PHYTOREMEDIATION POTENTIAL OF GUINEA GRASS (*PANICUM - MAXIMUM*) IN THE RECOVERY OF ENGINE OIL POLLUTED SOIL IN IMO STATE, NIGERIA.

[1]Ubuoh, E.A.,[2]Ezenwa, L, [3]Ogbuji S. I.
[1&2] Department of Environmental Management and Toxicology(EMT), College of Natural Resources and Environmental Management(CNREM), Michael Okpara University of Agriculture, Umudike(MOUAU) Abia State, Nigeria,
[3]Department of Geography and Environmental Management, Faculty of Social Sciences, Imo State University, Owerri, Imo State, Nigeria.
Corresponding Author :*ubuohemmanuel@yahoo.com*, *+23408037639777*

Abstract*: In recent decades, serious contamination of soils by human activities have been reported. It is therefore a matter of urgency to develop a new and efficient technology like phytoremediation , an emerging cleanup technology that uses plants and grasses to remove contaminants from the environmental media. Based on this , fieldwork was carried out on engine oil polluted soil in Nekede Mechanic village , Owerri , Imo State. Phytoremediation on the removal of engine oil from the polluted soil was assessed using guinea grass (Panicum -Maximum)for a period of five weeks.Soil parameters like pH, Electrical conductivity and temperature were analyzed. The percentage oil loss from the soil, total heterotrophic counts (THC), hydrocarbon utilizers count (HUC), and plan growth indices (PGI) were investigated. The results obtained showed significant (P≥0.05) increase in leaf index from 63cm^2 of 43% in the polluted planted soil, 85.5-183.3cm^2 of 55% in an unpolluted planted soil. The shoot height showed 23.36% and 27.7% increase in polluted planted soil and unpolluted planted soil respectively. There was 70% oil loss in polluted control soil and 100% oil loss in polluted planted soil.Total heterotrophic count also showed an increase count in polluted planted sample with 23.5 x 10c/u./g of soil studied. The hydrocarbon utilizing bacteria was identified as species of Bacillus, Pseudomonas and Micro-cocus. Result also showed that the engine oil polluted soil has effect on the soil chemical constituents with increase in pH level (6.0), decrease in EC and temperature in polluted soil than polluted soil control. From results obtained , guinea grass (Panicum Maximum) showed great potential for use in bioremediation of the engine oil polluted soil.*

Keywords: Phytoremdiation, polluted soil, guniea grass, engine oil.

1.0 Introduction

Synthetic chemicals foreign to a particular ecological system and has a biological activity can be called xenobiotic compounds. Some microorganisms have the ability of breaking down the xenobiotic compounds partially or entirely(Godheja *et al*, 2016).Condemed engine oil used by mechanics are foreign to soil ecosystem.

Engine oil is a toxic environmental contaminant (Dominguez-Rosado and Pichtel, 2004). Condemed engine oil can enter into the environment through improper disposal by mechanics when servicing cars can pollute soil (Nwachukwu et al., 2010). Soil pollution through such can pose large environmental threat (Wyszkowski and Ziolkowska, 2008), that agricultural land may generally reduce plant growth (Nwaogu *et al.,* 2008), due to reduction in soil fertility and soil microflora population (Torstenssen *et al.,* 1998). Wyszkowski and Ziolkowska (2008) also reported that the addition of diesel oil to the soil led to a significant reduction of organic carbon content of the soil.

Condemedengine oil when present in the soil can creates an unsatisfactory condition for soil quality, which is due to poor aeration in the soil, immobilization of soil nutrients and lowering soil pH (Atuanya, 2000; Dick,2007). Furthermore, Anoliefo *et al.* (2001) observed that disposal of used lubricating oil on farmland have been practiced by mechanists servicing cars, lorries and generators that degraded soil quality. This has been confirmed by Odiegba and Sadiq (2002) who explained that pollution from spent oil poses environmental problems in Nigeria and is more widely spread than crude oil pollution, and can cause physical damages to organisms and plants impairing natural processes such as oxygen replenishment and photosynthesis (Koma et al, 2001). In the same vein, certain microbes on continuous exposure to xenobiotics develop the ability to degrade the same as a result of mutations. Mutations resulted in modification of gene of microbes so that the active site of enzymes is modified to show increased affinity to xenobiotics (US DOE, 2002; Subramanian*et al*. 2006; Walter , 2011).

Plants have also been known to take up various organics and either degrade or process them for use in physiological processes (Vouillamoz and Milke, 2001). Barter(1999)has presented a good overview of phytoremediation of contaminated soils. Phytoremediation is then the use of plants and their associated microorganisms to degrades, remove, destroy, contain, or render contaminants harmless in environment. Some plants aid in degradation indirectly by supporting microbial population, other

plants take up inorganic contaminants from soil and concentrate them in plant tissues or roots to become hyperaccumulators. Therefore phytoremediation employs human initiative to enhance the natural attenuation of contaminated sites and is a process that is intermediate between engineering and natural attenuation. Omokhodiori (1999) specifically, indicated that the pollution effects of mechanic village activities in Nigeria have received limited attention even though these activities have been shown to produce petroleum based wastes (Nwachukwu *et al.,* 2010).

Therefore, this project focused on the efficacy of phytoremediation on the treatment of engine oil polluted soils in Nekede mechanic village through the use of guinea grass (*Panicum maximum*) to ascertain its influence on crops and microorganisms for the way forward in soil quality management and sustainability.

1.1 Study area: The study area is Nekede mechanic village in Owerri West Lccal Government Area of Imo State Nigeria .It lies within latitude $5^0\ 41^1$ and $5^0\ 31^1$ North. Longitude $6^0\ 53'\ 7^0\ 35'$ East., rainfall ranging between 2000mm- 3000mm (Angela *et al*,2011) . The minimum and maximum rainfall amounts for this area are 172mm and 256mm, respectively. Population of mechanics in Nekede mechanicshowed 1664 mechanics thatspread across 12 roads in the study area (Angela *et al,* 2011), and results obtained showed that over 1.4 million liters of spent engine oil was produced annually in the village. About 60% of the mechanics disposed spent engine oil on the soil, within their immediate environment, while others used it for other purposes, such as pest control, sharpening of blades and reuse in heavy trucks among others. Another 88.3% of mechanics were ignorant of environmental impact of inappropriate spent engine oil disposal(Angela *et al,* 2011). The total annual volume of spent engine oil produced stood at 1,469,678.08 liters or 7348.39 drums of oil (Angela *et al,* 2011). The geology of the area consists of plain soil, which is about 0.05 – 2.0 mm in size. This type of soil has good drainage and is well aerated, causing it to dry out quickly (Onweremadu and Duruigbo, 2007). In addition, the agricultural land has humus soil.

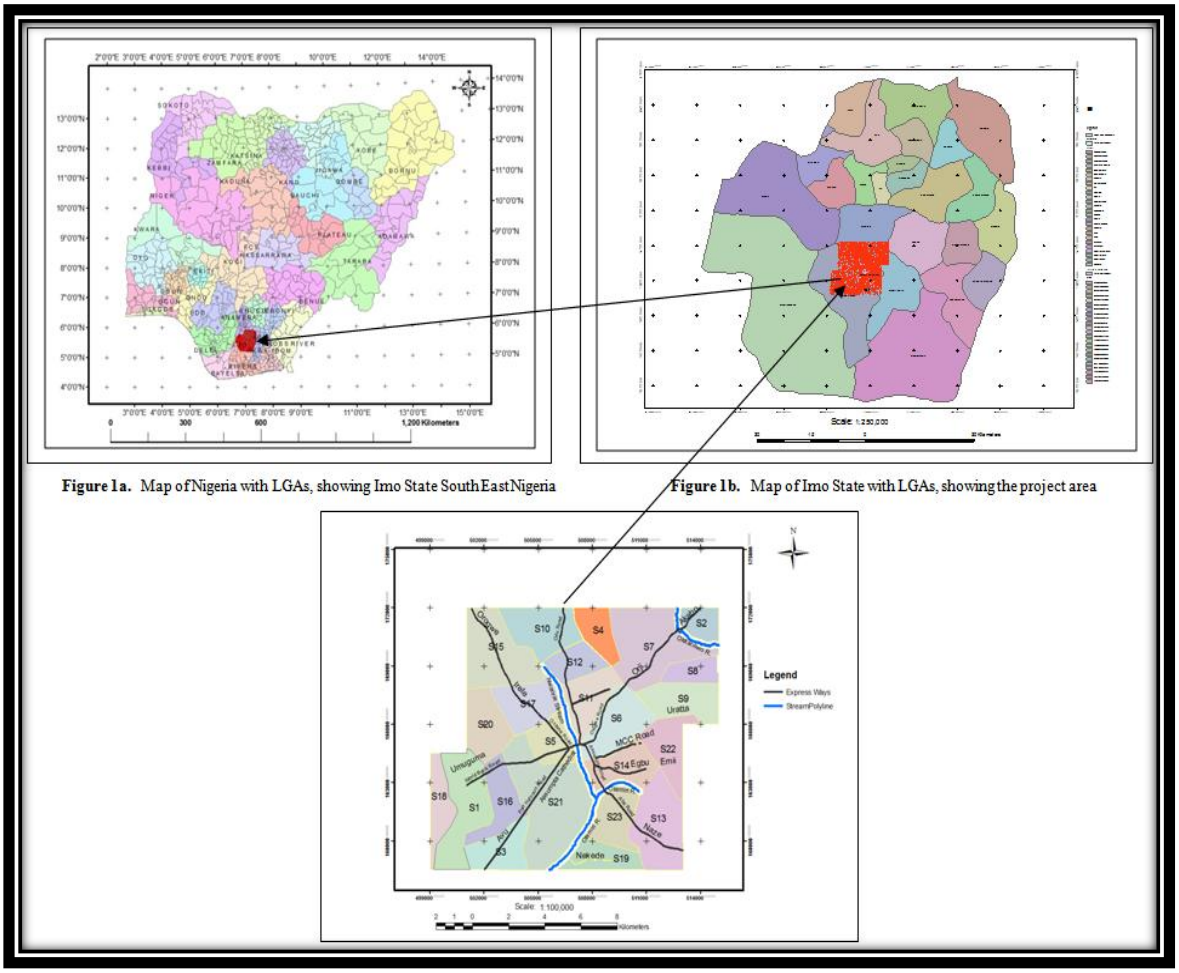

FIG. 1: Map of Nigeria Showing Imo State and L.G.As
Source: Akajiaku and Igbokwe,2014.

1.2 Sampling Techniques : Guinea grass (panicum maximum) was chosen due to large surface area provided for microbial growth by their extensive and widely fibrous root system.Seedlings of *panicum maximum* was collected from those growing in uncontaminated soil in the wild within Federal College of land Resources Technology (FECOLART), Owerri.The soil used for experiment was collected from a fallow patch of uncontaminated soil within FECOLART. Soil sample were air dried, sieved through a 2mm merged to remove stones and debris and to ensure uniformity, sieved samples were stored in the plastic buckets until the measurement of pH, electrical conductivity , temperature and microbes

1.3 Experimental Design and Samples Analyses : The spent engine oil used in polluting the sand was obtained from a car servicing workshop along Owerri -Aba, Owerri. 1.5kg of dry soil was weighed into each of the 10 plastic buckets.Then 8 buckets were polluted with 75g of oil and mixed thoroughly. This was done to obtain 5% spent engine oil in 1500g of soil. Then the remaining two served as control. The buckets were then kept in a greenhouse with approximate 12hours daylight with intermediate moisturing with water. Seedlings of *Panicum maximum,* obtained as described above were planted in an unpolluted soil in a small plastic container. The seedlings were than moisture with tap water and kept under 90% shading for 24 hoursand after which they were transported with tiny polythene into the experimental bucketscontaining spent engine oil polluted soil.After transporting, the plastic buckets were kept under about 90% shading for one week, followed by 55% shading for another two weeks and then transferred to a green hose .They were watered intermittently to keep the soil water content near field capacity. The leachaete from the bottom of the bucket was collected using the cover of the bucket and poured back into the buckets to avoid leakage of engine oil added to the soil.

The pH and conductivity of the soils was diiluted with distilled water in a ratio of 1:2.5 (l0g of soil in 25ml of water) and after 30minutes, the pH and conductivity respectively.Determination Of Soil Temperatureof was done by dipping a thermometer into the soil samples (5cm).Determination of*Panicum maximum*include the height, leaf number and leaf area measurement . The plant height was measured between 2 weeks for 16weeks using a meter rule. The leaf area was obtained in duplicates by placing the leaf on a graph paper of one square centimeter ($1cm^2$). The squares enclosed by the margin were counted after the trace. The squares which were divided by the leaves area were counted if they are greater than or equal to 0.5 cm^2 . Those that were less than $0.5cm^2$ were ignored (Akujobi et al, 2011). The mean of the duplicate figures was taken as the leaf area. The leaf numbers were obtained by visual counting of the leaves. All the parameters were obtained at 2 weeks interval for 16 weeks.The bacterial isolates were tested for their ability to utilize engine oil using the turbidity method as described by Ukaegbu-Obi and Mbakwem-Aniebo (2014). The bacterial isolates were cultured in nutrient broth and incubated at 28+20 C for 24 hours. Aliquot (0.1ml) of the young culture in nutrient broth grown was inoculated into each test tube containing 9.9ml of sterile mineral salt broth and 0.1ml of crude oil. A control test tube containing 9.9ml of sterile mineral salt broth plus 0.1ml of crude oil remained uninoculated. The tubes were incubated at room temperature for 7 days. The growth of the inocula was determined by visual observation of the mineral salt broth turbidity, as compared with the uninoculated control tube. Percentage oil loss in Soil was calculated by:

$$\% = \frac{Weight\ of\ oil\ removed}{Original\ \ Weight\ of\ oil} x\ 100 \ldots.\ \text{equation (1)}$$

1.4Data Analysis: The Data collected were analyzed using descriptive Statistics such as Arithmetic means and percentages. Correlation Analysis and Statistical packages for the Social Science (SPSS) as well as mean comparisons were made using the least Significant Difference (LSD) at ($P \leq 0.05$) level of probability for the purpose of drawing statistical inference and conclusion.

1.5Results and Discussion

From Table 1, the pH of the polluted planted soil sampled ranged from 5.8 to 6.0, the pH of the polluted control also ranged from 5.0 to 7.5. In unpolluted control and planted unpolluted control, the pH ranged from 3.7 to 6.1 and 5.8 to 6.3 respectively. The result shows that the soil is highly acidic in unpolluted soil which easily allow the mobility of the pollutants and slightly acidic in polluted soil with engine oil used for planting the grass. These results are in conformity with the findings of authors like Romkens and De Vries (1995), Ma and Roa (1997) who observed thal low pH allows the mobility of metals the result in bioavailability in soil. The growth and activity of soil microorganisms are very much dependant on the soil pH (Kalita and Devi, 2012). The soil pH regulates the solubility, mobility, and the availability of the ionized forms of contaminants (JRB Associates, Inc., 1984). According to the findings of Kalitha and Devi (2012), significant degradation of petroleum hydrocarbons takes at pH 4.5 and 7.5 and the pH of the soil at the end of the study falls with this range.

Table 1: Changes in the pH of polluted and nonpolluted Soils during Treatments with grass

Treatmentpolluted Soil soil control/plant	unpolluted +plant	polluted + plant	polluted	unpolluted
WK1	7.5	5.4	5.8	6.3
WK2	5.3	4.5	5.8	6.3
WK3	6.1	3.7	5.9	6.1
WK4	5.4	4.5	6.0	5.9
WK5	5.0	3.8	6.0	5.8
WK6	5.2	6.1	6.0	5.9
Mean	**5.75**	**4.66**	**5.9**	**6.05**

Key - WK= WEEK.

Soil electrical conductivity (EC) is a measure of the amount of salts in soil (salinity of soil). It is an From Table 2, the result showed that electrical conductivity in polluted soil control ranged from 106-920 µ/Scm, unpolluted plant ranged between 160-860 µ/Scm, pollutedsoil and plant ranged between 163-810 µ/Scm and plant in unpolluted soil ranged from 067-905 µ/Scm. Planting on the polluted soil resulted in reduced conductivity of soil samples from 860µ/Scm to 210 µ/Scm in 1500g contaminated soil planted with *Panicum maximum*. This subsequently increased during the period of study to 750 µs/Scm on the last week of the study (Table 2).

Table 2: Changes in the Electrical Conductivity (µ/Scm) of polluted and nonpolluted Soils during Treatments.

Treatment Soil soil control	Polluted plant + plant	Unpolluted	Polluted	plant+UnpolIuted
WKl	860	530	210	142
WK2	920	860	226	067
WK3	140	275	163	075
WK4	106	160	810	905
WK5	800	845	780	820
WK6	300	440	750	830
Mean	521	472.5	489.8	506.5

Result of temperature in polluted control soil ranged between 32-41^{o}C with wk5 recording the lowest value and wk1 and wk3 having the highest value respectively with the mean value of 35^{o}C, unpolluted soil control recorded temperature range of 32-43^{o}C with wk5 having the lowest value and wk3 recording the highest value with the mean temperature 36^{o}C, temperature in polluted soil with plant ranged btween 28-36 with the mean value of 30^{o}C and unpolluted soil and plant having the temperature range of 25-36.1^{o}C with the mean value of 29^{o}C (Table 3). The overall result indicated that polluted soil control recorded the mean value > unpolluted control> polluted soil with plant >unpolluted soil with plant. The temperature of the soil samples for each week of the study were approximately within the same range

Table 3: Temperature (^{O}C) of the soil samples over 5 weeks.

Period	Polluted control	Unpolluted control plant	Polluted +Unpolluted+ plant Plant	
WK0	36	36	36	36.1
WK1	41	39	32	33
WK2	38	32	28	29
WK3	41	43	28	25
WK4	34	33	26	26
WK5	32	32	28	27
Mean	**37**	**36**	**29.66**	**29.35**

The total heterotrophic microbial population response to plant exudates in soil planted with Panicum maximum is 23.5 x 10^{5}cfu/g in contaminated soil. This increase continued to 54 x10^{5}cfu/g soil in the fourth week of incubation and then decrease in the last week of study (Table 4). The degree in microbial population is suspected to be caused by vaiations in chemical characteristics of

the soil. The same results were supported by following authors.

Table 4: Total heterotrophic microbial count ($x10^5$ cfu/g) of treated soil samples

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5
Polluted control	$23.5x10^5$	$23\ x10^5$	$30\ x10^5$	$49.6\ x10^5$	$44.4\ x10^5$
Unpolluted control	$84\ x10^5$	$164.4\ x10^5$	$130\ x10^5$	$100x10^5$	$90\ x10^5$
Polluted+plant	$23.5\ x10^5$	$26\ x10^5$	$46.5\ x10^5$	$54\ x10^5$	$40.5\ x10^5$
Unpolluted	$84x10^5$	$30.5\ x10^5$	$45x10^5$	$108\ x10^5$	$150\ x10^5$ c

Bacteria are considered to be very important for soil fertility. The variation in bacterial population may be caused by nutritional and environmental changes, chemical pollution etc. Any adverse impact of chemical on soil characteristics and microorganism may lead to ultimate loss of soil fertility (Ubuoh *et al*., 2012).From Table 5, it is observed that there was a progressive multiplication of microbial counte in polluted soil with plant between week 1 and week 3. This could be explained by the fact that some microbes may survuive well through the inhibition of pollutants in the soil. In week 4 there was a drastic reduction in microbes and this could be due to excessive inhibition of pollutants in the cells. In five week the builtby microbes became necessary due to adaptation to the environment and the absoption of some of the pollutants by the grass. In the control soil samples of the hydrocarbon utilizing microorganisms increased then decreased on the last day of study. The sample containing unpolluted soil and plant increased in week three to $478\ x\ 10^4$ cfu/g while in the polluted soil and plant sample, there was increase in the hydrocarbon utilizing microorganisms.

Table 5: Total hydrocarbon utilizing microbial count ($x10^4$ cfu/g) of treated soil samples

Treatment	WeeK1	WeeK2	WeeK3	WeeK4	WeeK5
Polluted control	$120\ x10^4$	$470\ x10^4$	$478\ x10^4$	$30\ x10^4$	$121\ x10^4$
Unpolluted control	$31.8x10^4$	$42x10^4$	$38.2\ x10^4$	$38\ x10^4$	$34.5\ x10^4$
Polluted+plant	$120\ x10^4$	$384\ x10^4$	$452\ x10^4$	$35\ x10^4$	$215.5\ x10^4$
Unpolluted	$31.8\ x10^4$	$456\ x10^4$	$68\ x10^4$	$342\ x10^4$	$292.5\ x10^4$

Result in Table 6,total fungal count vairied between wk1-wk5 in polluted control soil, in that fungal were increasing in population showing ability of the fungi to inhibit, and adapt to unfavourable condition with the highest value of $7.1x\ 10^4$ during wk5tht fungi was able to use the waste oil as an active ingredient. This means that in unpolluted soil except wk3 with $17\mathbf{x10^4}$, there was drastic reduction in the fungal count. In polluted soil and Panicum Maximuem, there was a progressive increase in the fungal count because the plant was able to extract pollutant from the soil to pavn unpolled for the survival and multiplication of fungal count especially on wk5, and the same variation was found in unpolluted soil**.**

Table 6: Total Fungal Count ($x10^4$ cfu/g) of treated soil samples

Treatment	WK1	WK2	WK3	WK4	WK5
Polluted control	$2.15\ x10^4$	$5.3\ x10^4$	$6.5\ x10^4$	$7\ x10^4$	$7.1\ x10^4$
Unpolluted control	$5.5\ x10^4$	$4.5\ x10^4$	$17\ x10^4$	$7.5\ x10^4$	$7\ x10^4$
Polluted+plant	$2.15\ x10^4$	$9.5\ x10^4$	$18\ x10^4$	$19.33\ x10^4$	$20\ x10^4$
Unpolluted	$5.5\ x10^4$	$4.5\ x10^4$	$11\ x10^4$	$12\ x10^4$	$22.5x10^4$

Table 7 shows that polluted soil encouraged the survival of heterotrophs between wk 1-3 and fluctuated between wk4-5 showing the utilization of engine in the oil, with unpolluted soil having normal soil heterotrophs. Heterotrophs was found to reduce in polluted soil alongside plant with an increase in unpolluted soil without plant.

Table 7: Percentage Heterotrphs identified in polluted and None-Polluted soil Sampled

Treatment	WK1	WK2	WK3	WK4	WK5
Polluted control	$51\ x10^4$	$74\ x10^4$	$73\ x10^4$	$6\ x10^4$	$27\ x10^4$
Unpolluted control	$4\ x10^4$	$3\ x10^4$	$3\ x10^4$	$38\ x10^4$	$4\ x10^4$
Polluted+plant	$3\ x10^4$	$4\ x10^4$	$6\ x10^4$	$5\ x10^4$	$4\ x10^4$
Unpolluted	$19\ x10^4$	$18\ x10^4$	$13\ x10^4$	$28\ x10^4$	$5\ x10^4$

From Table 8, *Panicum maximum* in polluted soil showed an increase in shoot height from 50 cm in week one of the study to 63.75cm in the final week, while on unpolluted it showed increased shoot from

60cm during the first week to 83cm at the final week. This shows that if used oil as zenobiotic in soil is allowed can result to soil degredation that may affect soil microbes that aid soil during litter decomposition for humus formation.

Table 8: Shoot height (cm) of *Panicum maximum in polluted and unpolluted soils*

Period	Polluted+plant	Unpolluted+plant
WK1	50	60
WK2	60	80
WK3	62.75	81
WK4	63	82
WK5	63.75	83

The leaf area of *Panicum maximum* also increased from 63cm^2 in week one to 96 cm^2 in week five for polluted soil and 82.5cm^2 in week one to 183.3cm^2 in week five for unpolluted soil. This result implies the stunted growth of the plant due to bioavailabilty and biotranformation of engine oil in thw soil (Table 9).

Table 9: Leaf area of *Panicum maximum in polluted and unpolluted soils*(cm^2)

Period	Polluted+plant	Unpolluted+plant
WK1	63	82.5
WK2	76.16	113.9
WK3	84.6	117
WK4	85.5	147.5
WK5	96	183.3

From Table 10, the percentage reduction of engine oil ranged between 20-30%, with wks 1 and 2 having the lowest percentage and wk 4 recording the highest in polluted soil without Panicum maximum, and with Panicum maximum the percentage reduction ranged between 30-100% showing the potential of the grass to remedy contaminated soil, hence 70% oil loss in the polluted controlafter the study and 100% oil in soil planted with Panicum Maximum at the end of the 4wks of study.

TABLE 10: Percentage oil loss from polluted and unpolluted soils(%)

Period	Polluted control	Polluted+plant
WK1	20	40
WK2	20	60
WK3	45	80
WK4	70	100

From Table 11, total of three species bacteria were isolated and identified during the study. These include Bacillus species, pseudomonas species and micrococcus species.From the result , Bacillus Species and Micrococcus species were consistently found in all the sample showing adaptability of the two species to unfafourable condution aand also inability of pseudomonas species to survive in unpolluted environment. The result is consistent with the finding of Santhoshkumar *et al* (2015) who identified as *Bacillus subtilis, Pseudomonas putida, Pseudomonas aeruginosa* in chlorpyrifos polluted soil in India. These have helped in breakdown hydrocarbon in the polluted soil.This agrees with the findings of (Tesar *et al.,* 2002) who reported that a broad phylogenetic range of bacteria including species/strains of *Achromobacter,Acidovorax, Alcaligenes, Arthrobacter, Bacillus,Corynebacterium, Flavobacterium, Micrococcus,Mycobacterium, Norcadia, Pseudomonas,Rhodococcus, Sphinogomonas* and *Xanthomonas* have been identified in the breakdown of hydrocarbons.

Table 11: Bacteria species isolated from soil samples

Bacteria	Polluted control	Unpolluted control	Polluted control	+Unpolluted plant
Bacillus species	+	+	+	+
Pseudomonas species	+	-	+	+
Micrococcus species	+	+	+	+

+ = Present- = Absent

2.0Conclusion

Phytoremediation on the removal of engine oil from the soil was assessed using guinea grass for a period of fiveweeks. This study showed that guinea grass (*Panicummaximum*) has potentials to tolerate contaminated oil polluted soil, because 100percentage loss oil in polluted soil. The result showed decreased shoot height of *Panicum maximum* over their control in uncontaminated soil. However, at the beginning of the experiment, plant growth was adversely affected resulting in reduction of biomassyield .of the *panicummaximum*.A total of three species were isolated and identified as hydrocarbon utilizing bacteriafrom the soil samples during the study. These are *Bacillus species, Micrococcus species* and *Pseudomonas Species.* This study concludes that theisolated*Bacillus species,* Micrococcus species and Pseudomonas Species possess the capacity to tolerate and growin the presence of engine oil polluted soil in the mechanic village marks them out as good candidates for thebioremediation of polluted environment.

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