significant role in the compositions of the oil's physical and chemical properties. Additionally, the volatility of the oil is determined by the variation in the quantity of hydrogen and carbon atoms and their spatial arrangement [2]. It is broadly used in the industries, companies, homes, communities and other institutions as transport fuels and primary energy source for regular human activities [3]. Regardless, the excessive exploitation of crude oil give rise to

pollution through oil spillage and extraction activities, tanker accidents, pipeline vandalism, subterranean storage tank leaks, industrial operations at oil refinery locations and oil well blowouts [4]. Oftentimes, pollution of the soil and marine bodies with crude oil occurs in municipal areas and some rural communities where petroleum and natural gas are obtained [5].

Crude oil contaminated agricultural soil affects plants hostilely by limiting the accessibility of water and critical nutrients (oxygen, nitrogen, and phosphorus) required for plant growth and survival, due to the hydrophobic properties of oil [6]. However, some plants through rhizoremediation can stabilize oil contaminated sites by stimulating the growth and activities of degrading microbes in the rhizosphere, exuding phenolic compounds from their roots and converting petroleum hydrocarbons into more useful elements like carbon dioxide, water, methane and fatty acids [7].

According to Yuniati [8] and Bashir [9], crude oil degradation depends on the nature and number of hydrocarbons present in the oil, its toxicity level and bioavailability of the contaminant, the microbial population of the contaminated site, environmental parameters and the duration of pollution. Therefore, this research investigated the levels of total hydrocarbon content (THC) in the leaves of three crops (*Allium cepa, Telfairia occidentalis and Zea mays*) grown on soil polluted with the same volume (100ml) of crude oil.

2. METHODOLOGY

2.1 Study Area

The research was carried out at the Federal University of Technology, Owerri (FUTO), in Imo State, one of the oil-rich states in south eastern Nigeria. It is situated between latitudes 5°20'N and 5°25'N and longitudes 7°00'E and 7°05'E, as clearly shown in Figure 1. It has a tropical, humid climate with heavy rainfall between the months of April and August, an average rainfall of about 2500mm. Flat terrain and typical rainforest plants characterize the region.

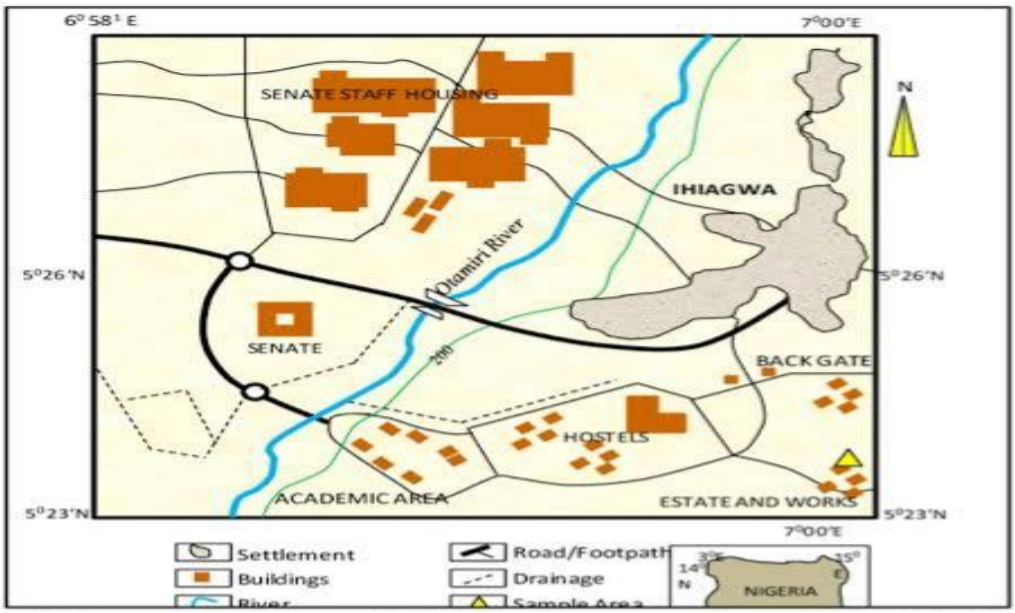

Figure 1: Map showing FUTO environment (Source: Onyekuru *et al*. [10]).

2.2 Sample Collection

Given that there is no known history of crude oil contamination at our study location, soil samples were randomly obtained using a sterilized soil auger from the Teaching and Research farm, School of Agriculture and Agricultural Technology (SAAT) Research farm FUTO. The materials were dried in the air and sieved through a 2mm mesh sieve. A proportion was then sent to the lab for analysis within an hour of collection after being weighed into perforated sterile seed bags containing 5kg of soil each. Bonny light crude oil used for this study, was procured from Akiri in Oguta, Imo State, Nigeria, and was gathered in sterile containers.

2.3 Exposure to crude oil

The experiment was conducted in two (2) distinct groups, as per the methodology used by FiriAppah *et al.* [2]. The first group was polluted two weeks after planting (PA) using 100ml of crude oil along with 50ml of sterile water distributed evenly to the soil and around the plants (ring application), while the second group was polluted prior to planting (PB) using 100ml of crude oil and the soil mixed to homogeneity with a spatula. No crude oil was used in the unpolluted group, which functioned as the control group. Depending on when each group's pollution was initiated, the test plants were collected after 28 days.

2.4 Soil analysis of non-planted polluted soil and unpolluted soil samples

Soil analysis was carried out on the non-planted polluted soil sample and the unpolluted soil sample at day zero (0), 14 and 28 of pollution to determine the soil total hydrocarbon contents.

2.5 Determination of Total Hydrocarbon Content in polluted soil samples

A modified approach published by Akpan and Usuah [11] was used to determine the total hydrocarbon content in the polluted soil samples at 2-week intervals for each polluted group. The hydrocarbon content in the oil-polluted soil was extracted using 10 ml of n-hexane after 10g of soil samples were measured into a 50 ml flask. In order to completely separate the oil

from the soil sample, the mixture was agitated violently on a magnetic stirrer for 30 minutes and then let to stand for 10 minutes. A Whatman filter paper was used to filter the solution, and the filtrate was then diluted by adding 1 ml of the extract to 50 ml of n-hexane. Utilizing n-hexane as a blank, the absorbance of this solution was measured in a spectrophotometer at 480 nm. It was then represented as mg/kg of total hydrocarbon content.

2.6 Determination of Total Hydrocarbon Content in polluted test plants

This was determined using UV-Vis spectrophotometric method as described by Numbere [12]. The polluted test plants' fresh leaves were dried in an oven at 60°C for 24 hours to remove the moisture. After being crushed, the dried samples were each weighed at 2g in a glass beaker with 2ml of n-hexane. Using a glass rod and vigorous swirling, the materials were homogenized over the course of around 30 minutes. After that, the samples were run through a glass funnel filled with cotton wool, silica gel, and anhydrous sodium sulfate to filter out any remaining particles. Following filtration, n-hexane was used as a blank and 10ml of the filtered organic extract was placed into a 10ml sample vial for spectrophotometer analysis at (wavelength 610nm). The amount of total hydrocarbons was given in mg/kg.

2.7 Statistical Analysis

All data samples from each experimental group in triplicates were analyzed statistically using Minitab 2017 one way analysis of variance (ANOVA) and the mean values separated with Tukey's pair wise test. Results were displayed as mean and standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Soil Total Hydrocarbon Content

Findings from this research as expressed in figure 2, indicated a huge statistical difference (P=0.05) between the total hydrocarbon content values of the unpolluted soil and the non-planted polluted soil samples subjected to this analysis on day zero (UPS = 96.38mg/kg; PS = 1082.80mg/kg). However, results obtained on day 14 as represented in figure 3 showed significant difference between the unpolluted soil sample and all the planted polluted soil samples for *Telfairia occidentalis* (TOPB, TOPA), *Allium cepa* (ACPB, ACPA), *Zea mays* (ZMPB, ZMPA) as well as, the non-planted polluted soil while no significant difference was recorded amongst all the polluted soil samples. Figure 4 represented total hydrocarbon content for soil samples at day 28. All polluted soil samples were significantly different from the unpolluted soil sample but, TOPB soil had reduced hydrocarbon content value (381.00mg/kg) when compared to the other polluted samples (PS = 883.14mg/kg, TOPA = 789.51mg/kg, ACPB = 695.90mg/kg, ACPA = 670.29mg/kg, ZMPB = 820.66mg/kg, ZMPA = 845.30mg/kg). No significant difference was observed amongst PS, TOPA, ZMPB and ZMPA. Also, ACPB and ACPA soil samples were insignificantly different from each other. Noticeably, the planted polluted soil samples (TOPB, TOPA, ACPB, ACPA, ZMPB and ZMPA) consistently showed a decline in their THC levels compared to the non-planted polluted soil.

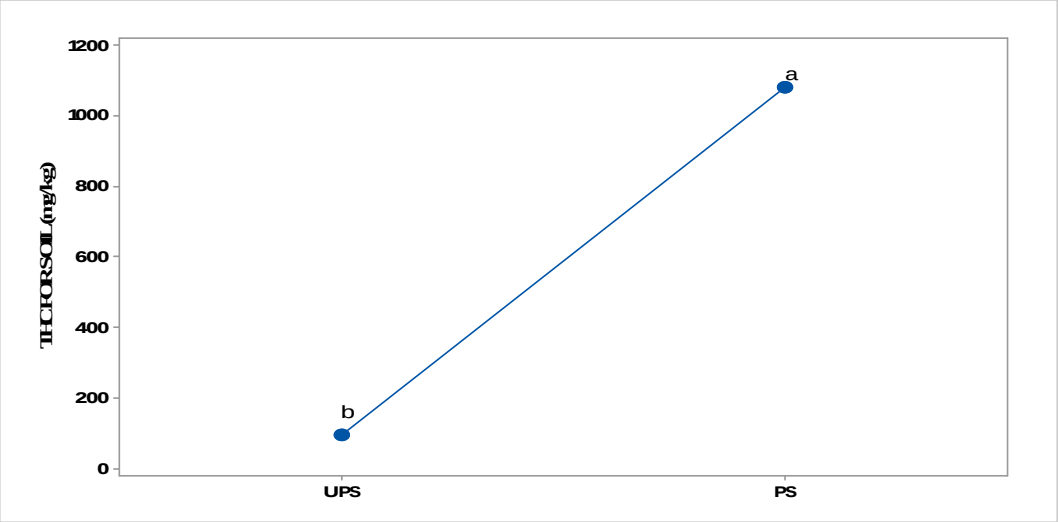

Figure 2: Total Hydrocarbon Content for polluted and unpolluted soil samples at Day zero

The values reflect the mean and standard deviation of three replicates. Means differ considerably at ($p < 0.05$) when they do not share a letter.

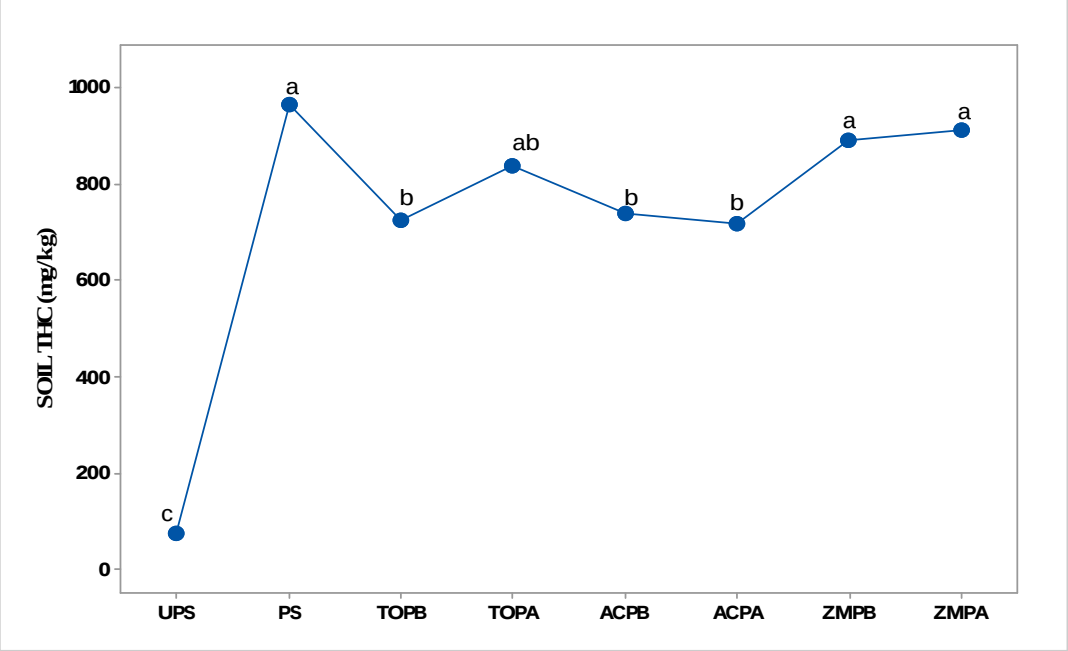

Figure 3: Total Hydrocarbon Content for polluted and unpolluted soil samples at Day 14 according to their pollution time

Unpolluted soil, Polluted soil, other polluted soil samples from TOPB – Telfairia occidentalis Polluted before Planting, TOPA – Telfairia occidentalis Polluted 2 WAP, ACPB – Allium cepa Polluted before Planting, ACPA – Allium cepa Polluted 2 WAP, ZMPB – Zea mays Polluted before Planting, ZMPA – Zea mays Polluted 2 WAP. The values reflect the mean and standard deviation of three replicates. Means differ considerably at ($p < 0.05$) when they do not share a letter.

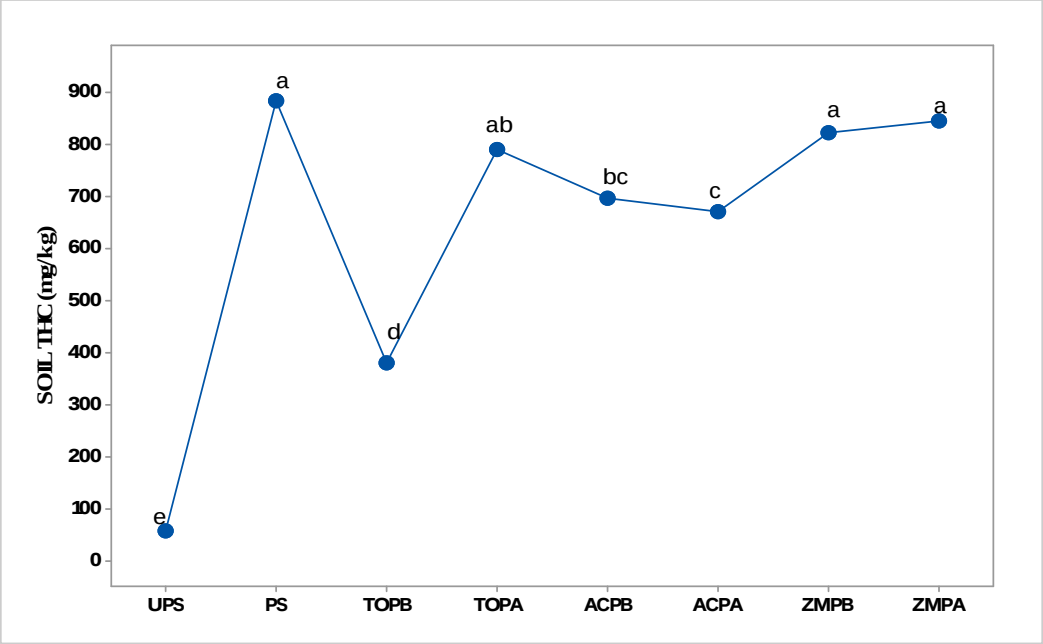

Figure 4: Total Hydrocarbon Content for polluted and unpolluted soil samples at Day 28 according to their pollution time

Unpolluted soil, Polluted soil, other polluted soil samples from TOPB – Telfairia occidentalis Polluted before Planting, TOPA – Telfairia occidentalis Polluted 2 WAP, ACPB – Allium cepa Polluted before Planting, ACPA – Allium cepa Polluted 2 WAP, ZMPB – Zea mays Polluted before Planting, ZMPA – Zea mays Polluted 2 WAP. The values reflect the mean and standard deviation of three replicates. Means differ considerably at ($p < 0.05$) when they do not share a letter.

3.1.2 Total hydrocarbon content of the leaves of test plants

As shown in both figure 5 and 6, TOPB had the highest total hydrocarbon content value of 14.47mg/kg and 36.73mg/kg for days 14 and 28 respectively while ZMPB had the least value of 5.38mg/kg at day 14 and ZMPA, the least total hydrocarbon content value of 7.76mg/kg at day 28. However, there were no significant differences recorded in ACPB, ACPA, ZMPB and ZMPA total hydrocarbon content values at days 14 and 28 respectively. Equally, TOPB and TOPA total hydrocarbon content values were insignificantly different at day 14 but showed statistical difference ($P<0.05$) at day 28. Also, the THC values of TOPB and TOPA were significantly higher than the THC values of ACPB, ACPA, ZMPB and ZMPA at days 14 and 28 respectively.

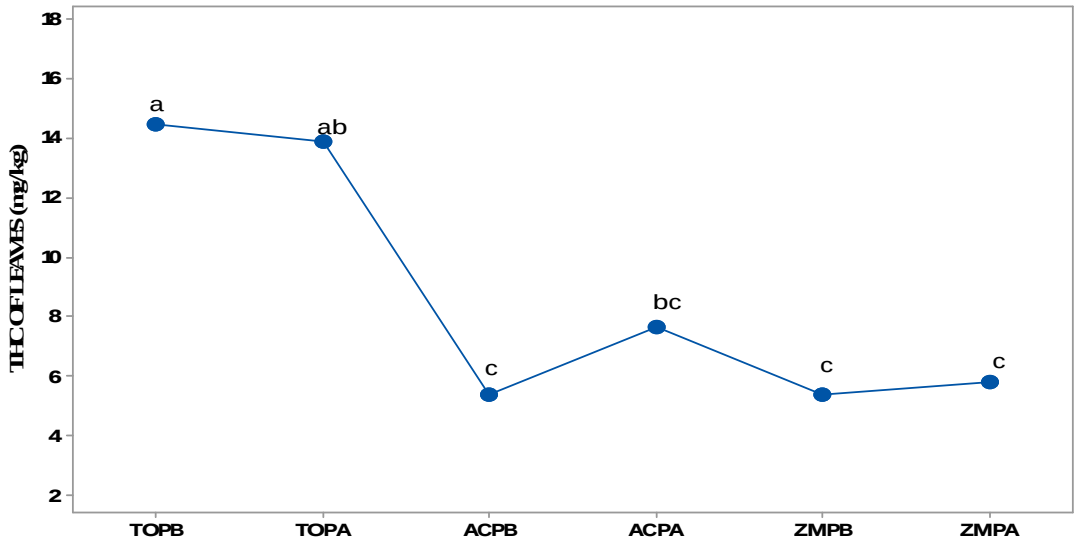

Figure 5: Total Hydrocarbon Content for polluted test plants at Day 14 according to their pollution time

TOPB – Telfairia occidentalis Polluted before Planting, TOPA – Telfairia occidentalis Polluted 2 WAP, ACPB – Allium cepa Polluted before Planting, ACPA – Allium cepa Polluted 2 WAP, ZMPB – Zea mays Polluted before Planting, ZMPA – Zea mays Polluted 2 WAP. The values reflect the mean and standard deviation of three replicates. Means differ considerably at ($p < 0.05$) when they do not share a letter.

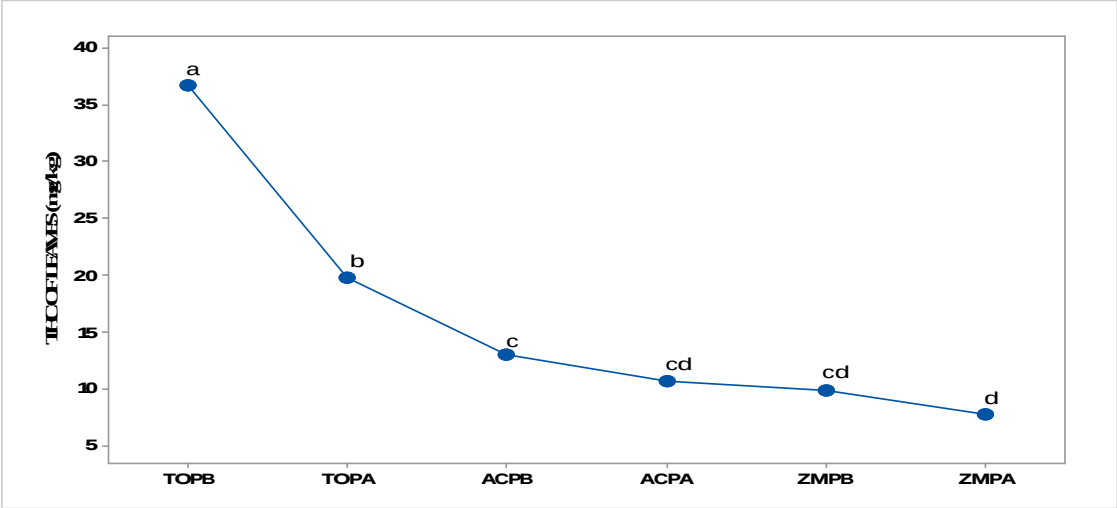

Figure 6: Total Hydrocarbon Content for polluted test plants at Day 28 according to their pollution time

TOPB – Telfairia occidentalis Polluted before Planting, TOPA – Telfairia occidentalis Polluted 2 WAP, ACPB – Allium cepa Polluted before Planting, ACPA – Allium cepa Polluted 2 WAP, ZMPB – Zea mays Polluted before Planting, ZMPA – Zea mays Polluted 2 WAP. The values reflect the mean and standard deviation of three replicates. Means differ considerably at ($p < 0.05$) when they do not share a letter.

3.2 Discussion

Values for total hydrocarbon content of soil increases with crude oil pollution. This is justified by the significantly higher THC values obtained in the polluted soil samples when contrasted with the soil sample that was not polluted. Relative work done by Ekemube *et al*. [13] affirmed our report, as the soil THC levels were directly proportional to the volumes of crude oil applied to the soil. High THC levels in polluted soils negatively affect soil textural class, aeration, soil nutrients and water flow; which equally impede plant growth and activities of soil macro and microorganisms. Additionally, Chukwumati and Asiegbu's research showed that as distance from crude oil contaminated regions increased, the amount of THC in plant tissues and soil samples dropped [14].
It was observed that soil samples of ACPB and ZMPB showed significant reduction in THC values when compared with ACPA, ZMPA and TOPA soil samples, and this could possibly be caused by the adaptive abilities of the plants; as influenced by the mode of crude oil application. However, the reduced soil THC levels showed an attempted soil remediation approach. Hence, the lowest THC value obtained in TOPB soil sample signifies that the highest soil remediation attempt was attained by *Telfairia occidentalis* polluted before planting. The lower THC values recorded for all the planted polluted soil samples in this study strongly asserted the fact that biodegradation of petroleum hydrocarbon occurs more in agricultural soil than in non-agricultural soil, probably due to phytoremediation capability of some plants. In 2010, Edwin-Wosu & Albert [15] conducted a comparative study, where a particular legume, *Leucaena leucocephala* accumulated higher levels of hydrocarbon from crude oil polluted soil than the other legume, *Bauhinia monandra.*

The massive accumulation of crude oil by TOPB and its survival till the end of the experiment showed the high capability of the crop to remediate oil polluted soil. Our result is in tandem with the report of Akpokodje & Uguru [16] which showed the ability of *Telfairia occidentalis* to degrade soil THC from 964.35mg/kg to 82.67mg/kg, even far better than *Abelmoschus esculentus* which lowered soil THC from 964.35mg/kg to 104mg/kg at 14 weeks interval. Still in support of this research finding, Idisi & Uguru [17] stated that, the ability of guinea grass to grow and accumulate hydrocarbon in an oil site; qualifies it as a potential phytoremediator. In 2022, Orji-Oraemesi & Njoku [18] recommended the use of cowpea plant to remediate crude oil polluted soil within a short time frame. Another study demonstrated that hydrocarbon contamination significantly slowed the growth rate of *Vigna unguiculata* in a concentration-dependent way [19]. Nevertheless, TOPA struggled greatly to survive with the accumulated THC level but eventually died at the end of the experiment and this might be attributed to its method of pollution.
The lower THC values observed in ZMPB, ZMPA, ACPB and ACPA could possibly explain that *Zea mays* and *Allium cepa* strived poorly in crude oil polluted soil, as the plants exhibited stunted growth, leaf wilting, chlorosis, necrosis and even death. This agrees with the work of Adesina & Adelasoye [20], where poor performance of maize and cowpea grown on crude oil polluted agricultural soil was reported. However, in some occasions, they survived in spent-engine oil contaminated soil through phytoextraction of heavy metals. With the aid of organo-mineral fertilizer (OMF), both *Panicum coloratum* and *Zea mays* strived well on soil tainted with hydrocarbons and even extracted heavy metals from the soil [21].

4 CONCLUSION

The lower THC values recorded for all the planted polluted soil samples in this study strongly asserted the fact that biodegradation of petroleum hydrocarbon occurs more in agricultural soil than in non-agricultural soil, probably due to phytoremediation capability of some plants or the endophytic bacteria they harbour. In addition, the varying THC levels observed in the leaves of crops used for this study, further confirmed the fact that bioaccumulation of

hydrocarbons is proportional to the plants phytoremediation capability and the method of pollution. However, it is further advised that soil-plant-animal feeding pathways be continuously monitored in all the oil-producing states in Nigeria due to the level of THC recorded in TOPB and other polluted plant samples in order to prevent future exposure of the dwellers in these regions to hydrocarbons.

REFERENCES

1. Singh SN, Kumari B, Mishra S. Microbial degradation of alkanes. In: Microbial degradation of xenobiotics. 1st ed. Berlin Heidelberg: Springer; 2012.
2. FiriAppah CF, Okujagu DC, Bassey SE. Effects of crude oil spill in germination and growth of *Hibiscus esculentus* (Okra) in Bayelsa State Niger Delta Region of Nigeria. The International Journal of Engineering and Science. 2014;3(6):30-40.
3. Turner NR, Renegar DA. Petroleum hydrocarbon toxicity to corals: A review. Marine Pollution Bulletin. 2017;2(119):1-16.
4. Ogbulie TE, Nwigwe HC, Okpokwasili GC, Iwuala MOE. Comparative study on the effect of symbiotic interaction between plants and non-indigenous isolates on crude oil remediation. Analele Universităţii din Oradea- Fascicula Biologie Tom. 2011;18(1):15-22.
5. Nwakanma NMC, Ikegwu E, Osaigbovo EJ. Genotoxic effects of spent engine oil (seo)-polluted soils on *Vernonia amygdalina* Del. International Journal for Research in Applied Science and Engineering Technology. 2018;6(12):564-570.
6. Ogbo EM, Zibigha M, Odogu G. The effect of crude oil on growth of the weed (*Paspalum scrobiculatum* L.) –phytoremediation potential of the plant. African Journal of Environmental Science and Technology. 2009;3(9):229-233.
7. Ogbulie TE, Nwanebu FC, Nwachukwu AA. Assessment of the total petroleum hydrocarbon content of agricultural soil polluted with different volume of crude oil during plant-microbe interaction. Annals of West University of Timişoara, ser. Biology. 2014;17(1):13-24.
8. Yuniati MD. Bioremediation of petroleum-contaminated soil: A review. Earth and Environmental Science. 2018;118(1):1-7.
9. Bashir MT. Environmental, Public Health and Socio- Economic Issues of Oil Spillage in Niger Delta, Nigeria. International Journal of Engineering Research and Technology. 2021:10(1):62-66.
10. Onyekuru SO, Nwankwor, GI, Okeke OC, Okoye C, Opara KD. An assessment of the pollution potential of hand pump wells from septic tank units in the hostel areas of the Federal University of Technology, Owerri, Nigeria. Futo Journal Series. 2017;3(1):138-160.
11. Akpan GU, Usuah PE. Phytoremediation of diesel oil polluted soil by fluted pumpkin (*Telfairia Occidentalis* Hook F.) in Uyo, Niger Delta Region, Nigeria. Journal of Environment and Earth Science. 2014;4(1):6-15.
12. Numbere AO. Bioaccumulation of total hydrocarbon content by three mangrove species (*Rhizophora, Laguncularia, Avicennia*) in the Niger Delta, Nigeria. Journal of Petroleum and Environmental biotechnology. 2019;10(1):1-6.
13. Ekemube AR, Emeka C, Nweke B, Atta TA, Opurum NA. Evaluation of simulated petroleum hydrocarbon on the physicochemical properties of soil. Research Journal of Ecology and Environmental Sciences. 2022;2(4),195–210.
14. Chukwumati JA, Asiegbu GC. Accumulation of hydrocarbons and some heavy metals on sediments and plants from crude oil polluted mangrove ecosystem in Okrika, Nigeria. World Journal of Advanced Research and Reviews. 2023;17(3):298-306.
15. Edwin-Wosu NL, Albert E. Total petroleum hydrocarbon content (TPH) as an index assessment of macrophytic remediation process of a crude oil contaminated soil environment. Journal of Applied Sciences and Environmental. 2010;14(1):39-42.

16. Akpokodje O, Uguru H. Phytoremediation of petroleum products contaminated soil. Archives of Current Research International. 2019;18(1):1-8.
17. Idisi J, Uguru H. Petroleum hydrocarbons content in the soil and its accumulation in grasses from oil spill site at Isoko North LGA of Delta State, Nigeria. Direct Research Journal of Agricultural and Food Science. 2020;8(5):144-150.
18. Orji-Oraemesi CM, Njoku KL. Study of the time-efficacy and rate of phytoremediation of crude oil polluted soil by *Vigna Unguiculata* (L) Walp. International Journal of Environmental Quality. 2022;48(1):41-57.
19. Ismail HY, Farouq AA, Rabah AB, Muhammad AB, Aliyu RU. Effect of soil contamination with crude petroleum on cowpea: An insight into the prospects of crop production in Nigerian frontier basins. Journal of Environmental and Agricultural Studies. 2021:2(2):50-62.
20. Adesina FP, Akinnfesi OJ, Akinyemi MI, Adesina TF. Occurrence and uptake of heavy metals in sol and plant (tomato) assocated with crude oil contamination. Trends in Sciences. 2022;19(16):1-11.
21. Anukwa FA, Onuoha EM, Nkang A, Nkereuwem J. Phytoremediation potential of *Zea mays* L. and *Panicum coloratum* L. on hydrocarbon polluted soils. International Journal of Botany. 2021;17(1):1-7.