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BIOREMEDIATION ACTIVITIES OF *Pseudomonas putida* AND *Staphylococcus aureus* ON SOIL CONTAMINATED WITH SPENT MOTOR ENGINE OIL IN ABAKPA-NIKE, ENUGU STATE NIGERIA

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AUTHOR'S CONTRIBUTION

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Biodegradation by natural population of microorganisms represents one of the cheap primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment. The effectiveness of *Pseudomonas putida* and *Staphylococcus auerus* in remediation of soil contaminated with spent engine oil was investigated using standard methods. The result indicates significant variation in bacteria count between spent engine oil contaminated soil and control soil in 3rd to 6th week of bioremediation ($p<0.05$). pH of control soil was significantly ($P<0.05$) different from bio-remediated soil in the 1st, 4th, 5th and 7th week of bioremediation. Percentage organic matter content of control soil also differed significantly from bio-remediated soil in the 1st, 2nd, 3rd, 6th and 7th week of the experiment while the organic matter content of both samples did not show any significant difference in the 4th and 5th week ($P<0.05$). There was no statistically significant difference between concentrations of Pb, Cu, and Zn in bio-remediated soil when compared with control soil ($P<0.05$). Similarly total organic carbon in bio-remediated soil was not significantly different from the control soil ($p=0.001$). *Pseudomonas putida* and *Staphylococcus aureus* are effective in the clean-up of spent engine oil contaminated soil.

Keywords: Biodegradation; engine oil; *Pseudomonas*; *Staphylococcus*; bioremediation.

1. INTRODUCTION

Petroleum-based products are the major source of energy for cars, industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products [1,2]. Also intentional spills by roadside motor mechanics occur on daily bases though such activity receives very low attention [1].

Used motor oil is the brown-to-black oily liquid removed from a motor vehicle, when the oil is changed [3,4]. Used motor oil contains additional chemicals that are produced or build up in the oil, as a result of engine lubricating function at high temperatures and pressures, inside an engine as it runs [5,1]. It also contains metals such as aluminum, chromium, copper, iron, lead, manganese, nickel, silicon and tin that comes from engine parts, as they wear down [6].

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The technology commonly used for soil remediation includes mechanical, burying, evaporation, dispersion, and washing. However, these technologies are expensive and can lead to incomplete decomposition of contaminants [7]. The best approach is the process of bioremediation, defined as the use of microorganisms to detoxify or remove pollutants owing to their diverse metabolic capabilities [7,8]. It is an emerging method for the cleaning and degradation of many environmental pollutants including the products of petroleum industry, in addition, bioremediation technology is believed to be noninvasive and relatively cost-effective [1].

One important requirement is the presence of microorganisms with the appropriate metabolic capabilities [2]. Microbial degradation is the major and ultimate natural mechanism by which one can clean up the petroleum hydrocarbon pollutants from the environment [9,10,11]. In bioremediation, the contaminated site is exposed to a population of microorganisms which undergoes metabolic activity to transform or mineralize organic contaminants into less harmful, non-hazardous substances which are then integrated into natural biogeochemical cycles [1,12]. Oil degrading microorganisms tolerate high concentrations of hydrocarbons and have a high degradation capability [13,14].

Abakpa-Nike is an agrarian community in Enugu state with 60 per cent of the population relying on cultivated farm produce as source of income. Unfortunately availability of land for cultivation of crops is also very scarce due to urbanization and politics of land allocation brought about by the land use act. Some of the few available plots of land end up been used as auto-mechanic workshops for a long period of time. Such lands are constantly spilled and contaminated with spent motor engine oil during the motor repairing activities of these auto mechanics. The amount of spent engine oil contamination of the soil is alarming and has constituted a nuisance resulting in abandonment of such natural farmlands after it has been used as motor mechanic workshops. It is not only that these soils are contaminated with the spent oil but also some of the constituents of the used motor oil such as metals can dissolve in water and move through the soil easily, and may be found in surface water and ground water. Thus, metals from used oils can build up in plants, animals, soil, sediments and non-flowing surface water [15]. Heavy metals and chemicals in used motor oil are absorbed and distributed into various tissues of human, plants and animals by their movement in the environment, which can result in serious health problem, such as anemia, tremor and consequently, resulting in death. Other health hazards which can result from used motor oil include mutagenicity and carcinogenicity [15]. It is therefore imperative that regular bioremediation of oil contaminated abandoned plots of lands be done to recover such lands back for farm use and to detoxify and make it safe for human habitation. Oil spills at auto-mechanic workshops have been left uncared for over the years, and its continuous accumulation is of serious environmental concern because of the hazard associated with it.

A lot of works have been reported on bioremediation of hydrocarbon pollutants in other places, but to the best of our knowledge, this is the first experimental study of the bioremediation activity of *Pseudomonas putida* and *Staphylococcus aureus* on soil contaminated with spent motor engine oil in Abakpa-Nike Enugu State. This study amongst others intends to investigate the effectiveness *Pseudomonas putida* and *Staphylococcus aureus* in bioremediation of soil contaminated with used motor engine oil.

2. MATERIALS AND METHODS

2.1 Composition of Assay Medium for Culturing of Bacteria

Mineral medium were prepared as was described earlier (Bushnell and Haas, 1941) for both enrichment and isolation of hydrocarbon degraders by adding 0.2g, Mg_2SO_4; 0.02g, $CaCl_2$; 1.0g, KH_2PO_4; 1.0g, K_2HPO_4; 1.0g, NH_4N0_3; and 0.05g, $FeCl_3$ in one liter of distilled water. The liquid BHM were supplemented with 15g/l of agar prior to autoclaving. After sterilization, the media were allowed to cool at 45^0C and poured into sterile Petri dishes in an aseptic condition.

2.2 Collection of Soil Samples

Top soil (0-30 cm) contaminated with spent motor oil was collected from different sites at Abakpa-Nike auto-mechanics workshops Enugu State Nigeria, and was stored at 4°C until used (this constituted the test soil sample). Another 500 g of soil sample was collected from the state ministry of environment. This portion from the ministry of environment was used as control throughout the research as it was not contaminated with oil.

2.4 Soil Sterilization

Soil sterilization was conducted in the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology. The method according to Akwaji et al. (2016) was used for soil sterilization. Briefly described: The loamy soil sample

was collected at about 30 cm depth, heat sterilized in a cut covered metal drum using firewood at 100°C for twenty (20) minutes and allowed to cool for twelve (12) hours. The heated soil sample was sieved through a 2 mm mesh size sieve to remove debris and stones. Thereafter the soil sample was poured into sterile polyethylene bag and kept on the shelves until use.

2.5 Physicochemical Characteristics of Soil Samples

Physicochemical analysis of contaminated soil sample before and after bioremediation was done at the research laboratory of the Project Development Institute (PRODA) Enugu State Nigeria.

Particle Size Analysis/Distribution: This was done according to the method described by Mabachu et al. (2017). Briefly: fifty gram of the heat sterilized soil was weighed, after which it was passed through a 2 mm sieve and weight of the mass of sample > 2 mm taken. To 20 g of the weighed sieved soil, 25 mL of sodium diphosphate ($Na_2P_2O_7$) solution was added; left for about 8 hours and thereafter, 200 mL of water was added and shaken. The suspension was passed through a sieve set arranged in order of decreasing pore size, and placed directly above a 1 litre-measuring cylinder. Sieves were then dried in an oven at 105 ^{0}C until a constant weight was achieved with each sieve weighed before and after sieving the suspension. Bulk Density was done according to Bahunguna et al. [16]. Briefly: An already heat sterilized dried soil sample whose constant weight has been achieved was used. Known weight of the soil sample was then gently poured little at a time into a measuring cylinder while gently tapping the cylinder to compact it in order to measure the volume. Exchangeable Cations was determined using the method described by Mabachu et al. [17]. Briefly: Five (5) g of the soil was weighed and placed in a 100 mL polyethylene bottle. To this, (25) mL of ammonium acetate solution was added and the mixture shaken for 1 hour for filtration of the supernatant directly into a 100 mL volumetric flask through a filter paper held in a funnel inserted in the neck of the flask, leaving the soil in the bottle. Twenty (20) mL of 95% ethanol was then added to the bottle and shaken. This was allowed to settle and the supernatant filtered into the same 100 mL flask as before. The extract was made-up to 100 mL with distilled water and the concentrations of exchangeable cations (Ca2+, Mg2+, Na+, Mn2+ and K+) determined using Atomic Absorption Spectrophotometer Model: BUCK Scientific ACCUSYS 211.

Soil pH: This was done according to Bahunguna et al. [16] by preparing a 10% (w/v) suspension of air-dried soil in double distilled water. Thereafter it was mixed thoroughly, allowed to settle for 1 hour and filtered through a Whatman filter paper. The pH for all the soil filtrates was then checked using a calibrated pH meter. Total Organic Carbon was done according to the method of Ayandele [18] using soil samples already sieved through 1 mm sieve. One (1) g of the sieved soil sample was placed in a 100 mL flask and to it, 10 mL potassium dichromate and 20 mL sulphuric acid was then added and shaken very well. The mixture was allowed to cool on asbestos sheet and the volume was made up of 100 mL with distilled water and kept overnight. The optical density was then measured at 660 nm wavelengths using a spectrophotometer. Total Organic Nitrogen content of the contaminated soil was determined by Macro-kjeldahl method as described in Oyeleke and Manga [19]. Available Phosphorus was determined using Vanado-molybdo- phosphoric acid colometric method as described by Rabah and Ibrahim [20] by using ammonium molybdate which forms molybdo-phoshoric acid under acidic condition. The intensity of the yellow colour was measured using spectrophotometer at 490 nm. The soil conductivity was determined according to the method of Aligwekwe [21]. In achieving this, 10 g of the soil sample was weighed and dissolved in 100 mL distilled water. The conductivity cell was rinsed with three (3) portions of the sample and then immersed in sufficient volume of the sample. The conductivity meter was then turned on and the conductivity of the sample recorded. The soil heavy metal concentration was determined according to the method of Kumar et al. [22]. Cd, Pb, Cu, Zn, Cr, and Fe were estimated by weighing 0.5 g of the dried soil and digested with conc. HNO_3, H_2SO_4 and H_2O_2 in the ratio 2:6:6. The blanks were run in a set, and the heavy metals present in the samples determined using Atomic Absorption Spectrophotometer Model: BUCK Scientific ACCUSYS 211.

2.6 Isolation of *Pseudomonas putida* and *Staphylococcus aureus*

The bacteria used in this study were isolated from an old automobile workshop at Abakpa-Nike Enugu State South East Nigeria. Isolation of bacteria was done using serial dilution technique by agar plate method of soil sample on Bushnell- Haas Mineral medium (BHM). Ten grams (10g) of contaminated soil sample was suspended in 100 ml sterile distilled water and vortexed for 5 minutes for even distribution. Serial dilution was carried out up to 10 fold before plating. Using a sterile pipette, 0.1 ml from the serially diluted tubes was placed onto a BHM plate in duplicates and spread gently using sterilized bent glass rod. 1 ml of filtered sterilized

engine oil was added onto a sterile filter paper (Whatman No.1) that was fixed on the lid of the Petri dishes. The engine oil (which serves as a carbon source) was absorbed via vapour phase transfer. The plates were incubated for four (4) days at room temperature (28°C), and observed for bacterial growth.

2.7 Purification of Isolates

Well developed isolate colonies from above were picked with a sterile inoculating loop and further purified on freshly prepared solid BHM agar plates amended with engine oil by streaking distinct colonies and incubating for 48 hrs at room temperature (28°C) to observed growth. Purified isolates were preserved by inoculating onto nutrient agar slants in bijoux bottles containing 10% glycerol and subsequently stored as stock cultures in the refrigerator at 4^{o}C.

2.8 Identification and Characterization of *Pseudomonas putida* and *Staphylococcus aureus*

The bacteria isolates obtained were characterized and identified by their Cellular Morphology (microscopically using cellular characteristics), and Biochemical Test (Gram Staining, Catalase Test, Citrate Utilization Test, Oxidase Test, Methyl Red and Voges Proskauer (MR-VP) Test, Indole Test, Growth in 6.5% NaCl Broth, Growth in 7.5% NaCl Broth, NaCl test, Sugar Fermentation test, Triple Sugar Iron Agar test and Coagulase test) was done according to the method by Fawole and Oso, [23].

2.9 Experimental Procedure for Bioremediation

In carrying this out, 250 mL of stock Mineral Salt Medium (MSM) broth was prepared as described earlier. Either of isolated *Pseudomonas putida* or *Staphylococcus aureus* kept as stock in the refrigerator was then inoculated into individual broth and incubated at 37°C for 8 hours after sterilization. Thereafter, the broths were introduced collectively into the filtered sterilized spent engine oil contaminated soil samples and mixed thoroughly. A control sample was set aside containing only the contaminated soils. The soil samples were then monitored for parameters, which included pH, total bacterial count, percentage moisture content and percentage organic matter for 49 days at 7 days (weekly) interval.

2.10 Monitoring of Parameters

pH: Changes in pH of the control and sterilized spent engine oil contaminated soil was monitored over the course of the bioremediation period. In doing this, the method of Ekundayo and Osunla (2016) was adapted. Briefly: known gram of the soil samples were taken and distilled water introduced into them. The mixtures were then shaken vigorously to obtain homogenized solution. The pH electrode was standardized using buffer solutions of pH 4, 7 and 9 after which pH readings were taken on the pH meter scale by dipping the glass electrode into the soil solution.

Total Bacterial Count: This was determined quantitatively by taking 1 g each of the control and bio-remediated soil samples and then making serial dilutions up to 10-5. Sterile molten nutrient agar was then poured aseptically into each Petri dish and the plates swirled gently to mix the inoculum and agar properly. On solidification the plates were incubated upside down at 37 ^{0}C for 24 hours. The numbers of colonies on each plate was then counted with the aid of colony counter and expressed as CFU/mL (Ekundayo and Osunla 2016).

Percentage Moisture Content: This was carried out with the aid of a Moisture Analyzer (RADWAG PCM 50/1 402496). Each of the soil sample (control and bio-remediated) was weighed into a pre-weighed aluminum dry-dish used for the analyzer. On completion, the percentage moisture content of the soil sample was displayed on the screen of the machine and the result recorded (Okerentugba and Ezuronye, 2003).

Percentage Organic Matter: This was achieved by determining the mass of an empty, clean and dry porcelain dish and recorded. Thereafter, the entire oven-dried test specimen from the moisture content experiment was placed in the porcelain dish, and the mass of the dish and soil specimen was determined and recorded. The dish was then placed in a muffle furnace and the temperature in the furnace gradually increased to 440 0C and left overnight. The porcelain dish was then carefully removed using tongs and allowed to cool at room temperature after which the mass of the dish containing the ash was determined and recorded. The dish was then emptied and cleaned (AOAC 2005).

2.11 Statistical Analysis

Each set of the experimental data was collected in duplicates and the analytical results were taken as the mean of the duplicate measurements. The standard deviations and statistical significance (5% level of significance) was determined with IBM SPSS Statistic 20 using (analysis of variance) ANOVA (One-way ANOVA).

3. RESULTS

The bioremediation activities of *Pseudomonas putida* and *Staphylococcus auerus* on soil contaminated with spent motor engine oil was studied and the results are here presented. Table 1 shows the physicochemical properties of test soil before bioremediation. The soil texture was clay-loamy, while the bulk density was 1.41±0.00 and particle size distribution between silt (3.35±0.01%) and sand (73.30± 0.00%). It was observed that the soil samples before bioremediation have a pH mean value of 6.52±0.00, indicating that the soil was slightly acidic. Electrical conductivity of the soil was 33.25±0.00 (µs/cm) slightly high probably because of the presence of exchangeable cations (Ca^{2+}, Mn^{2+}, K^{+}, Na^{2+}, and Mg^{2+}). Assay for metals indicated the presence of Cd, Pb, Mg, K, Cu, Zn, and Fe, with mean values of 0.02±0.00, 0.4±0.00, 0.72±0.00, 0.62±0.00, 1.23±0.01, 2.10±0.00 and 11.04±0.00 respectively. Organic matter and carbon were slightly high with mean values of 25% and 74% respectively.

3.1 Bacteria Count of Soil Samples during Bioremediation

The weekly analyses of bacteria count during the bioremediation process are shown in Table 3. The result shows that total bacteria count increased from 1st week of soil remediation to the 5th week and decreased in 6th and 7th weeks. This is in contrast to bacteria count in the control soil sample where the mean count decreased from 1st week to the 3rd week. After the 3rd week, bacteria count in the control soil sample rose to 65.00±8.70 X 10^5CFU/mL and later reversed to decline to 33.25±10.6 X 10^5CFU/Ml in the 7th week of the bioremediation process. In the first week of the bioremediation, there was no significant difference (P = 0.011) between control soil bacteria count and bio-remediated soil. There were significant differences (P<0.05) between control soil bacteria count and bio-remediated soil bacteria count in 2nd, 3rd, 4th, 5th and 6th weeks of bioremediation. In the 7th week of experiment, there was no statistical difference between bacteria count for control and bio-remediated soil samples.

Table 1. Physicochemical properties of soil sample before bioremediation.

Soil parameters(units)	Values of physicochemical properties
Particle size:	
Silt (%)	3.35±0.01
Sand (%)	73.30± 0.00
Soil texture	Clay-loamy
Bulk density (g/cm^2)	1.41±0.00
Soil pH	6.52±0.00
Total organic carbon (%)	25±0.00
Total organic matter (%)	74±0.00
NO_3^{2-} (mg/kg)	19.0±0.13
PO_3^{2-}(mg/kg)	26.50±0.07
Exchangeable cations	
Ca^{2+}(mg/kg)	9.16±0.74
Mn^{2+}(mg/kg)	7.25±0.21
K^{+}(mg/kg)	2.14±0.01
Na^{2+}(mg/kg)	6.76±0.12
Mg^{2+} (mg/kg)	6.5±0.00
Electrical conductivity (µs/cm)	33.25±0.00
Metals	
Cd (mg/kg)	0.02±0.00
Pb (mg/kg)	0.4±0.00
Mg (mg/kg)	0.72±0.00
K (mg/L)	0.62±0.00
Cu (mg/kg)	1.23±0.01
Zn (mg/kg)	2.10±0.00
Fe (mg/kg)	11.04±0.00

Values are means of two replicate reading and standard deviation of soil parameters

Table 2. Cellular morphology and Biochemical characteristics used for identification of *Pseudomonas putida* and *Staphylococcus aerus*

Bacteria isolates	Cellular characteristics		Biochemical characteristics											Tentative identification
	Gram's Reaction	Cell shape	Catalase test	Citrate test	Oxidase test	Indole test	Growth in 7.5% NaCl	Growth in 6.5% NaCl	Coagulase test	Methyl red test	Voges Proskauer test	Triple sugar iron test	Sugar fermentation test	
												H_2S	Mannitol	
B1	-	Rod	+	+	+	-	-	-	-	-	+	-	+	Enterobacter sp.
B2	-	Rod	+	+	+	+	-	-	-	ND	ND	+	+	Pseudomonas sp.
B3	+	Cocci	+	+	-	-	+	+	+	+	+	-	+	Staphylococcus

Key: B1-B3, = bacteria isolates, ND = Not determined. + = positive, - = Negative

Table 3. Total bacteria count of soil samples during of bioremediation

Soil samples	Duration of bioremediation (weeks)/Total bacteria count (10^5CFU/mL)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Control	14.60±6.26[a]	12.00±4.77[a]	7.50±2.13 [a]	65.00±8.70[a]	36.67±9.75[a]	34.33± 21.22[a]	33.25±10.6[a]
Bioremediated	9.01±2.87[a]	45.30±11.2[b]	107.60±45.34[b]	167.00±11.23[b]	101.5±32.21[b]	93.60± 23.21[b]	35.40±30.81[a]

Values are means of duplicate reading and standard deviation of bacteria counts from the bio-remediated and control soil samples. Values in the same column with different superscript are significantly different at P <0.05.

Table 4. pH, percentage organic matter and moisture content of soil samples during bioremediation

Duration	pH		% organic matter		% moisture content	
(week)	**Control**	**Bioremediated**	**Control**	**Bioremediated**	**Control**	**Bioremediated**
1	6.25±1.01[a]	7.18±0.09[b]	93.20±0.14 [b]	57.38±0.02 [a]	2.65±0.38 [a]	6.72±0.39 [b]
2	6.60±0.21 [a]	6.42±0.34 [a]	28.67±0.83 [a]	32.65±1.23 [b]	2.52±0.08 [a]	3.23±0.18 [b]
3	6.20±0.32 [a]	6.38±0.01[a]	32.26±1.05 [a]	34.23±0.31[b]	3.72±0.05 [b]	1.76±0.02 [a]
4	6.31±0.00 [a]	5.67±0.06 [b]	73.64±0.83 [a]	74.34±0.06 [a]	4.69±0.51 [b]	3.67±0.02 [a]
5	6.21±0.21 [a]	5.83±0.53 [b]	68.77±0.42 [a]	69.87±0.21[a]	4.73±0.63 [b]	3.59±0.11 [a]
6	8.87±0.16 [a]	8.86±0.00 [a]	23.80±0.12 [a]	26.43±0.32 [b]	5.67±0.04 [b]	4.43±0.41 [a]
7	8.10±0.13 [b]	7.55±0.07 [a]	25.23±0.36 [a]	31.56±1.67 [b]	2.53±0.06 [a]	6.43±0.25 [b]

Values are means of duplicate reading and standard deviation of physicochemical parameters determined after bioremediation. Values along the same row for each parameter at different weeks with different superscript are significantly different at P <0.05.

3.2 Percentage Moisture, Organic Matter Content and pH of Soil Samples during Bioremediation

The pH, percentage organic matter and moisture content of soil samples during bioremediation are presented in Table 4. From the table the pH of the bio-remediated soil decreased from the 1st week of the experiment to the 5th week. It went up to average of 8.86 in the 6th week and decreased again to average of 7.55±0.05, whereas the pH of the control soil samples remained similar from 1st week through to 5th week of bioremediation. In the 6th and 7th weeks of bioremediation the control pH increased to an average of 8.87±0.16 and 8.10±0.13 respectively. There was significant difference between pH of control soil samples and bio-remediated soil in the 1st, 4th, 5th and 7th week (P<0.05).

Percentage organic matter content of control soil samples also differed significantly with that of bio-remediated soil samples in the 1st, 2nd, 3rd , 6th and 7th

weeks of the experiment while the organic matter content of both samples did not show any significant difference in the 4th and 5th weeks of sampling. The organic matter content was highest in the control soil sample at the beginning of the experiment whereas that of the test soil was highest at the 4th week of bioremediation. Moisture content of both samples showed varied average values with time. At the 1st to 2nd weeks of experiment, the moisture content of the control soil samples remained similar whereas the moisture content of bio-remediated soil decreased from an average of 6.72 ±0.39 to 1.76±0.02% in the 3rd week. After the 3rd week the moisture content of bio-remediated soil started increasing and reached an average of 6.43±0.25% in the 7th week of bioremediation. There were significant differences (P<0.05) between the moisture content of the control soil samples and the moisture content of bio-remediated soil in all the weeks of sampling.

Table 5. Select Physicochemical Parameters of Spent Engine Oil Contaminated Soil Sample after Bioremediation

Soil Parameters (Units)	Soil sample	
	Control	Bioremediated
pH	8.85±0.15[a]	8.15±0.08[b]
Heavy metals		
Cd (mg/kg)	ND	ND
Pb (mg/kg)	0.307±0.00 [a]	0.306±0.00 [a]
Cu (mg/kg)	1.066±0.00 [a]	1.067±0.01 [a]
Zn (mg/kg)	0.104±0.00[a]	0.104±0.00 [a]
Fe (mg/kg)	9.40±0.44[b]	0.022±0.01[a]
Total organic carbon (%)	13.03±0.04[a]	13.08±0.06 [a]
Total organic matter (%)	43.83±1.12[b]	36.24±1.07[a]
NO_3^{2-} (mg/kg)	25.67±0.13[a]	25.66±0.03[a]
PO_3^{2-} (mg/kg)	13.96±0.06[a]	15.66±0.07[b]

Values are means of duplicate reading and standard deviation of physicochemical parameters determined after bioremediation. Values along the same row for each parameter at different weeks with different superscript are significantly different at P <0.05. ND = not determined.

3.3 Select Physicochemical Parameters of Spent Engine Oil Contaminated Soil Sample after Bioremediation

Table 5 is showing the select physicochemical parameters of spent engine oil contaminated soil sample after bioremediation. From the result there was a significant difference (P <0.05) between pH of control soil samples and pH of bio-remediated soil samples (Table 5). Among the metals assayed, Fe concentration in the control soil samples was higher than that of bio-remediated soil samples (P<0.05). There was no statistically significant difference between concentrations of Pb, Cu, and Zn in bio-remediated soil samples when compared with control soil samples (P<0.05). Similarly total organic carbon in bio-remediated soil samples was not significantly different from the control soil samples. Our study observed a significant difference between total organic matters in bio-remediated soil samples when compared to control soil samples. Assay for cations indicates that there was no difference between remediated soil and control for the presence of NO_3^{2-} ion, whereas there was significant difference in the occurrence of PO_3^{2-} as more of it was found in bio-remediated soil than the control. Unfortunately we could not determine the average concentration of Cd in both samples due to equipment spoilage.

4. DISCUSSION

Bioremediation of soil contaminated with spent engine oil was studied and the result was quit revealing. Our findings showed that *Pseudomonas putida* and *Staphylococcus aureus* affected or caused changes in the investigated soil parameters (pH, percentage organic carbon, percentage organic matter content, percentage moisture content, cations, and heavy metals) of spent engine oil contaminated soil. A pH of neutral scale has been shown to favour growth of many bacteria population [24,25]. Before bioremediation the soil sample was slightly acid probably as a result of the presence of hydrocarbons in the soil, which increases free cations causing the soil to have properties of a weak acid. pH also influences the solubility and accessibility of soil components which indirectly influence biological activities in the soil (Onojake and Osuji 2012, [26]). The pH recorded in this study is inline as has been previously reported by Haritash and Kaushik [27] as the optimal soil pH for efficient bioremediation, which is between 5.5 and 8.8 [25]. There was gradual decrease in the heavy metal content of bio-remediated soil when compared with the controls in line with previous report of Song et al. [28].

Other physicochemical parameters such as soil texture and bulk density are important factors for bioremediation since they help to determine soil aeration, movement of nutrients through soil pores, and water holding capacity [29]. Increased aeration will directly influence microbial growth, which can enhance the biodegradation of petroleum compounds (Luepromchai et al 2007; [30]). The bacteria count for the bio-remediated soil increased as compared to the control probably because the bacteria were able to use the carbon from the petroleum compound as a source of metabolic precursor, which enhance their reproduction and hence increase in their population

[5]. The decrease in the total organic carbon recorded for the bio-remediated soil samples compared with the control sample are an indication of effective hydrocarbon degradation as a result of increased microbial activities. Use of hydrocarbon as the sole source of carbon by hydrocarbon degraders help in the clean-up of oil components in the process [31]. Nrior and Echezolom (2017) made similar observations and concluded that the microbial count of crude oil contaminated soils during bioremediation increases within the first 20 days.

Bacteria are the most predominant group in metabolism of hydrocarbons. They possess a broad array of physiological and metabolic properties as well as a complex enzymatic system that enables them to utilize a wide range of aliphatic and aromatic compounds as their sole carbon source [30,32].

The selection of proper microbial strains is the key step to a successful bioremediation. For a pollutant to be eliminated, it is very important to select microbial inoculant isolated from contaminated sites [33].

5. CONCLUSION

In conclusion, *Pseudomonas putida* and *Staphylococcus aureus* caused changes in the investigated soil parameters (pH, percentage organic carbon, percentage organic matter content, percentage moisture content, cations, and heavy metals) of spent engine oil contaminated soil when compared with control soil. There was significant difference in bacteria count between spent engine oil contaminated soil and control soil during the course of bioremediation ($p<0.05$). pH of control soil varied significantly ($P<0.05$) from bio-remediated soil during and at end of bioremediation. Percentage organic matter content of control soil also differed significantly from bio-remediated soil in while the organic matter content of both samples did not show any significant difference ($P<0.05$). There was no statistically significant difference between concentrations of Pb, Cu, and Zn in bio-remediated soil when compared with control soil. Similarly total organic carbon in bio-remediated soil was not significantly different from the control soil. Therefore microbial degradation of petroleum hydrocarbons can be considered as a key component in the cleanup strategy for the petroleum hydrocarbon remediation. Bioremediation using *Pseudomonas putida* and *Staphylococcus aureus* on spent motor engine oil contaminated soil can improve the soil status. Therefore, based on the present research, bioremediation using *Pseudomonas putida* and *Staphylococcus aureus* should be regarded as a key component in the cleanup strategy for oil spill dispersants pollution.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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