INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

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OIL DEGRADATION ASSESSMENT OF BACTERIA ISOLATED FROM USED MOTOR OIL CONTAMINATED SOILS IN OTA, NIGERIA

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ABSTRACT
The discharge of used engine oil from vehicles by motor mechanics is a major source of oil pollution in this studied community. This study intended to isolate and assess bacteria capable of effectively degrading and cleaning up used engine oil in this locality and also to ascertain the influence of temperature and nutrient concentrations on the rates of engine oil degradation by these bacterial isolates. Oil-contaminated soil samples were randomly collected from six auto-mechanic workshops at depths 0-15 and 15-45cm. At each mechanic workshop, pristine soil samples were collected at about 100m away from each workshop to serve as reference points. Samples were analyzed microbiologically and chemically following standard microbiological and chemical methods. The ability of the culture isolates to degrade engine oil was tested both as pure culture as well as mixed bacterial consortium at different temperatures and nutrient concentrations. The population of oil degrading bacteria was found to be higher in contaminated soils as compared to non-contaminated soils. Engine oil degraders isolated were *Pseudomonas aeruginosa, Alcaligenes faecalis, Bacillus* sp. and *Serratia* sp. Of the pure culture isolates, *Pseudomonas aeruginosa* was observed to degrade the highest amount of engine oil (55.11%) over a period of 30 days at 32^{o}C. However, the mixed bacterial consortium of the isolates proved to be more effective, degrading 58.23% of engine oil within 30 days under the same conditions. All the isolates showed optimum degradation potential at 32°C except *Serratia* sp. which was optimum at 28^{o}C and its degradation rate increased with increase in nutrient concentration. This study revealed the presence of bacteria capable of metabolizing engine oil in the studied environment. The biodegradative ability of these bacteria could be effectively enhanced by supplementation with appropriate concentration of sources of essential nutrients such as nitrate, phosphate and sulphate.

KEYWORDS: Environmental pollution, engine oil, heterotrophic bacteria, oil-degrading bacteria, physico-chemical factors, bioremediation.

INTRODUCTION

Environmental pollution with petroleum and petroleum products has been recognized as one of the most serious current problems especially when associated with accidental spills on large-scale. The presence of different substrates and metabolites in hydrocarbon contaminated soils has no doubt provided an environment for the development of a quite complicated microbial community (Udeani *et al*., 2008; Butler and Mason, 1997). The discharge of used engine oil from automobiles, industrial machines, plants and electric power generators are the main sources of oil pollution in Ota, a western Nigeria. Oil released into the environment affects many plants, animals, microorganisms and humans within the oil impacted environment. Additionally, prolonged exposure to oil as well as high concentration of oil could cause the development of liver or kidney disease, possible damage to the bone marrow and an increased risk of cancer (Igwo-Ezikpe *et al*., 2009; Lloyd and Cackette, 2001; Mishra *et al*., 2001; Deni, and Penninck, 1999). Engine (motor) oils are lubricants for various internal combustion engines. In addition to lubricating moving parts, engine oil also cleans, inhibits corrosion, improves sealing and cools the engine by carrying heat away from moving parts (Nwoko *et al*., 2007). Notwithstanding these important functions, engine oils also impact our environment negatively. While new engine oils contain more volatile and water soluble hydrocarbons that would be more of acute toxicity to organisms, used engine oils contain metals and heavy polycyclic aromatic hydrocarbons (PAHs) that could contribute to chronic hazards such as mutagenicity and carcinogenicity (Mandri and Lin, 2007; Boonchan *et al*., 2000). The use of physico-chemical methods for pollution control have often worsened the problem rather than reducing or eliminating it. There has been an intensified effort to search for effective and efficient methods for pollutant removal from contaminated sites in recent years. A promising approach being researched so far is the bioremediation method since the physico-chemical methods to remove hydrocarbon pollutant is rather too expensive and does not give a total elimination of the pollutant (Walker and Crawford, 1997; Bushnell and Haas, 1941). The economic viability and environmental soundness of bioremediation makes it an attractive method for the remediation of oil-contaminated environments. It is simply the improvement of live organisms such as fungi, bacteria and plant to detoxify hydrocarbon and organic contaminants. As a cheaper and effective method, the use of microbes in pollution abatement is now receiving an increasing attention (Clementina and Omoanghe, 2008;

Daane *et al*., 2001; Dinkla *et al*., 2001; Lalithakumari, 2001; Deni, and Penninck, 1999).

According to Harder (2004), bioremediation accounted for 5 to 10 percent of all pollution treatment and has been used successfully to clean up used engine oil illegally dumped. Upon this background, we isolated engine oil degrading bacteria and assessed their potentials for engine oil degradation.

MATERIALS & METHODS

Study Site

The study was carried out in Ota, western Nigeria. Indiscriminate disposal of used engine oil is the major source of oil pollution in this locality as transportation is one of the main sources of income. Six mechanic workshops (about 4 km apart) contaminated with used engine oil were randomly selected for this study.

Sample collection

Oil contaminated soils were collected around six randomly selected mechanic workshops. Soil samples were also collected from non-contaminated reference areas at about 100 m from the contaminated sites. At each sampling point, four soil samples were collected; two samples each from depths 0 – 15 and 15 – 45 cm respectively using a sterile cutlass and a hand auger. Samples were immediately taken to the laboratory for analysis.

Physico–chemical Analysis

The mean temperatures of the samples were determined using a mercury thermometer while the pH of the samples were determined using portable pH meter with combined glass and calomel electrodes as previously reported (Nwachukwu, 2000). Phosphate, sulphate and nitrate concentrations were determined spectrophotometrically using the method of APHA (1998). The moisture content of the soil samples was determined using moisture analyzer. The residual engine oil was extracted from the soil sample using n-hexane: dichloromethane system (1:1) and quantified gravimetrically (Le Dreau *et al*., 1997). To achieve this, 10gm of homogenized soil sample was weighed into a 75ml beaker and 50ml of n-hexane: dichloromethane was added to extract the residual engine oil in the soil sample. After shaking vigorously, the mixture was allowed to stand for 5 minutes and then filtered through whatman No1 filter paper into 75ml beaker of known weight (W_1) as residual oil extract (ROE). The residual oil extract was placed in an oven at 80^0C for 5-10 minute to evaporate the solvent system (Nwachukwu, 2000). The combined weight of the residual oil and the beaker was taken and recorded as W_2. The residual oil content (ROC) was then obtained by difference in mass ($W_2 - W_1 = ROC$).

Determination of bacterial counts:

Heterotrophic bacterial counts were determined by plating serially diluted samples on nutrient agar. Oil degrading bacteria were enumerated on minimal salt agar using sterile motor oil as carbon and energy source as previously reported (Amund *et al*., 1994).

Isolation of oil degrading bacteria and determination of their oil degrading potential:

Oil degrading bacteria were isolated from samples by the enrichment culture technique using sterile motor oil as carbon and energy source (Amund *et al*., 1987). To do this, 1.0gm of thoroughly mixed soil sample was inoculated into sterile minimal salt broth containing 10% V/V sterile motor oil as the sole carbon and energy source (enriched culture medium) and incubated at 28°C for 7 days. Two millilitres (2ml) of the enriched culture was aseptically transferred into a fresh enrichment culture medium and incubated for another 7 days. The same process was repeated for the third enrichment culture medium. Bacterial isolates were obtained by plating 0.1ml from the third enrichment culture onto nutrient agar. Colonies were subcultured severally on the basis of their colonial characteristics to obtain pure culture isolates (Nwachukwu and Akpata, 2003). The pure culture isolates were identified following the method in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

To determine the oil-degrading potential of the pure and mixed culture isolates, 20 gm portion of steam sterilized soil containing 4 ml of sterile engine oil were set up. Each batch of four 20gm portions was inoculated with a standard suspension (approximately 2.01×10^5cfu/ml) of each pure isolate or a mixture of the pure isolates. Non-culture inoculated sample served as control. The set-ups were incubated at 28°C for 30 days. Sub-samples (2.0 gm each) were withdrawn at day zero and at 5 day intervals for analysis. Oil-weight loss following bacterial degradation was assessed by gravimetric method after extraction with n-hexane: dichloromethane (1:1). Values obtained were expressed as percentages of the amount of oil in the sample at day zero (Akoachere *et al*., 2008).

Influence of temperature and nutrient concentration on oil degradation:

The experiment was set up in the same way as in the determination of oil degrading potential of isolates above, but different samples were incubated at various temperatures of 26^oC, 28^oC, 32^oC and 37^oC. The percentage of oil degraded at 5 days interval over a period of 30 days was calculated. To determine the effect of nutrient concentration, 25ppm nitrate/12.5ppm phosphate/25ppm sulphate, 40ppm nitrate/20ppm phosphate/40ppm sulphate, 50ppm nitrate/25ppm phosphate/ 50ppm sulphate, and 60ppm nitrate/30ppm phosphate/60ppm sulphate were incorporated into soils containing sterile engine oil and then seeded with standard suspension of isolates. The amount of oil degraded (%) at 5 days interval over a period of 30 days was calculated (Akoachere *et al*., 2008).

Statistical analysis

All data collected were analysed using the SPSS 17.0 statistics and Microsoft Excel 2010.

RESULTS & DISCUSSION

The physico-chemical parameters of the contaminated and non-contaminated soil samples analysed are presented in Table 1. As shown in Table 1, all pristine samples collected have zero residual oil content, confirming that the pristine samples were not polluted with oil. In order to develop bioremediation strategy applying indigenous and adapted bacteria for effective and efficient clean-up of used engine oil indiscriminately dumped in our environments, we isolated bacteria capable of engine oil degradation from soil samples collected from mechanic workshops in Ota and assessed their engine oil degradative

potentials. Past studies have shown that bioaugmentation for bioremediation is best done with the use of indigenous microorganisms as compared to imported or foreign microorganisms (Onifade *et al*., 2007; Odu, 1972). This could be attributed to the fact that indigenous microorganisms are best adapted to indigenous environmental conditions (Desai and Vyas, 2006). Figure 1 depicts the distribution of heterotrophic bacteria in contaminated and non-contaminated soil samples from various sampling points. The relatively low heterotrophic bacterial counts (Figure 1) recorded in this study for most oil-contaminated soils compared to that of non-contaminated soils agreed with the previous report by Jensen (1975), and could be attributed to the toxic or unfavorable effect of oil contamination. In Figure 2, engine oil degrading bacteria were found to be higher in oil-contaminated soils than the non-contaminated soils. This finding corroborates the reports of Hubert *et al.* (1999) and Michalcewicz (1995). These higher populations of oil degrading bacteria recorded could be due to the stimulatory effect of additional carbon and energy source in the form of lubricating oil. The high oil degraders observed at depth 0 – 15cm (Figure 2) is an indicative of the fact that most engine oil degrading bacteria are aerobic and this is in agreement with previous works by Akaochere *et al.* (2008), Malatova (2005) and Walker and Crawford (1997). However the presence of anaerobic hydrocarbon utilizers in the soil have been reported (Desai and Vyas, 2006).

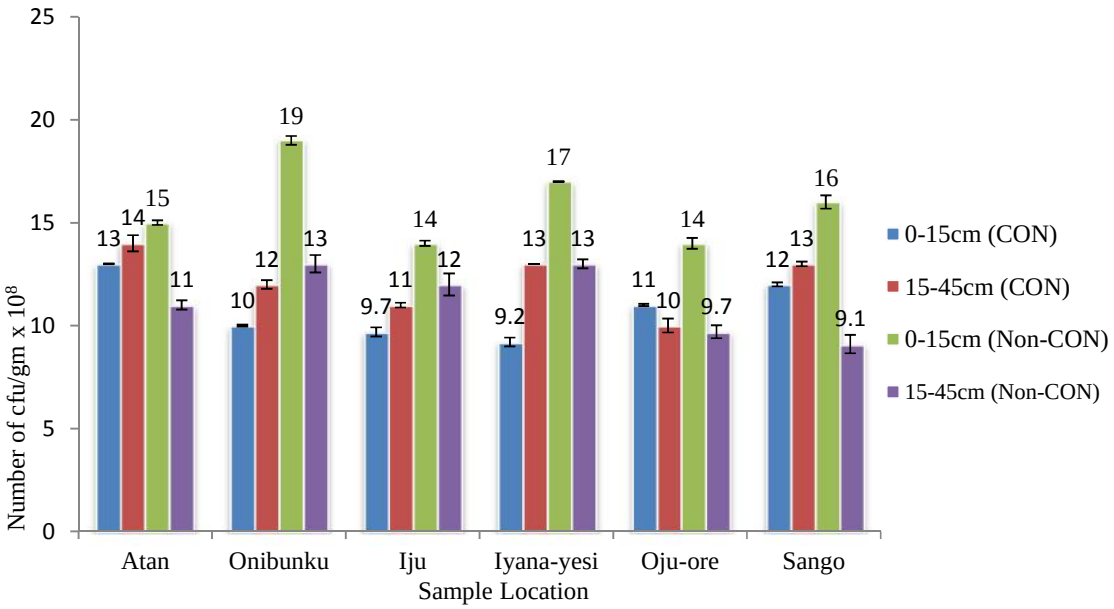


FIGURE 1: Distribution of heterotrophic bacteria in contaminated (CON) and non-contaminated (Non-CON) soils around some mechanic workshops in Ota.

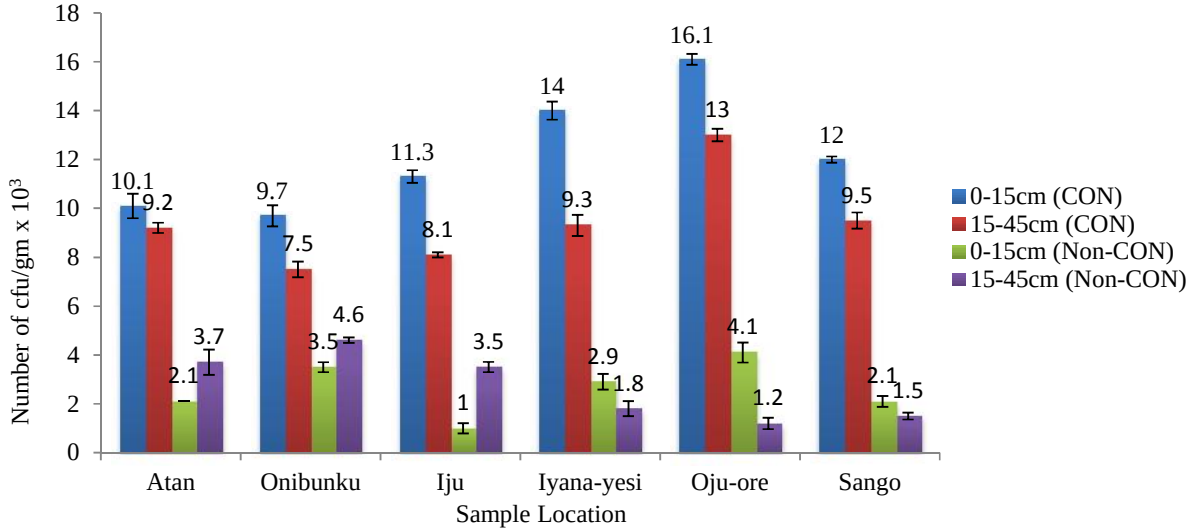
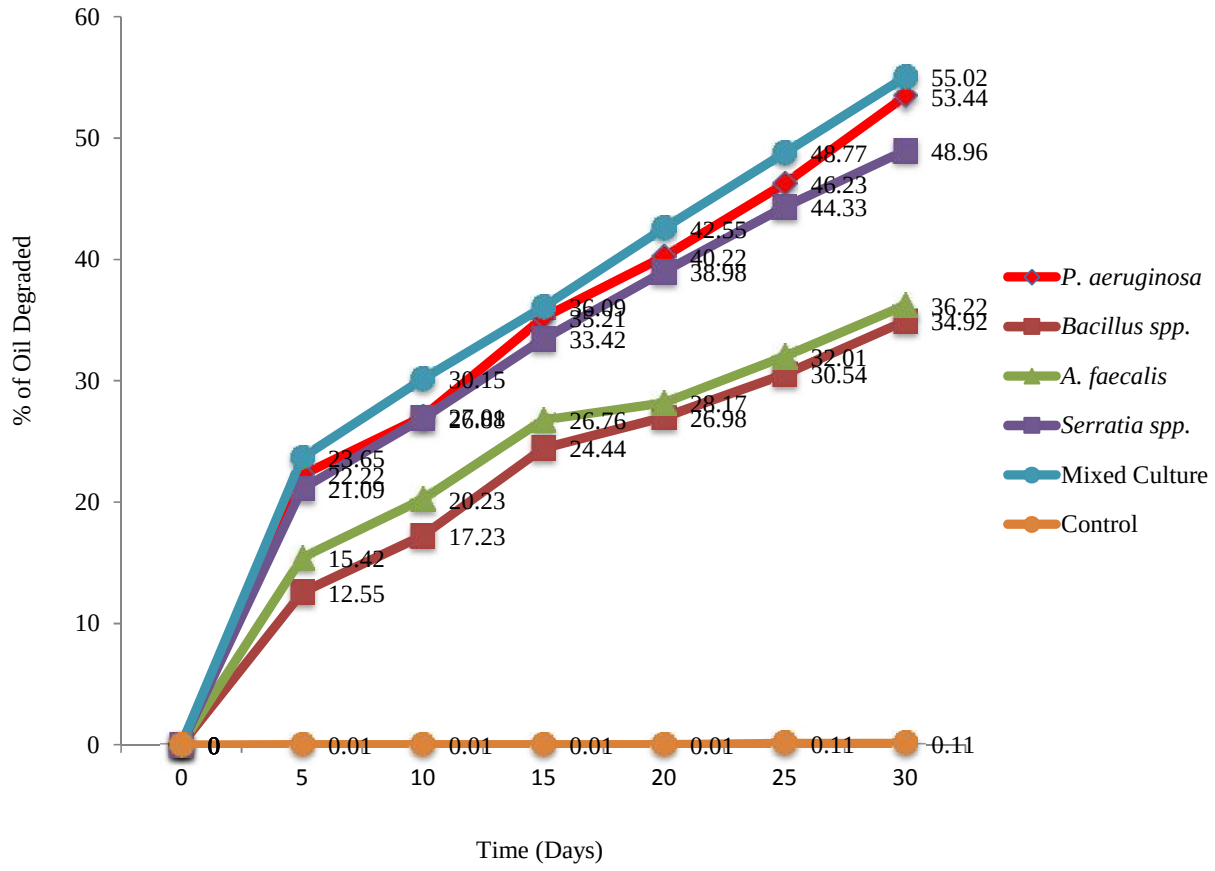


FIGURE 2: Distribution of engine oil degrading bacteria in contaminated (CON) and non-contaminated (Non-CON) soils around some mechanic workshops in Ota.

Oil-degrading bacteria isolated from the soil samples analysed were *Pseudomonas aeruginosa, Bacillus* sp., *Alcaligenes faecalis and Serratia* sp. This implies that the soils have the ability of undergoing self-purification in the case of minor oil contamination. Microorganisms capable of hydrocarbon utilization are widely distributed in nature and have been found in areas not directly contaminated with hydrocarbons (Atlas, 1981) as evidenced in this study. Of the pure isolates, *Pseudomonas aeruginosa* degraded the highest amount of engine oil (53.44%) within a period of 30 days, while *Bacillus* sp. degraded the least amount of engine oil (34.92%) under the same conditions (Figure 3). The mixed bacterial culture, however, proved to be the best degrader of engine oil under all conditions tested (55.02, 58.23 and 75.33% respectively for Figures 3, 4 and 5).This is in accordance with other reports (Facundo *et al*., 2001; Kulwadee *et al*., 2001; Amund *et al*., 1994; Obire, 1988) that confirmed microbial consortia as better degraders than pure isolates. In a mixed culture, some species utilize intermediates of degradation of the original hydrocarbon produced by other members of the culture leading to a complete degradation of the oil (Facundo *et al*., 2001; Atlas, 1981).

FIGURE 3: Thirty days monitoring of oil degradation by pure and mixed bacterial isolates.



The influence of environmental factors has been reported to limit the degradation of pollutants by microorganism (Barthen and Atlas, 1977). The degrading potentials of the isolated engine oil degrading bacteria were observed to be optimum at temperatures of 28^{o}C and 32^{o}C (Figure 4), which is in agreement with the report by Desai and Vyas (2006). But further increases in temperature showed a drop in oil degrading potential of the isolates. This may probably resulted from a gradual inactivation of the enzymes responsible for the degradation of the pollutants as earlier reported by Achuba *et al.* (2008). In Figure 5, the degradation rates of the isolates were observed to vary with nutrient concentrations. *Serratia* sp. degrading potential increases with increase in nutrient concentration, while the degrading potentials of other bacterial isolates relatively alternated with increase in nutrient concentrations. However, the application of nutrients generally enhanced the degradative ability of the isolates tested.

ISSN 2250 – 3579

TABLE 1: Physico-chemical Parameters of Contaminated and Non-contaminated Soil Samples

Sample Location	Depth (cm)	Moisture Content (%)		Residual Oil Content (mg/kg)		NO_3^- Concentration (%)		PO_4^{-3} Concentration (ppm)		SO_4^{-2} Concentration (ppm)	
		Cont.	Non-Cont.	Cont.	Non-Cont.	Cont.	Non-Cont.	Cont.	Non-Cont.	Cont.	Non-Cont.
Atan	0-15	5.10±0.3	3.70±0.4	10.10±0.2	0	2.03±2.01	2.04±1.83	4.42±1.02	4.14±1.10	274.48±1.65	276.88±1.30
	15-45	7.25±0.7	4.45±0.3	2.02±0.5	0	2.04±1.90	2.03±1.75	4.06±1.05	4.15±1.07	179.36±1.80	215.12±1.64
Onibunku	0-15	5.45±0.5	6.85±0.4	5.21±0.3	0	2.04±2.00	2.04±1.96	4.18±1.14	4.06±1.04	281.20±1.52	182.40±1.73
	15-45	7.25±0.8	5.60±0.6	5.03±0.6	0	2.04±1.87	2.04±1.64	4.04±1.25	4.04±1.13	260.72±2.01	218.96±1.90
Iju	0-15	2.75±0.3	8.65±0.2	9.01±0.7	0	2.04±1.74	2.04±1.89	4.03±1.09	4.67±1.15	188.88±1.58	189.84±1.49
	15-45	5.35±0.2	7.90±0.7	3.22±0.4	0	2.03±1.82	2.04±1.65	4.03±1.11	4.10±1.08	274.40±1.48	203.12±1.75
Iyana-yesi	0-15	6.01±0.5	4.85±0.4	9.11±0.5	0	2.04±1.96	2.04±1.35	4.18±1.03	4.07±1.19	180.72±2.00	198.24±1.43
	15-45	8.25±0.6	10.95±0.8	3.01±0.3	0	2.03±1.68	2.04±1.57	4.04±1.10	3.99±1.02	251.12±1.39	199.12±1.94
Oju-ore	0-15	5.20±0.3	11.15±0.5	4.21±0.5	0	2.04±1.60	2.04±1.49	4.10±1.18	4.19±1.04	206.64±1.73	197.92±2.01
	15-45	8.70±0.9	12.15±0.7	2.12±0.4	0	2.03±1.53	2.04±1.72	4.01±1.21	4.20±1.06	267.60±1.29	193.04±1.98
Sango	0-15	4.05±0.4	2.15±0.3	6.03±0.7	0	2.04±1.92	2.04±1.55	4.00±1.30	4.02±1.17	263.52±1.64	174.24±2.00
	15-45	5.55±0.2	5.25±0.5	4.14±0.5	0	2.03±1.75	2.04±1.48	3.98±2.01	4.02±1.12	252.96±1.29	194.08±1.91

Key
Cont. = Contaminated
Non-Cont. = Non-Contaminated

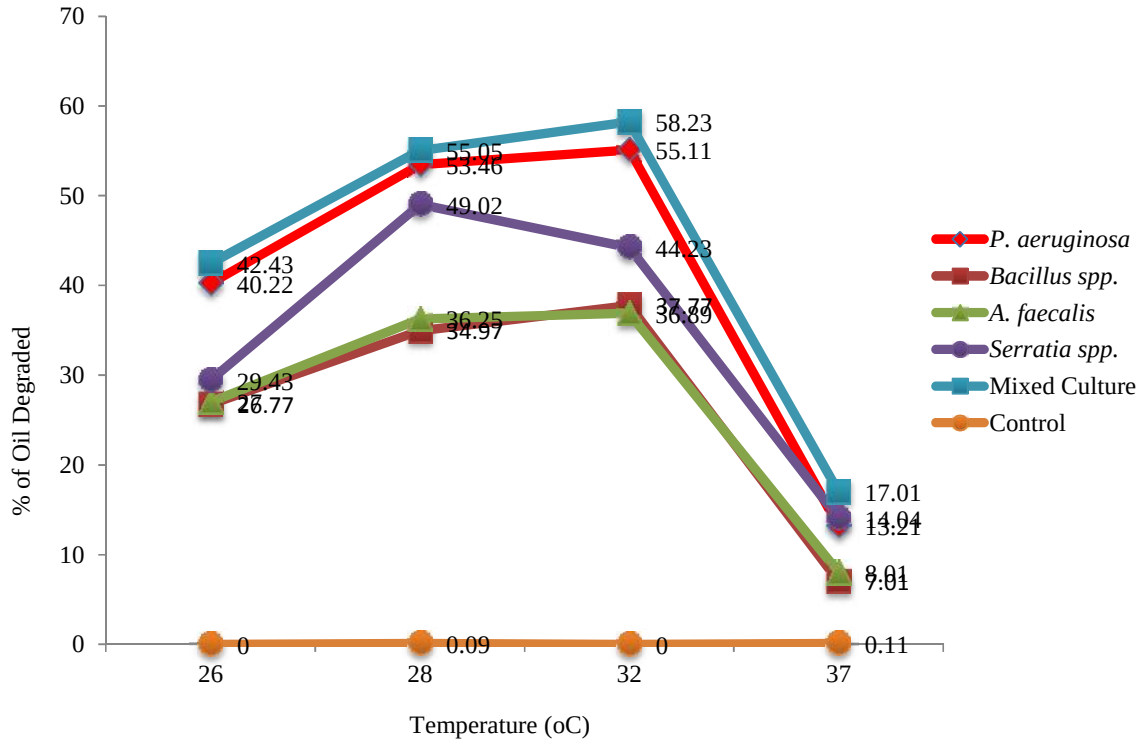


FIGURE 4: The influence of temperature on the degradation patterns of bacterial isolates after 30 days of monitoring.

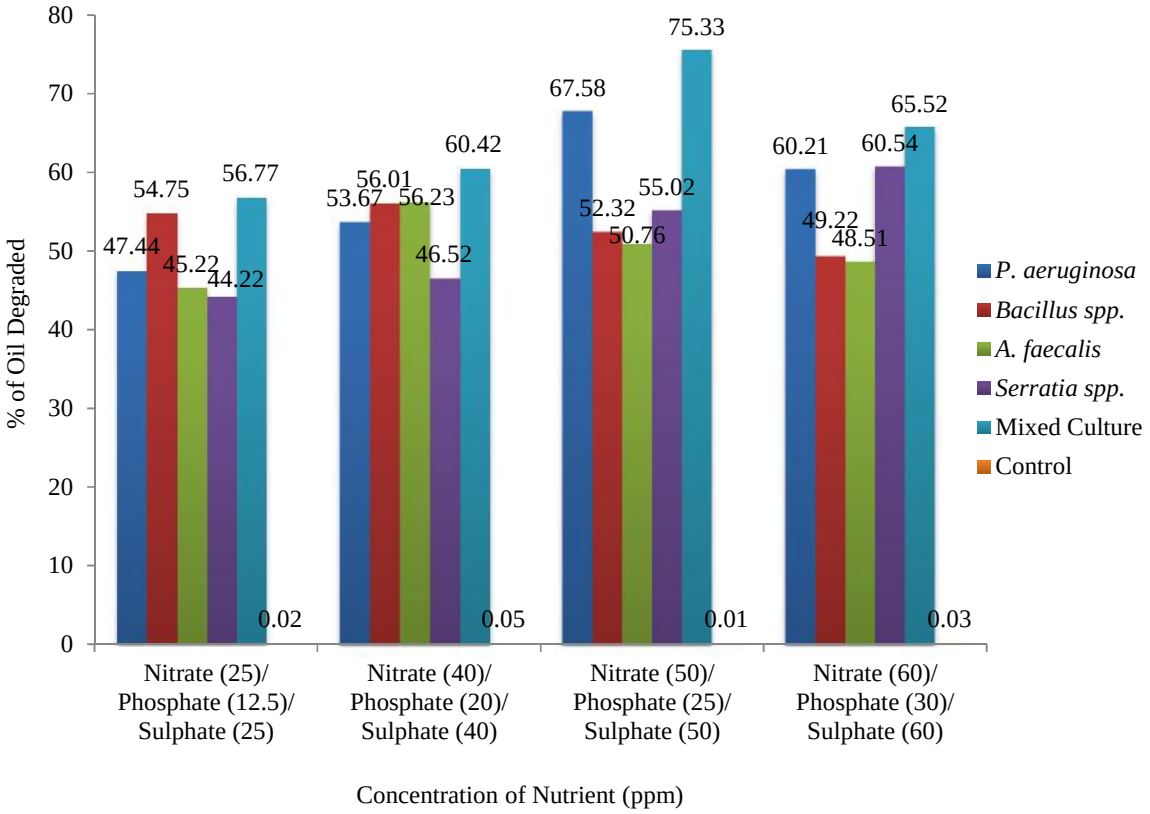


FIGURE 5: The influence of nitrate, phosphate and sulphate concentrations on the degradation patterns of the isolates after 30 days of monitoring.

CONCLUSION

We observed the presence of bacteria capable of metabolizing engine oil in the studied environments. This indicates that the studied environments have the ability to undergo natural attenuation and clean-up of engine oil over time in the case of minor oil pollution or pollution with similar contaminants. This is however subject to subsequent discontinued introduction of these pollutants into the same environment. Additionally, application of appropriate concentrations of nutrient sources such as nitrate, phosphate and sulphate could accelerate biodegradation of engine oil pollutant in soil. Also the bacterial isolates obtained from this study could be exploited for oil spill clean-up in similar environments. However, environmental consciousness should be instilled into automobile mechanics to avoid indiscriminate disposal of used engine oil and researchers should work towards recycling of spent engine oil.

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