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Full Length Research Paper

Lubricating oil-degrading bacteria in soils from filling stations and auto- mechanic workshops in Buea, Cameroon: occurrence and characteristics of isolates

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The discharge of used crankcase oil from vehicles is a major source of oil pollution in Buea. The objectives of this study were to isolate and characterize bacteria capable of effectively degrading and cleaning up waste engine oil in this locality and also to ascertain the influence of some environmental factors on the rates of degradation of these isolates. Seventy-two soil samples collected from lubricating oil dump sites (3 auto-mechanic workshops and 3 petrol filling stations, comprising impacted soils) and uncontaminated plots (non-impacted soils) (controls) were analysed for oil-degrading and heterotrophic bacteria following standard microbiological and biochemical methods. The ability of cultures to degrade lubricating oil was also tested individually and in mixed bacterial consortium at different temperatures and nutrient concentrations. Results were analysed using the chi-squared test. P values of < 0.05 were considered significant. Heterotrophic bacterial counts were significantly higher (P < 0.05) in non-impacted than in impacted soils. Conversely, the population of oil degraders was significantly lower (P < 0.05) in non-impacted than in impacted soils. Oil degraders isolated included *Pseudomonas fluorescens*, *Bacillus mycoides* and *Serratia marcescens*. Of the pure isolates, *Serratia marcescens* degraded the highest amount of oil (36.2%). However, a mixed culture of the isolates proved to be more effective, degrading 38.1% of oil within 20 days. All the isolates exhibited highest degradation at 32°C; and degradation rates of *Pseudomonas fluorescens* and *Bacillus mycoides* increased with increase in nutrient concentration. This study, the first of its kind in Buea, revealed the presence of oil-degrading bacteria in soils as well as the physico-chemical requirements of these bacteria for optimum degradation. This finding could be exploited in case of oil-spill clean-up campaigns.

Key words: environmental pollution, oil-degrading bacteria, heterotrophic bacteria, physico-chemical factors, bioremediation, Cameroon.

INTRODUCTION

With an ever increasing world's population, there is a concomitant increase in the demand for petroleum and petroleum products, which apparently constitutes a source of environmental pollution (Raven et al., 1993). Oil pollution is a major environmental concern in many countries, and this has led to a concerted effort in studying the feasibility of using oil-degrading bacteria for bioremediation. The discharge of used engine oil from vehicles is the main source of oil pollution in the environment of Buea. The soil is habitat to many living organisms; any change in their number or form may upset or cause a total collapse of the ecosystem. The effect of oil spills on soil leads to an enrichment of the oil–degrading microbial

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population. However, a decrease in microbial population exposed to crude oil and its products have also been documented. Atlas (1981) reported that certain crude oils contain toxic components that are bacteriostatic. These inhibitory effects have also been reported to depend on concentrations (Benka-Coker, 1989). No single micro organism has been found to be able to completely degrade a petroleum hydrocarbon molecule. However, different species or strains of the same specie may be capable of degrading different groups of hydrocarbons, found in oil (Facundo et al. 2001). Different naturally occurring species of *Pseudomonas* are known to contain plasmids with the relevant genes for the degradation of different hydrocarbons (Jawetz et al., 1991).

Pollution control strategies involving physico-chemical methods have often aggravated the problem rather than eliminated it (Walker and Crawford, 1997). Biodegradation is an attractive method for the remediation of contaminated sites because of its economic viability and environmental soundness (Dinkla et al., 2001). The use of microbes therefore in pollution abatement either through natural selection or recombinant DNA technology is receiving increasing interest as this is cheap and most effective (Deni and Pennick, 1999; Daane et al., 2001; Lalithakumari, 2001) In bioremediation, the contaminated site is exposed to an 'army' of microorganisms which gobble up the poison and leave behind harmless substances such as carbondioxide and water.

In spite of the increasing number of auto-mechanic workshops in Buea with their attendant and indescrimate dumping of waste engine oil in the environment, we are not aware of any study that has attempted to isolate and characterise microorganisms capable of effectively degrading and cleaning up this contaminant, especially in a situation of major spillage. Also noting that the extent of biodegradation and the rate at which it occurs depend on the interactions between the environment, number and type of microorganisms present (Atlas, 1981), and the chemical structure of the contaminant (Walker and Crawford, 1997), the present study was therefore undertaken with a view to: isolating and characterizing oil-degrading and heterotrophic bacteria in both oil contaminated and non–contaminated soils; assess the oil degrading potentials of the isolates and determine the influence of some physico-chemical parameters on the rate of oil degradation by these microorganisms.

MATERIALS AND METHODS

Study site

The study was carried out in Buea, South West Province of Cameroon, located about 1000 km above sea level, with low temperatures (16°C) and a relatively high humidity. Although Buea is a semi-rural area with few industries and business activities, indiscriminate disposal of crankcase oil is the major source of oil pollution in this locality.

Sample collection

Oil contaminated soils were collected around various petrol filling stations and auto-mechanic workshops. Samples were also collected from non-contaminated reference areas about 100 m from contaminated sites. At each sampling point, four samples were collected at depths of 0 - 15 cm and 15 - 45 cm using a hand auger followed by bulking. Samples were immediately transported to the laboratory for analysis. Samples for physico-chemical studies were ground, sieved and air-dried in the laboratory.

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 motor oil as carbon source (Amund et al., 1987). Pure cultures of bacteria were identified based on colonial morphology and biochemical characteristics following the scheme of Koneman et al. (1992) and API 20NE. Two ml of the enriched culture was transferred into a fresh enriched culture medium and incubated. Pure cultures were obtained by plating 0.1 ml of culture from the second enrichment culture onto nutrient agar.

To determine the oil-degrading potential of the pure isolates, 15 g portion of steam-sterilized soil containing 3 ml of lubricating oil were set up. Each batch of four 15 g portions was inoculated with a standard suspension (approximately 1x 10^5 CFU/ml) of each pure isolate or a mixture of the pure isolates. Uninoculated samples served as controls. Samples were incubated at 28 $\pm$ 2°C for 20 days. Sub-samples (2.0 g each) were withdrawn at time zero and at 5 day intervals. Oil-weight loss following bacterial degradation was assessed by gravimetric method after extraction with carbon tetrachloride. Values obtained were expressed as percentages of the amount of oil in sample at time zero.

Determination of bacterial counts

Heterotrophic bacterial counts were determined by plating serially diluted samples on nutrient agar. Oil-degrading bacteria were enumerated on minimal salts agar using motor oil as carbon source as previously reported (Amund et al., 1993).

Determination of physico-chemical characteristics of samples

Nitrate concentration was determined following a previous method (Bless, 1984) using a Gallenkamp OS1 spectrophotometer. The phosphate content of soils was also determined spectrophotometrically using the method of Bless (1984). The oil content was determined by gravimetric method after extraction with carbon tetrachloride. Each experiment was repeated 4 times.

Influence of temperature and nutrient concentration on oil degradation

The experiment was set up as in the determination of the oil-degrading potential of isolates, but different samples were incubated at various temperatures of 32, 35 and 37°C respectively. The percentage of oil degraded at 5 days interval over a period of 20 days was calculated. To determine the effect of nutrient concentration, 2 ppm PO_4^{-3}/30 ppm NO^-_3, 4 ppm PO_4^{-3}/ 50 ppm NO^-_3 and 6 ppm PO_4^{-3}/ 70 ppm NO^-_3 were incorporated into soils containing lubricating oil and then seeded with standard suspension of isolates. The amount of oil degraded (%) at 5 days interval over a period of 20 days was calculated.

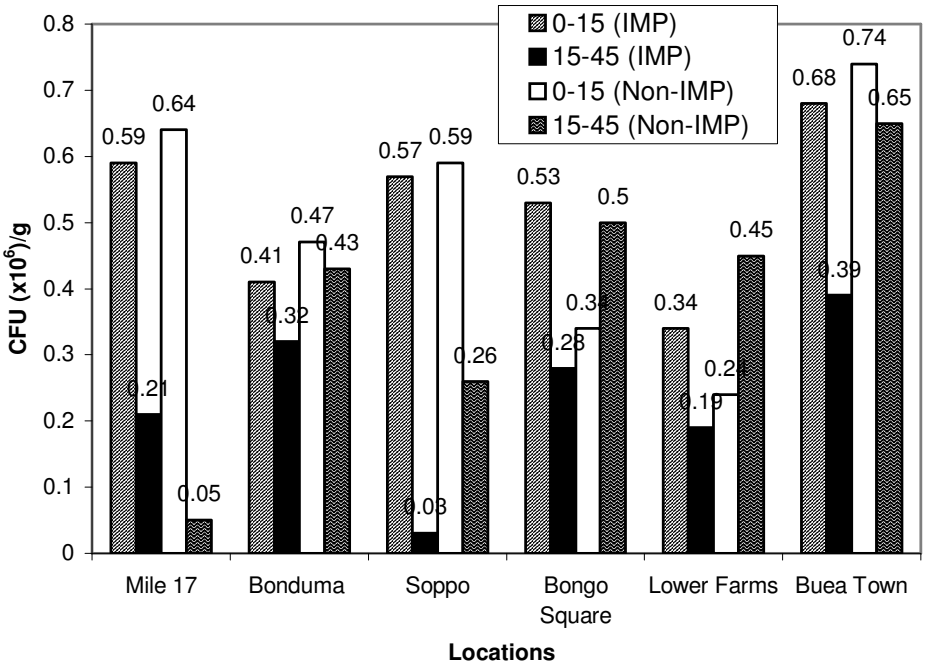

Figure 1. Distribution of heterotrophic bacteria at different locations.

Statistical analysis

The Chi - square test was employed to compare bacteria population and their ability to degrade oil. P values of < 0.05 were considered significant.

RESULTS

Microbiological characteristics of samples

Heterotrophic bacterial counts were generally higher in uncontaminated samples (5 x 10^4 CFU/g) than in contaminated soils (Figure 1). Counts of oil-degrading bacteria ranged from 6 x 10^4 to 49 x 10^4 CFU/g in contaminated samples and from 0 to 14 x 10^4 CFU/g in uncontaminated soils and this difference was significant ($p < 0.05$) (Figure 2). Generally, there was a reduction in counts with increase in depth observed for both heterotrophic bacteria and oil-degraders. Oil-degrading bacteria isolated from contaminated and uncontaminated samples included *Pseudomonas fluorescens*, *Serratia marcescens* and *Bacillus mycoides,* with *Serratia marcescens* being the most predominant at all depths (Figure 3).

The degradation pattern of isolates over a 20 day period was identical, as the amount of oil degraded increased with time (Figure 4). *S. marcescens* exhibited the highest oil-degrading potential (36.23%) than *P. fluorescens* (26.26%), and *B. mycoides* (18.36%). However, a mixed population of these bacteria exhibited the highest degrading potential (38.17%).

Physico-chemical characteristics of samples

Contaminated soils exhibited significantly lower ($P < 0.05$) moisture content which ranged from 23.7 to 56.5% compared with 32.2 to 76.7% for uncontaminated samples (Table 1). Values were observed to increase with depths. Analysis of samples for lubricating oil content revealed values ranging from 0.03 to 2.2 mg/g in contaminated soils. No oil was detected in uncontaminated samples. There were no significant differences ($P > 0.05$) in the nutrient content (NO_3^- and PO_4^{-3}) of contaminated and uncontaminated soils (Table 1).

Influence of physico-chemical parameters on degradation of lubricating oil

With the exception of *B. mycoides* which showed highest degradation (33.33%) at 35°C, all the other isolates as well as the mixed culture exhibited highest degradation at 32°C (Figure 5). The degradation potential of *S. marcescens* and the mixed culture dropped with an increase in nutrient concentration up until phosphate (4 ppm)/ nitrate (50 ppm) and thereafter increased with an increase in phosphate/nitrate concentration (Figure 6). *B. mycoides* exhibited an increase in degradation which dropped slightly with further increase in nutrient concentration. The degradation potential of *P. fluorescens* increased with increase in nutrients.

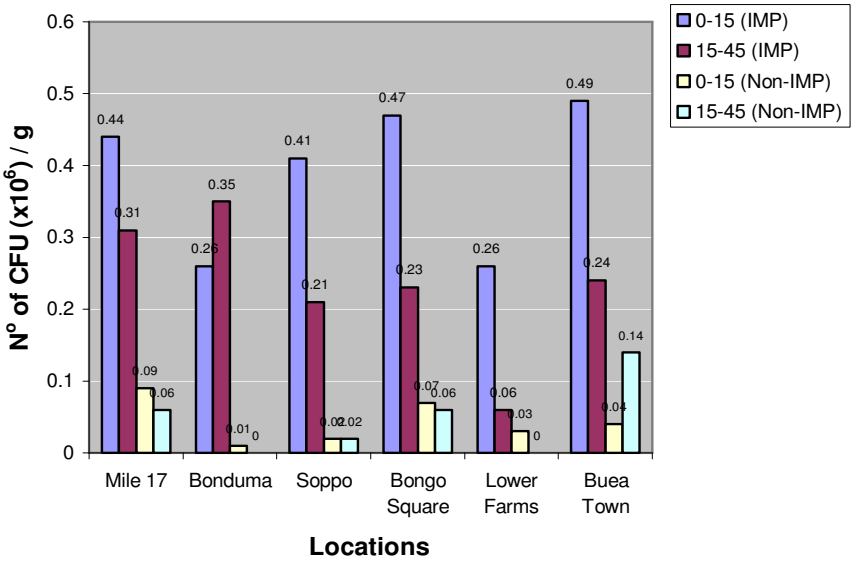

Figure 2. Distribution of hydrocarbon-utilising bacteria at various locations.

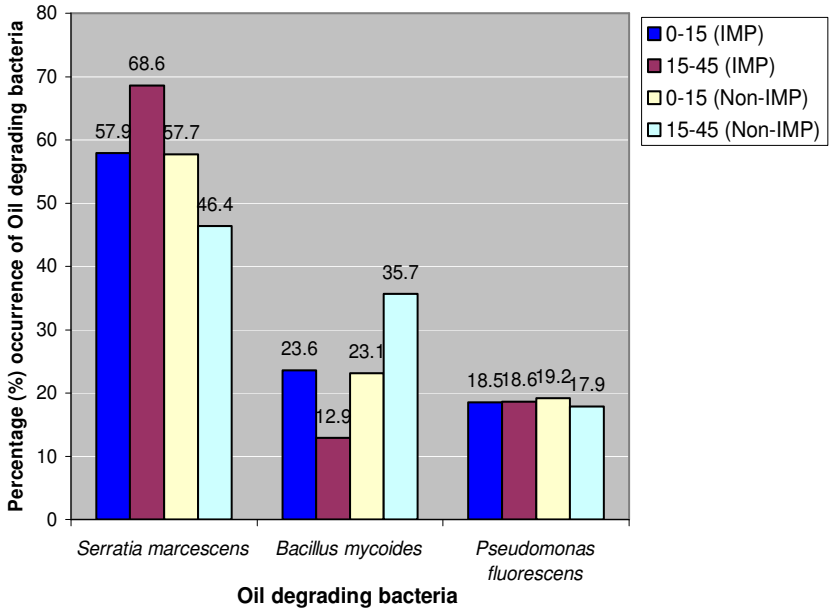

Figure 3. Percentage occurrence of oil degrading bacteria on both Oil Impacted and Non Impacted soils from various depths.

DISCUSSION

Our data show an obvious influence of waste engine oil discharge on the microbiological and physico-chemical properties of soil. The relatively low heterotrophic bacterial counts observed in oil contaminated soils can be attributed to the toxic or unfavourable effect of oil contamination (Jensen, 1975). The finding of the presence of higher oil-degrading bacterial populations in contaminated soils corroborates the results of Hubert et al. (1997) and Michalcewicz (1995) that attributed these high microbial populations to the stimulatory effect of additional carbon and energy source in the form of lubricating oil. Counts of oil-degraders were observed to decrease significantly ($P < 0.05$) with an increase in depth of sample collection. This can be attributed to a decrease in lubricating oil content with increasing depth. A decrease in substrate will therefore result in a drop in the population of oil-degraders. In addition, oil degraders are mostly aerobes (Walker and Crawford, 1997) and will

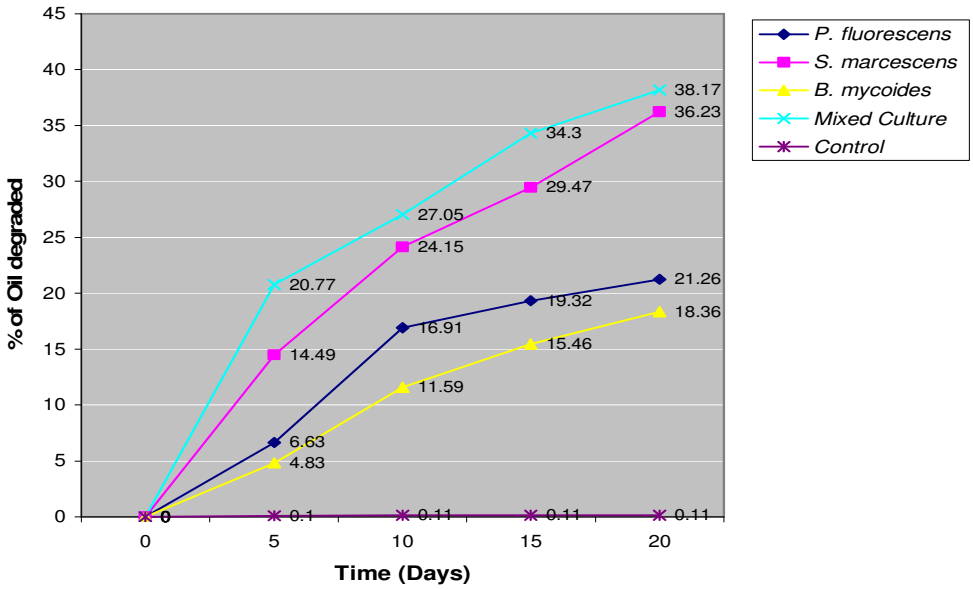

Figure 4. 20 Day monitoring of oil degradation by pure and mixed isolates.

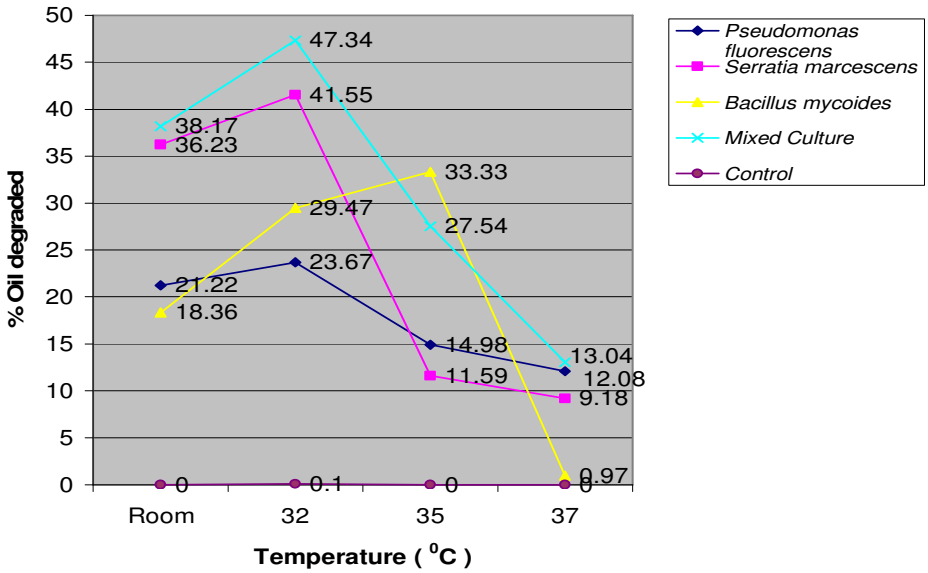

Figure 5. The influence of temperature on the degradation patterns of isolates after 20 days of monitoring.

be more abundant in surface soils than in sub surface samples. Oil-degrading bacteria isolated from both contaminated and uncontaminated soils included *P. fluorescens, S. marcescens* and *B. mycoides.* This implies that the soils have the ability of self-purification in case of major spillage. *S. marcescens was* the most predominant in all the samples. Micro-organisms capable of hydrocarbon utilization are widely distributed in nature and have been found in areas not directly contaminated with hydrocarbons (Atlas, 1981).

Of the pure isolates, *S. marcescens* degraded the highest amount of oil (36.2%) while *B. mycoides* degraded the least amount. The mixed culture, however, degraded even higher amounts of lubricating oil. Several reports (Obire, 1988; Amund and Nwakaye, 1993; Amund et al., 1993; Facundo et al., 2001; Kulwadee et al., 2001) have confirmed microbial consortia as better degraders than pure isolates. In a mixed culture, some species utilise intermediates of degradation of the original hydrocarbon produced by other members of the culture leading to a complete degradation of the oil (Atlas, 1981; Facundo et al., 2001). Thus, a mixed culture is a better innoculum for

Table 1. Physico-chemical characteristics of contaminated and uncontaminated soils.

Location	Depth (cm)	Moisture (%)		Oil content (mg/g)		PO_4^{-3} concentration (ppm)		NO^-_3 concentration (ppm)	
		Contaminated	Uncontaminated	Contaminated	Uncontaminated	Contaminated	Uncontaminated	Contaminated	Uncontaminated
Mile 17 PFS	0-15	45.7 (±7.4)	54.5 (±5.2)	1.6 (±0.03)	0	1.70 (±0.4)	1.30 (±0.24)	23.2 (±2.3)	38.2 (±3.1)
	15-45	56.5 (±9.8)	60.5 (±4.58)	0.8 (±0.14)	0	1.01 (±0.22)	0.81 (±0.13)	33.5 (±15.6)	30.4 (±2.9)
Bonduma AMW	0-15	38.1 (±14.2)	48.4 (±2.5)	2.2 (±0.31)	0	0.53 (±0.06)	0.89 (±0.02)	49.6 (±2.6)	38.7 (±6.7)
	15-45	39.9 (±4.1)	46.7 (±9.5)	0.2 (±0.0)	0	0.76 (±0.38)	1.13 (±0.04)	51.4 (±2.0)	36.2 (±8.6)
Soppo AMW	0-15	34.8 (±4.3)	48.4 (±3.0)	1.8 (±0.3)	0	0.81 (±0.33)	0.53 (±0.04)	46.2 (±6.2)	36.3 (±0.7)
	15-45	23.7 (±5.0)	57.6 (±6.0)	0.7 (±0.2)	0	0.62 (±0.19)	0.87 (±0.02)	30.0 (±5.7)	41.3 (±3.1)
Bongo Square PFS	0-15	29.4 (±10.3)	38.2 (±10.7)	0.8 (±0.3)	0	1.49 (±0.07)	0.75 (±0.04)	41.1 (±6.2)	38.3 (±2.2)
	15-45	35.8 (±0.4)	42.3 (±0.69)	0.03 (±0.03)	0	1.21 (±0.29)	0.67 (±0.04)	45.4 (±6.2)	37.0 (±4.5)
Lower Farms PFS	0-15	44.6 (±7.7)	54.4 (±2.5)	1.3 (±0.6)	0	1.08 (±0.34)	1.26 (±0.35)	56.6 (±9.6)	31.4 (±3.4)
	15-45	36.5 (±7.4)	76.7 (±5.2)	0.04 (±0.01)	0	0.64 (±0.24)	1.05 (±0.07)	23.8 (±1.8)	33.2 (±2.6)
Buea Town AMW	0-15	47.6 (±5.00)	66.8 (±1.7)	1.5 (±0.4)	0	0.78 (±0.23)	0.68 ±0.05)	47.1 (±4.2)	17.0 (±5.8)
	15-45	40.9 (±0.25)	44.3 (±3.0)	0.1 (±0.02)	0	0.55 (±0.66)	0.63 (±0.04)	41.4 (±12.2)	21.3 (±0.9)

AMW, Automechanic workshop; PFS, Petrol filling station. (±) Stardard deviation of physico-chemical parameters in oil contaminated soils at various depths.

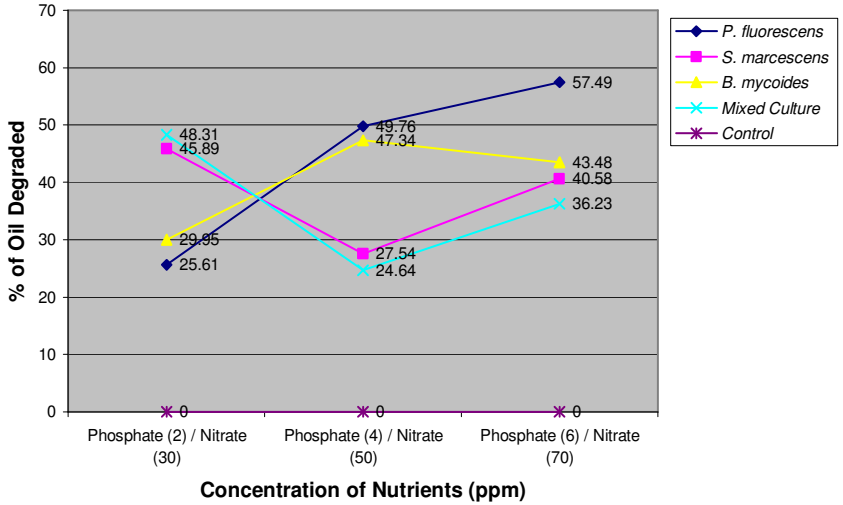

Figure 6. The influence of Phosphate and Nitrate concentrations (in ppm) on the degradation patterns of isolates after 20 days of monitoring.

oil spill clean-up.

Soils contaminated with petroleum products have been shown to have large increases in nitrogen and phosphate content (Odu, 1972; Amund et al., 1993). This contradicts the observations made in this study, where by the nitrate and phosphate contents were not significantly different ($p < 0.05$) between contaminated and uncontaminated soils. We may not be able to advance a definite reason for this, but speculate that it may be related to the extent of contamination as well as some soil and microbial properties. There was a significant reduction in the moisture content of oil contaminated soils, which can be attributed to the oil rendering the soil hydrophobic thus reducing its water holding capacity (Dibble and Bartha, 1979; Amund et al., 1987).

The influence of environmental factors rather than genetic capability of a microorganism have been reported to limit the degradation of pollutants (Barther and Atlas, 1977; Jackson and Jackson, 2000). The activity of oil-degraders (with the exception of *B. mycoides* whose maximum activity was at 35°C) and that of the mixed culture increased from room temperature to a maximum of 32°C. Further increases resulted in a drop in the degradation rates. This may likely be due to the denaturation of enzymes which catalyse these reactions. The oil-degraders required different nutrient concentrations for maximum activity. The degradation rates o*f S. marcescens* and the mixed culture dropped with increase in nutrients, although further increases in nutrients resulted in increased degradation rates. The degradation rate of *B. mycoides* rose with increased nutrient concentration up to a maximum of 47.34% and dropped thereafter. *P. fluorescens* is the only isolate for which an increase in degradation rate compared linearly with increased nutrient concentration. Thus, isolates exhibited varying requirements of physico-chemical factors for optimum degradation.

Conclusion

We conclude that oil-degrading bacteria are abundant in soils in Buea. This can be exploited for large oil-spill clean-up campaigns. This study also provides information on the physico-chemical requirements for optimum degradation by these bacteria.

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REFERENCES

Amund OO, Adebowale AA, Ugoji EO (1987). Occurrence and characteristics of hydrocarbon utilizing bacteria in Nigerian soils contaminated with spent motor oil. Indian J. Microbiol. 27: 63-87.

Amund OO, Nwokaye N (1993). Hydrocarbon degradation potentials of yeast isolates from a polluted lagoon. J. Sci. Res. Dev. 1: 61-64.

Amund OO, Omole CA, Esiobu N, Ugoji EO (1993). Effects of waste engine oil on soil physico-chemical and microbiological properties. J. Sci. Res. Dev. 1: 65-68.

Atlas RM (1981). Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiol. Rev., 45: 180-209.

Barther R, Atlas RM (1977). The microbiology of aquatic oil spills. Adv. Appl. Microbiol. 22: 225-266.

Benka-Coker MOO (1989). Studies on the effects of crude oil and heavy metal contamination of soil and water on the microbial population at the Kokori flare site, Bendel State, Nigeria. PhD thesis submitted to the University of Benin, Nigeria, p. 137.

Bless AJN (1984). Principles of analytical methods of used soils and plant analysis. International courses on soil and plant analysis (ICSPA), Egypt. p. 174.

Daane L, Harjono I, Zylstra GJ, Haggblom MM (2001). Isolation and characterisation of polycyclic aromatic hydrocarbon – degrading bacteria associated with the rhizosphere of salt marsh plants. Appl. Environ. Microbiol. 67: 2683-2691.

Deni J, Penninck MJ (1999). Nitrification and autotrophic nitrifying bacteria in hydrocarbon – polluted soil. Appl. Environ. Microbiol. 65: 4008-4013.

Dibble JT, Bartha R (1979). Effect of environmental parameters on biodegradation of oil sludge. Appl. Environ. Microbiol. 37: 729-739.

Dinkla IJT, Gabor EM, Janssen DB (2001). Effects of iron limitation on the degradation of toluene by Pseudomonas strains carrying TOL (pWWO) plasmid. Appl. Environ. Microbiol. 67: 3406-3412.

Facundo J M-R, Vanessa H-R, Teresa ML (2001). Biodegradation of diesel oil in soil by a microbial consortium. Water Air Soil Pollut. 128: 313-320.

Hubert C, Shen Y, Voordouw G (1997). Comparison of toluene – degrading microbial communities from soil at different concentrations of toluene. Appl. Environ. Microbiol. 63: 3064-3070.

Jackson ARW, Jackson JM (2000). Environmental Science. In: The natural environment and human impact. 2nd Ed. Prentice Hall, New Jersey, p. 405 .

Jawetz E, Melnick JL, Adelberg EA, Brooks GF, Butel SJ, Ornston LN (1991). Pseudomonads, Acinetobacter and uncommon gram-negative. In: Jawetz E, Adelberg EA, Brooks GF, Butel SJ, Ornston LN editors. Review of Medical Microbiology, 19th edn. Appleton and Langue Publishing Prentice Hall, Los Altos, California, USA, p. 224-229.

Jensen V (1975). Bacterial flora of soil after application of oily waste. Oikos. 26: 152-158.

Koneman EW, Allen DS, Janda NW, Schrenchekberger CP, Washington C (1992). Colour atlas and textbook of diagnostic microbiology 4th Ed. J.B. Lippincott Company, Philadelphia, PA, pp. 185-252.

Kulwadee T, Vithaya M, Prayad P, Attawut I (2001). Isolation and characterisation of crude oil degrading bacteria in Thailand. International Conference on 'New Horizons in Biotechnology', April 18-21, Trivandruim, India.

Lalithakumari D (2001). Microbes in bioremediation of zenobiotics. International Conference on 'New Horizons in Biotechnology', April 18-21, Trivandrum, India.

Michalcewicz W (1995). The influence of diesel fuel oil on the number of bacteria, fungi, actinomycetes and soil microbial biomass. Rocz Panstw Zakl Hig. 46: 91-97.

Obire O (1988). Studies on the biodegradation potential of some microorganisms isolated from water systems of two petroleum producing areas in Nigeria. Niger. J. Microbiol. 1: 81-90.

Odu CTI (1972). Microbiology of soils contaminated with petroleum hydrocarbon I. Extent of contamination and some soil and microbial properties after contamination. J. Inst. Pasteur 58: 201-208.

Raven R, Berg LR, Johnson GB (1993). Environment. Saunders College Publishing, Philadelphia. USA, p. 569.

Walker MV, Crawford RL (1997). Overview: Biotransformation and Biodegradation. In: Hurst CJ, Kundsen GR, Mcinerney MJ, Stetzenbach LD, Walter MV (eds). Manual of Environmental Microbiology. American Society for Microbiology Press, Washington, D.C. p 707-708.