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Hydrocarbon Utilization by Culturable Microbial Species in the Rhizosphere of *Eleusine indica* in Oil-Polluted Soils

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Abstract
The present study screened fungi species isolated from rhizosphere of Eleusine indica in some mechanic workshops in Benin City for hydrocarbon utilization. Ten different automechanic workshops were selected for the study; these were equally distributed among the 5 Local Government Areas (Egor, Oredo, Ikpoba-Okha, Ovia-North-East and Uhunmwode) in Benin City. Rhizospheric soils were obtained and analyzed for total hydrocarbon and microbial content. The results showed that total fungal colony count in the soils sampled ranged from 1.6 – 9.0 ×105 cfu/g whereas total bacterial colony count was 1.3 – 9.3 ×106 cfu/g. Total hydrocarbon contents of rhizosphere soils in the workshops ranged from 17.27ppm to as low as 0.27ppm. Saccharomycetes sp. and Aspergillus flavus were the dominant rhizospheric fungi species in the samples assayed. Five days after fungi inocula were introduced into the oil amended PDA medium THC in the Aspergillus flavus – inoculated oil-polluted media was 6.08ppm, representing 18.33% hydrocarbon utilization. Utilization by Aspergillus niger was highest (63.50%); whereas Saccharomycetes sp had the lowest percentage of utilization (0.16%). Mycelia weight of inoculated fungal species in oil-impacted PDA medium after 5 days was 0.95g in Aspergillus niger and 5.34g in Saccharomycetes sp. and 7.14g in Aspergillus flavus.
Keywords: *Eleusine indica*, Bioremediation, Rhizosphere, Root zone, Petroleum hydrocarbons

Introduction
As an important energy source in the world, availability of petroleum is a serious concern for many nations. By the end of 2007, the output of global petroleum had reached 2.7 billion barrels (1970–2007). In the processes of exploration, refining, transporting and marketing petroleum products, increase number of sites are polluted by petroleum hydrocarbons (PHCs). A most worrisome trend is the indiscriminate disposal of spent petroleum products like the lubrication oil in open spaces and gutters. Although the pollution of the environment from hydrocarbons in crude oil may occur only at sites of exploration, exploitation and transportation (pipelines), the incident of waste oil spills from indiscriminate disposal is widespread. Prevalence of spent engine oil in the environment is from artisans setting up their workshops as auto and generator mechanics, on the roadsides and any available open spaces in total disregard to regulations. As a result, they produce oil wastes and indiscriminately dump them within and around their workshops.

Oil in soil poses a grave challenge to the latter and its biotic components, including resident plant community. Although refined petroleum products, like kerosene and gasoline spread easily on water surfaces and percolate porous soils, posing the risk of fire out break and toxicity, the refined products are quickly evaporated and leave behind minute residue. On the other hand, Sivasubramaniam *et al.* [1] reported there are lesser fire risk and toxicity in heavier refined petroleum products which do not readily spread on water, they are not easily degraded, and may pose a huge challenge in remediation.

Oil-contaminated soil adversely affects the vegetation as well as the health of animals and human in oil-producing areas of the many countries like Nigeria. Over 2 decades, the need to stem the effects of oil contamination as well as provide sustainable means of its remediation has been on the front burner. Although physicochemical methods of oil cleanup had been provided including physical clearing and the use of dispersants, are dubbed unfriendly or almost unfriendly to the environment, there is a general clamour for the adoption of a biological means of soil reclamation particularly because it put no further pressure on the environment.

The principle behind the use of natural existing organisms in the soil to clean up contamination is that these microorganisms (bacteria, fungi and yeast) like other living things need nutrients like carbon, nitrogen, phosphate, water and environment for growth and survival [1], as such, the presence of these conditions, will aid some of the microorganisms to break down organic contaminants, using them as carbon source for their energy and growth. Soil bacteria such as *Pseudomonas putida* and *Bacillus subtilis*. use petroleum hydrocarbons as food and energy source, changing them into less toxic substances like CO_2, water and fatty acids. Nutrients for the subsistence of these relevant soil microorganisms are available in the soil. However, in most parts, soil microorganisms require some nutrients for improved growth and development when in association with some

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plant roots. These microorganisms are abundant in association with plants. This is because, naturally, as the plant roots grow into the soil, they release root exudates, which serves as food for microorganisms, thus attracting them to the site of the release [2]. The activities of these microbes lead to the detoxification of organic contaminants in the soil, hence a process known as rhizoremediation; a plant-microbe interaction involving the use of microorganism within the rhizosphere (area around the root) to remediate polluted soil or sediment [3, 4].
A variety of common weed species used in the cleanup of PHC compounds include *Festuca arundinacea* [5], *Lolium perenne* [6], *Cynodon dactylon* [7], and *Panicum virgatum* [8]. *Eleusine indica* has been report to show promising characteristics in remediation of pesticide-polluted soils [9]. The weed is seen as a common feature in the flora of most oil-contaminated sites including auto mechanic workshops [10, 11]. The success story recorded for bioremediation of oil-polluted soils by *E. indica* has been reported to be mainly because of its highly developed fibrous root system which allows for improved microbial activity [9, 12, 13]. *Eleusine indica* is a fast-growing C_4 grass which has exceptional vigour and quickly establishes themselves; thriving in full sunlight and wet areas [14]. The aim of the present study therefore is to determine dominant culturable species in the root zone of the plants, and then screen them, particularly the fungi species, for their ability to utilize petroleum hydrocarbons.

Materials and Methods

Study Site:

Whole plants of *Eleusine indica* were uprooted from within working environment of selected auto mechanic workshops. These plants were obtained within 3m radius of the core oil-polluted zone of the workshops. Ten different automechanic workshops were selected for the study; these were equally distributed among the 5 Local Government Areas (Egor, Oredo, Ikpoba-Okha, Ovia-North-East and Uhunmwode) in Benin City, the administrative headquarters of Edo state of Nigeria (Table 1).

Preparation of Rhizospheric Soil Sample

Eleusine indica plants were uprooted carefully from oil-polluted soil with the aid of a trowel, and roots with adhering soil samples were collected in sterile polyethylene bags, properly labeled and immediately taken to the laboratory. The roots were carefully removed, and shaken vigorously to remove superfluous soil. The rhizosphere soil sample was collected by gently scrapping of the soil particles closely adhering to the root surface by using a sterile brush and spatula, and then soil was air-dried for analysis. Soil was analyzed for polyaromatic hydrocarbon contents using the standard methods of Dean and Xiong [15]. Isolation and characterization of bacterial and fungal species was carried out using the methods of Cheesebrough [16].

Bench-top fungi remediation determination

This determination was conducted in 2 phases; the first was the complete mixing of PDA growth medium with pent engine oil (SEO) before inouculation of fungi isolates, whereas the second simply meant that PDA was poured unto SEO that was already in the petri dish.

Experiment-1 (Mixed)

Some 9.75g of PDA was dispensed into 250ml of deionised water. Some 25ml of SEO was measured and poured into an unsterilized agar. Agar-SEO mix was sterilized in an autoclave at 121^{o}C for 15mins. PDA mixed with spent engine oil (sterile) was allowed to cool and was dispensed into petri dish. The fungi inoculants were streaked on the surface of agar (mixed) and incubated at 27^{o}C for 5 days.

Experiment-2 (Not mixed)

When the 9.75g of PDA was dispensed into 250ml of deionized water, the mixture was autoclaved at 121^{0}C for 15mins. 1ml of SEO was dispensed into sterile petri dish and covered with agar (3mm thick). PDA was allowed to set after which the inoculants were streaked on the surface of agar (not mixed) and incubated at 27^{o}C for 5days.

After the 5 day period of observation, fungi growth was determined in terms of radial spread, whereas THC was determined at both the first and 5th day after inoculation to determine what quantity of hydrocarbons were actually scavenged by the fungi isolates.

Efficiency of THC utilization

Efficiency of hydrocarbon utilization was calculated as follows;

$$\frac{THC(N)_1 - THC(F)_5}{THC(N)_1} \times \frac{100}{1}$$

Where $THC(N)_1$ = THC of oil-polluted medium just before the introduction of the fungal inoculum
$THC(F)_5$ = THC of oil-polluted medium with fungal inoculum at day 5

Mycelial Weight

The Mycelial Weight at a specific time was calculated using the formula below;
Mycelial Weight (g) = wt. of inoculated PDA media - wt. of PDA media (no inoculum)

Table 1: Distribution of workshops visited

S/N	LGA cited	Name	Address	Age of workshop (years)	Periodicity of clearing weeds	Nature of auto repairs
1	Ovia NE	Belena Services Limited (BSL)	36, Benin/Lagos express way by total filling station, Oluku, Benin City	4	Monthly	PD
2	Ovia NE	Eddy Best (EB)	Beside Ineh Petro filling station, Isihor	4	2 times a year	PD
3	Oredo	Social Auto service (SAS)	1, Akenzua junction off plymount road	6	Every 3 months (use herbicides)	PD
4	Oredo	Johnbull Workshop (JBW)	36 Aurosa Street off igbesamwan road	15	once in 3 months	PE
5	Egor	The Two Young Stars (TYS)	28 textile mill road	3	Monthly	PE
6	Egor	God's Glory Workshop (GGW)	Agbontaen Street off Evbareke off textile mill road	10	Monthly	PE
7	Ikpoba-Okha	John Mechanic Workshop (JM)	Uwabi off upper sakponba road	21	Monthly	PE
8	Ikpoba-Okha	Akpu Workshop (AW)	195 upper sakponba road after jesus Christ junction	4	2 times a month	PD
9	Uhunmwode	Agbegi Mechanic Workshop (AMW)	opposite Oasis by Christ Chosen church HQ, before Benin City Bypass	11	Weekly	DE
10	Uhunmwode	Patrick Engineering Workshop (PE)	By Vicko Filling Station, Eyean	4	3 times a year	PD

PE repairs only petrol-engine vehicles, PD repairs only petrol-engine as well as diesel-engine vehicles, DE repairs only diesel-engine vehicles

Results

The results of the total colony count of microorganism isolation from the study sites have been represented on Table 1. Total colony count of isolated fungi species ranged from $1.6 \pm 0.3 \times 10^5$ cfu/g to $9.0 \pm 1.2 \times 10^5$ cfu/g, whereas the highest total bacterial colony count was $9.3 \pm 0.6 \times 10^6$ cfu/g (Table 2). At the Belena Services Limited (BSL) auto mechanic workshop, fungi species isolated included *Saccharomycetes* sp. and *Aspergillus flavus* (Table 3). However, at Johnbull Workshop (JMW), five individual fungi species were isolated isolates were viz; *Saccharomycetes* sp., *Mucor* sp., *Aspergillus flavus, Aspergillius niger* and *Trichoderma* sp. The most prevalent fungi species in all the sites visited were *Saccharomycetes sp.* and *Aspergillus flavus*, the least prominent fungi species were *candida* sp. and *Rhodoturula* sp. However most prominent among bacteria species isolated were *staphylococcus aureus* and *Bacillus* sp.

Table 2: Total colony count of microbial isolation from the study sites

Treatment	Fungi ($\times 10^5$ cfu/g)	Bacteria ($\times 10^6$ cfu/g)
BSL	2.7 ± 0.3	1.3 ± 0.2
EB	3.3 ± 0.6	3.4 ± 0.2
SAS	2.1 ± 0.5	9.3 ± 0.6
JBW	1.6 ± 0.3	1.6 ± 0.4
TYS	7.1 ± 0.1	2.1 ± 0.8
GGW	9.0 ± 1.2	1.5 ± 0.2
JMW	7.3 ± 0.9	2.3± 0.2
AW	9.0 ± 0.2	1.4± 0.4
AWM	2.9 ± 0.6	1.4 ± 0.6
PEW	6.9± 1.0	8.3 ± 1.2

BSL – Belena Services Limited; EB – Eddy Best; SAS – Social Auto Service; JBW – Johnbull Workshop; TYS – The Two Young Star; GGW – God's Glory Workshop; JMW – John Mechanic Workshop; AW – Akpu Workshop; AMW – Agbegi Mechanic Workshop; PEW – Patrick Engineering Workshop.The values presented are means ± standard errors.

Table 3: Rhizospheric soil microbial isolates of *Eleusine indica* collected from the designated spot in the mechanic workshop

Treatment	Fungi	Bacteria
BSL	*Saccharomycetes* sp., *Aspergillus flavus,*	*Pseudomonas* sp., *Staphylococcus aureus*
EB	*Mucor* sp., *Candida* sp., *Rhizopus* sp.	*Bacillus* sp., *Staphylococcus aureus*
SAS	*Aspergillus niger, Aspergillus flavus, Candida* sp., *Saccharomycetes* sp., *Penicillium* sp.	*Bacillus* sp., *Staphylococcus aureus*
JBW	*Candida* sp., *Mucor* sp., *Rhizopus oryzea, Trichoderma* sp.	*Staphylococcus epidymis, Bacillus* sp.
TYS	*Penicillin* sp., *Saccharomycetes* sp., *Aspergillius niger, Rhizopus oryzea, Mucor* sp.	*Staphylococcus aureus*, *Bacillus* sp., *Micrococcus* sp.
GGW	*Candida* sp., *Saccharomycetes* sp., *Penicillium* sp.	*Staphylococcus epidymis, Staphylococcus aureus*, *Micrococcus* sp., *Pseudomonas* sp.
JMW	*Saccharomycetes* sp., *Mucor* sp., *Aspergillus flavus, Aspergillius niger, Trichoderma* sp.	*Bacillus* sp., *Staphylococcus aureus*
AW	*Candida* sp., *Aspergillius niger, Aspergillius flavus, Trichoderma* sp.	*Bacillus* sp., *Staphylococcus aureus*
AWM	*Aspergillus niger, Saccharomycetes* sp., *Aspergillus flavus, Mucor* sp., *Rhodotula* sp., *Trichoderma* sp.	*Staphylococcus aureus*, *Bacillus* sp.,
PEW	*Aspergillus niger, Sacharomycetes* sp., *Rhizopus* sp.	*Staphylococcus epidymis, Staphylococcus aureus*

BSL – Belena Services Limited; EB – Eddy Best; SAS – Social Auto Service; JBW – Johnbull Workshop; TYS – The Two Young Star; GGW – God's Glory Workshop; JMW – John Mechanic Workshop; AW – Akpu Workshop; AMW – Agbegi Mechanic Workshop; PEW – Patrick Engineering Workshop.

Table 4: Total Hydrocarbon content of rhizospheric soil obtained from *Eleusine indica* at the designated sites

Treatment	Total Hydrocarbon Content (ppm)
BSL	0.27 ±0.08
EB	1.73 ±0.23
SAS	1.49 ±0.62
JBW	17.27 ±0.62
TYS	5.51 ±1.23
GGW	1.33 ±0.56
JMW	11.36 ± 2.36
AW	12.16 ±4.28
AWM	0.86 ±0.12
PEW	3.27 ±0.62

Note: BSL – Belena Services Limited; EB – Eddy Best; SAS – Social Auto Service; JBW – Johnbull Workshop; TYS – The Two Young Star; GGW – God's Glory Workshop; JMW – John Mechanic Workshop; AW – Akpu Workshop; AMW – Agbegi Mechanic Workshop; PEW – Patrick Engineering Workshop.Values presented are means ± Standard Error

Table 4 shows the total hydrocarbon content for rhizospheric soil, collected from *Eleusine indica* at mechanic workshops within Benin metropolis. From these mechanic workshops, soils in JBW had a total hydrocarbon content (THC) of 17.27 ppm, being the highest value for total hydrocarbon content for the rhizospheric soils collected for the study. This was followed by AW (12.16 ppm) and then JMW (11.36 ppm); whereas the mechanic workshop with the lowest THC value was BSL (0.27 ppm). During preliminary visits upon which the various 10 mechanic workshops were selected, it was observed that JBW had the highest numbers of cars under repair; whereas, car repair activities was least in BSL.

Five days after fungi inocula were introduced into the oil amended PDA medium, THC in the control was 6.11ppm when the oil was mixed with PDA prior to inoculation (OMP), and 8.46ppm when PDA was poured on oil before inoculation (POO) (Table 5). In the *Aspergillus flavus* – inoculated petri dish, THC was 6.08ppm in OMP, compared to 8.43ppm in POO. The lowest hydrocarbon content was obtained when *Aspergillius niger* was inoculated in OMP (2.23ppm). However the respective THC in POO was 8.41ppm. In terms of utilization

of hydrocarbons (efficiency), *Aspergillius niger* in OMP was the most promising with an efficiency of utilization of 63.50%, whereas *saccharomycetes* sp in OMP shows the lowest percentage of utilization (0.16).
Mycelia weight of inoculated fungal species in oil impacted PDA medium is presented in Table 6. After 5 days of inoculation, the weight *Aspergillus niger* was 0.95g followed by *Tricoderma* sp. (1.67g), *Rhizopus* sp. (2.50g), *Saccharomycetes* sp. (5.34g) and *Aspergillus flavus* (7.14g). However, mycelia weight of *Aspergillus niger* in POO was 4.03g, compared to 2.34g in *Rhizopus* sp. Comparatively, performance of fungi species were better in POO than in OMP. In the OMP, oil was mixed with PDA and then sterilized with heat. The effect of heat would have reduced THC just before inoculation.

Table 5: Total Hydrocarbon content and of oil- impacted Potato Dextrose Agar medium at 5 days after inoculation and efficiency of utilization by fungi

Fungi Inocula	**Total Hydrocarbon Content (ppm)**		**Efficiency of utilization (%)**	
	OMP	**POO**	**OMP**	**POO**
Control	6.11 ±1.20	8.46± 1.62	-	-
Aspergillus flavus	6.08± 0.96	8.43± 1.28	18.33	0.35
Aspergillus niger	2.23± 0.28	8.41± 0.96	63.50	0.59
Saccharomycetes sp.	6.10± 0.76	8.39± 0.78	0.16	0.82
Tricoderma sp.	4.10± 1.04	7.53± 1.02	32.90	10.99
Rhizopus sp.	4.97± 0.63	7.95± 0.48	18.70	6.03

OMP = Oil mixed with PDA prior to inoculation, POO = PDA was poured on oil before inoculationValues presented are means ± standard error for 3 replications

Table 6: Mycelia weight of inoculated fungal species in oil impacted Potato Dextrose Agar medium

Fungi Inocula	**Weight of mycelium (g) at 5 DAI**	
	OMP	**POO**
Aspergillus flavus	7.14 ± 0.28	3.12 ± 0.62
Aspergillus niger	0.95 ± 0.64	4.03 ± 0.58
Saccharomycetes sp.	5.34 ± 1.23	5.06 ± 1.02
Tricoderma sp.	1.67 ± 0.19	2.87 ± 0.73
Rhizopus sp.	2.50 ± 0.43	2.34 ± 0.43

OMP = Oil mixed with PDA prior to inoculation, POO = PDA was poured on oil before inoculation. DAI = Days after inoculation. Values presented are means ± Standard Error

Table 7: Radial spread of fungi Inocula after 5 days in oil impacted PDA medium

Fungi Inocula	**Radius (cm)**	
	OMP	**POO**
Aspergillus flavus	3.30± 0.62	1.10± 0.62
Aspergillus niger	4.62± 1.08	3.85± 0.84
Saccharomycetes sp.	4.25± 0.68	3.90± 1.02
Tricoderma sp.	3.60± 0.43	3.80± 0.92
Rhizopus sp.	4.25± 1.06	3.80± 0.73

DAI = Days after inoculation. Values presented are means ± Standard Error

In terms of radial growth of the inocula, *Aspergillus niger* covered 4.62cm radius, which indicates high growth rate of the Inocula on oil impacted medium (Table 7). *Saccharomycetes* sp. and *Rhizopus* sp. was having the same radius of 4.25cm but second in terms of centimeters cover on the surface of the medium in respect to growth. *Aspergillus flavus* was the lowest with 3.30cm radius. PDA poured on oil, the lowest radius was 1.10cm for *Aspergillus flavus* followed by *Tricoderma* sp. and *Rhizopus* sp. with 3.80cm each whereas the highest *Saccharomycetes* sp. (3.90cm) and *Aspergillus niger* (3.85) respectively.

Discussion

This research thus provides information on the evaluation of abundance of fungi species and hydrocarbon content of rhizosphere of *Elusine indica* in an oil polluted soil. Evidence has shown that the chemical conditions of the rhizosphere differ from those of the bulk soil, as a consequence of various processes that are induced by plant roots and/or by the rhizobacteria [17, 18, 19]. Plant-microbial interactions could stimulate the production

of compounds that could alter soil chemical properties in rhziosphere and enhance heavy metals accumulation in plants. This enhances hydrocarbon degradation in the root zone compared to bulk soil a number of factors contribute to heavy metal reduction in polluted soils, including soil physicochemistry as well as biological action, most importantly the activities of local resident plant species [12].

In the present study, the abundance of *E. indica* isolated from the mechanic workshops showed luster growth. There were no visible signs of plant stress occasioned by the polluted environment. Obviously, the enhanced plant-microbial interaction may have been one of the reasons for its subsistence as the study showed significant utilization of hydrocarbons by the fungi species isolated from the root zone. The rhizosphere substantially increases the surface area where active microbial degradation can be stimulated. The roots provide additional surface area for microbes to grow on and a pathway for oxygen transfer from the environment. Also degradation of the exudates can lead to co-metabolism of contaminants in the rhizosphere [20]. Although numerous researchers have established that the primary mechanism for the disappearance of both petroleum hydrocarbons and polycyclic aromatics (PAHs) is rhizodegradation [6, 11, 21, 22], the way the mechanism operates is poorly understood, at least in the tropics. There are some indications that the presence of hydrocarbons may even encourage the proliferation of hydrocarbon-degrading microorganisms in the rhizosphere [6, 23].

High root biomass as in *E. indica* translates to a larger rhizosphere, which stimulates soil microorganisms and their biodegradative activity by increase of diffusion, mass flow and concentration of nutrients, thus facilitating the degradation of crude oil hydrocarbons [24]. Irwin [25] reported that if site assessments reveal that species of indigenous microorganisms are unable to degrade target contaminants, exogenous microorganisms with the required biochemical capabilities can be introduced to successfully degrade specific waste compounds. Although this was not the case of the present study; however the possibility of the re-introduction of neighboring microorganism from bulk soils into rhizosphere soils may have been made possible by root exudates, in the rhizosphere region [26, 27].

Colony count in the rhizospheric soil of *Eleusine indica* at John Bull Workshop (JBW) showed reduced microbial count. JBW was the most contaminated site, evidenced by the increased number of cars repair activities as well as number of cars parked at the site. Obire and Nwaubeta [28, 29] corroborated that in increased oil in soil reduce microbial load. The reduction in microbial population that always occurs before its rise in number again when crude oil is added to the soil is being attributed to oil toxicity. Some microorganisms are killed or inhibited by toxic fractions in the oil, while other heterotrophic organisms degrading the oil are increasing in number. The toxicity of crude oil or petroleum products varies widely, depending on their composition and concentration. The scale of pollution depends on the quantity of oil and the damage done to the environment [31]. Obire and Anyanwu [30] also reported that the addition of oil to the soils resulted in selective increases and decreases in the numbers of fungal populations. The decrease in species diversity (fungal genera) with increasing concentration of added crude oil is as an indication of environmental stress of petroleum hydrocarbons.

Microbial isolates from the mechanic workshops sampled were *Pseudomonas* sp. *Bacillus* sp. *Staphylococcus* sp., *Micrococcus* sp., *Aspergillus niger, Aspergillus flavus, Rhizopus* sp., *Tricoderma* sp., *Penicilium* sp., *Candida* sp. and *Saccharomycetes* sp. These microbial isolates were earlier identified by Ekhaise and Nkwelle [32], who worked on microbiological and physicochemical analyses of oil-contaminated soil from major motor mechanic workshops In Benin City. The authors reported a number of hydrocarbon-utilizing bacteria and fungi genera including *Pseudomonas, Bacillus, Micrococcus, Flavobacterium, Klebsiella, Aspergillus niger, Aspergillus versicolor, Penicillum, Trichoderm* and *Rhizopus*.

The isolation of *Aspergillus flavus, Aspergillus niger, Penicillium* sp. and *Candida albicans* from the sample which persisted even after days of inoculation into oil-amended media supports the findings of Sutherland and da Silva *et al.* [33], who reported the degradation of polycyclic aromatic hydrocarbons (PAHs) by *Aspergillus niger* and *Penicillium janthinellum* among others. It also lend more weight to the studies made by Nkwelang *et al*. [34] on the diversity, abundance and succession of such isolated hydrocarbon-utilizing microorganism as *Pseudomonas* sp., *Bacillus* sp., *Acinetobacter* sp. *Aspergillus* sp, *Penicillium* sp, *Candida* sp., *Mucor* sp., *Rhizopus* sp., *Sporobolomyces*. As in the tropical soil polluted with oil sludge.

According to Ikhajiagbe *et al*. [22], plants and microorganisms can degrade petroleum hydrocarbons independently of one another but it is the interaction between them (i.e., the rhizosphere effect) which is the primary mechanism responsible for petrochemical degradation in phytoremediation efforts. Anoliefo and Ikhajiagbe [11] also opined that the capability for remediation of polluted soils resides with both organisms. However, a synergistic approach has been found to be a better strategy. Plants are able to provide root exudates of carbon, energy, oxygen, nutrients, as well as enzymes to microbial populations in the rhizosphere. This stimulates microbial activity within this region and hence enhanced degradation of the hydrocarbon mixture.

One of the possible survival mechanisms of *Eleusine* indica found within these mechanic workshops may have been their capability to degrade oil in soil [10, 35]. Contamination and perturbations in the environment can have significant effect on shoot growth, causing plants to allocate much of its resources to roots. This ability to change carbon fluxes in the plant is an essential tool for phytoremediation, and this may suggest the favourable

response of *Eleusine* indica plants collected to changes in the environment [36]. This finding also corroborates Merkl et al. [24] report on the tolerance of *E. indica* to crude oil treatments. Although the study did not include morphological assessment of the plants on the field; nevertheless, soils samples were collected from rhizospheric regions of complete plants. The researcher took the liberty of noticing that these plants looked as healthy as those in the unpolluted region of the mechanic workshop with the production of enhanced rhizospheric environment in these plants. The report of Oyedeji et al. [36] is corroborating. They reported that the presence of high root biomass as in *E. indica* translated to a larger rhizosphere, which stimulates soil microorganisms and their biodegradative activity by increase of diffusion, mass flow and concentration of nutrients, thus facilitating the degradation of crude oil hydrocarbons [24]. This can explain the lower levels of THC around the rhizospheric region of *E. indica* and *Eleusine*-planted soils in this research area.

Conclusion

Automobile wastes are becoming a visible problem especially in developing countries such as Nigeria. Therefore the usual improper disposal of these wastes now demands attention in order to protect the soil for agricultural purposes. The automobile mechanic workshop within the Benin metropolis and the disposal of the wastes into open vacant plots, farms and water drains pose an environmental risk considering the water table in the South – South Region of Nigeria and shallow bore-holes dug to get water for domestic use. They also render farms unfit for agricultural purposes since the contaminated soils inhibit plant growth and the inhabitant soil microbes respond to the presence of the heavy metals since plants have been identified to adapt to growth in most of these oil-impacted soils; an example being *Eleusine indica*. With myriad of microorganisms present in its rhizosphere, as with other adapted plant species, it is only possible to conclude that perhaps one of its mechanisms of survival in such environments may center on its synergistic association with microorganisms. These microbial species could serve as microbial indicator for metal pollution levels and/or also used in bioremediation where necessary. *E. indica* are widely distributed and have proved successful in phytoremediation of crude oil contaminated soils, they can be beneficial for many other tropical countries facing the problem of crude oil spillage.

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