Isolation of Bacteria From Engine Oil Contaminated Soils In Auto mechanic workshops in Gwagwalada, Abuja,FCT-Nigeria.

Ugoh, S.C. and Moneke, L.U.

Department of Biological Sciences, University of Abuja, PMB 117, Abuja,FCT ,Nigeria.
sylvaugoh@hotmail.com.

Abstract: Isolation of bacteria associated with engine oil contaminated soil was carried out. Five different mechanic workshops within Gwagwalada were selected and five soil samples were collected from each site. 5 grams of contaminated soil was added to 50ml of the enrichment medium and was incubated at 30^{o}C for 5 days. The soil samples from the mechanic workshop were enriched using Bushnell- Haas medium and then subsequently plated out on nutrient agar plates for 24 hours at 30^{o}C. Spread plate method involving the use of serial dilutions was employed for the isolation of the bacteria. The number of viable bacterial count were determined and expressed in colony forming units (cfu). The bacterial species isolated were *Pseudomonas sp.*, *Micrococcus sp.*, *Serratia sp.* And *Bacillus sp*. *Bacillus sp.* was the most dominant showing a 100% occurrence, followed by *Micrococcus* and *Pseudomonas sp*. each with 80% and lastly *Serratia* sp. with the least of 40% .On the whole the data suggests that of the isolates gotten, *Bacillus sp.* are most adapted to conditions present in soils contaminated with used engine oil and hence could be exploited in bioremediation activities.

[Ugoh, S.C. and Moneke, L.U. **Isolation of Bacteria From Engine Oil Contaminated Soils In Auto mechanic workshops in Gwagwalada, Abuja,FCT-Nigeria.** Academia Arena, 2011;3(5):28-33] (ISSN 1553-992X). http://www.sciencepub.net.

Keywords: Bacteria; Contaminated Soil; Engine oil; Bioremediation

1. Introduction

Used/waste engine oil is defined as used lubricating oils removed from the crankcase of internal combustion engines (Jain *et al*., 2009). Before they are used, they consist of hydrocarbons, (80 to 90% by volume) and performance enhancing additives (10 to 20% by volume). Engine oils are altered during use by vehicles, motor-bikes, generators and other machinery because of the breakdown of additives, contamination with the products of combustion and the addition of metals from the wear and tear of the engine. It is recognized that the major components consist of aliphatic and aromatic hydrocarbons such as phenol, naphthalene, benz (a)antracene, benzo (a)pyrene, fluoranthene, lead, cadmium and other potentially toxic metals (Jain *et al*., 2009).

Used motor oil can cause great damage to sensitive environments and soil microorganisms. Substantial volumes of soil have been contaminated by used oil in many countries of the world, especially industrialized nations. High concentration of aliphatic, polycyclic aromatic hydrocarbon and heavy metals contribute to the inherent toxicity of used oil (Vasquez– Duhalt and Bartha, 1989).

Large amounts of used engine oil are liberated into the environment when the oil from motor cars, motor-bikes, generators etc is changed and disposed into gutters, water drains, open vacant plots and farmlands, a common practice by motor and generator mechanics (Odjegba and Sadiq, 2002).

Spent engine oil, when present in the soil creates an unsatisfactory condition for life in the soil, which is due to the poor aeration it causes in the soil, immobilization of soil nutrients and lowering of soil pH (Atuanya, 1987). Various contaminants such as used engine oil and heavy metals have been found to alter soil biochemistry, which includes alteration in soil microbial properties: pH, O_2 and nutrient availability (Atuanya, 1987; Brookes, 1995 and Odjegba and Sadiq, 2002).

In spite of the increasing number of auto-mechanic workshops in Gwagwalada, with their attendants indiscriminately dumping waste engine oil in the environment, we are not aware of any study that has attempted to isolate and identify bacteria present in used engine oil contaminated soil environment here in Gwagwalada. The present study was therefore undertaken with a view to isolating bacteria in soil samples contaminated with used engine oil.

2. Materials and Methods

2.1 Sample collection

The study sites were 5 different mechanic workshops situated at different locations in the town. The locations include the mechanic workshops at Demonstration Secondary School road, SDP junction, Jibeco filling station, beside St. Mary's Hospital and the mechanic workshop along market road in Gwagwalada, Abuja.

Apart from visual observation, the attendants at the mechanic workshops were asked questions pertaining to the sites with heavy oil spillage. The sites with the oil spillage had a characteristic black color and the surfaces were hard. They also had no grasses growing on them. Soil samples were collected at each workshop by digging up the soil with a hoe and transferring directly into clean, sterile containers. Samples were collected at 5 different sites at each mechanic workshop. Also, pristine samples were collected from non contaminated reference areas using the botanical garden in the University of Abuja as control site. They were then carefully transferred to the University of Abuja microbiology laboratory for analysis. Physical properties of the soil such as texture, temperature and pH were examined.

2.2 Determination of PH, Temperature and Electrical Conductivity Of Contaminated Soil

Soil pH was measured using a pH meter. The soil sample was mixed with
distilled water and shaken properly. The pH meter was turned on and calibrated using buffers 4 and 10. This was done according to the manufactures instruction. The probe was rinsed thoroughly between buffers using de-ionized water. The pH meter was calibrated before each use. The calibration of the pH meter was confirmed by measuring the pH of the standard solutions in measure rather than calibrate mode. The meter which was used measures the pH taking into account temperature. The probe of the meter was submerged into the sample and the readings were taken. The readings were then recorded accordingly. The same procedure was followed in measuring for the E.C of the soil samples.

2.3 Isolation of Degrading Microorganisms

The culture media used for the isolation of engine oil degrading bacteria were Bushnell- Haas medium (Atlas, 1994) which is an enrichment medium for the isolation of engine oil degrading bacteria, and nutrient agar. The media were prepared according to manufacturer's instructions.

During inoculation, 5 grams of contaminated soil was added to 50ml of the enrichment medium and was incubated at 30^oC for 5 days. The enriched soil was shaken using a mechanical shaker according to Udeani *et al*., (2009). Turbid samples were then sub-cultured into solid nutrient agar plates by transferring 1ml of the enriched soil sample into 9ml of distilled water, agitating vigorously and making serial dilutions up to 10^5.

2.4 Total Bacterial Load

Samples were enumerated by making ten-fold dilutions of the soil samples from 1:10 to 1:1000000. 0.02ml of Dilutions 10^1,10^2,10^3,10^4 and 10^5 were transferred unto the solid nutrient agar plates. A clean sterile spreader was then used to spread the inoculum evenly throughout the medium. The plates were prepared and inoculated in duplicates. The inoculated plates were incubated at 35^oC for 24 hours and subsequently monitored for growth. The colonies of the isolates were counted using a colony counter and the heterotrophic bacterial counts of the contaminated and uncontaminated samples were compared. Isolated colonies were further purified by sub-culturing and identified using bio-chemical tests and microscopy.

2.5 Identification of Isolates

Each isolate was examined for its size, shape, margin, consistency, elevation, pigmentation, gram reaction and cell morphology. The isolates were characterized as described by Holt *et al*., (1994). Biochemical tests which were carried out include production of catalase, indole and oxidase enzymes. Motility test, spore production and oxidation/ fermentation of sugars were also carried out.

3. Results

A total of 5 samples were collected from 5 different mechanic workshops. Heterotrophic bacterial counts in the contaminated samples ranged from 1.5×10^4 to 7.6×10^4 colony forming units g^{-1} and from 6 to 14×10^4 colony forming units per gram of soil in the uncontaminated soil samples which were collected. Table 1 shows bacterial counts of soil samples obtained from the five different sites.

Table 1: Bacterial counts of soil samples from different sites in Gwagwalada town.

Sites	Total Bacterial Count
S_1	7.6×10^4
S_2	5.8×10^4
S_3	3.6×10^4
S_4	1.5×10^4
S_5	4.0×10^4

S_1=SDP junction mechanic site, S_2=Demonstration secondary school road mechanic site, S_3= Jibeco filing station mechanic site, S_4= Mechanic site beside St. Mary's hospital and S_5 = Mechanic site at Gwagwalada market road.

3.1 Physiochemical Characteristics of Contaminated soil

The Physiochemical characteristics of soil samples collected from different automobile workshops used for the study were analyzed and subsequently tabulated in Table 2. The various characteristics like texture, temperature, electrical conductivity and pH were taken into consideration for each of the samples which were named S_1, S_2, S_3, S_4 and S_5. The highest pH value (6.9) which was reported was in S_2 and the lowest (6.3) was in S_1. The electrical conductivity value was low in S_2 and S_5 but high in S_1, S_3 and S_4.

Table 2: Physiochemical properties of contaminated soils.

S/No	Properties	S_1	S_2	S_3	S_4	S_5
1.	Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
	Temperature	31.9°C	32.4°C	30.9°C	30°C	31.6°C
2.	pH	6.3	6.9	6.4	6.7	6.5
3.	Electrical Conductivity Ec(dSM.1)	1.25	0.20	1.30	1.28	0.60
4.						

3.2 Isolates obtained from used engine oil contaminated soil

The serial dilution technique was employed in isolating bacteria from engine oil contaminated soil samples. The isolates were then identified by morphological and biochemical characteristics. In totality, different species of bacteria were isolated. The probable bacteria isolated are *Micrococcus* spp., *Pseudomonas* spp., *Bacillus* spp. and *Serratia* spp. (Table 3). The biochemical reactions of the isolates were also shown in Table 4.

Table 3: Bacterial Isolates from engine oil contaminated soil

Isolates	S_1	S_2	S_3	S_4	S_5	Percentage Occurrence
Micrococcus	+	-	+	+	+	80%
Pseudomonas	+	+	+	-	+	80%
Serratia	+	+	-	-	-	40%
Bacillus	+	+	+	+	+	100%

{+ = Present,- = Absent}

Table 4: Biochemical characteristics of isolates

Identification parameters	Probable Identification			
	Micrococcus sp.	*Pseudomonas sp.*	*Serratia sp.*	*Bacillus spp.*
Motility	Non- motile	Motile	Motile	Motile
Indole	-	-	-	-
Oxidase test	+	+	-	+
Catalase test	+	+	+	+
Oxidation/ fermentation test				
Glucose	Non glucose fermenting	Non glucose fermenting	Glucose fermenting	Non glucose fermenting
Lactose	Non lactose fermenting	Non lactose fermenting	Non lactose fermenting	Non lactose fermenting
Spore test	-	-	-	+

{+=positive, - = negative}

3.3 Biochemical and morphological characteristics of isolates

Table 4 shows the biochemical characteristics of the isolated organisms. The *Pseudomonas* spp. which was isolated produced colonies which were small, round, slightly raised and produced a blue green water soluble pigment known as pyocyanin which diffused into the medium. The bacteria were also oxidase positive, oxidized glucose in the oxidation fermentation test and were indole negative. They were also non spore forming, gram negative, and catalase positive, motile and aerobic rods. Morphologically, the colonies of *Micrococcus* spp. which were isolated were pigmented in shades of yellow. Its cells were also rhizoidal, opaque, rough and raised. Its cells were spherical in shape occurring as irregular clusters and not in chains. This helps to differentiate them from other gram positive cocci. The bacteria were catalase positive, non- motile, aerobic, non – sporulating and gram positive. Another isolate, *Serratia* spp. produced red pigments on nutrient agar plates with a weak elevation after 24 hours of incubation. Biochemically, *Serratia* spp. is a rod shaped bacterium which reacted negatively to the gram stain and is also motile. On nutrient agar, the *Bacillus* spp. which were isolated produced cream, circular, entire, opaque, flat and rough edges. Microscopically, they were seen as gram positive long rods with a central spore.

The results of the bacterial count shows that the mechanic workshop at SDP junction had the highest count with 7.6 x 10^4cfu/ml followed by the workshop at demonstration road with 5.8 x 10^4cfu/ml, the next in line was the mechanic workshop at

Gwagwalada market road with 4.0 x 10^4cfu/ml while the mechanic workshops at Jibeco filling station mechanic site had bacterial density of 3.6 x 10^4cfu/ml and lastly the mechanic workshop beside St Mary's Hospital had the least with 1.5 x 10^4cfu/ml. The bacterial isolates from the soil contaminated with petroleum products showed that *Bacillus* spp. had the highest percentage occurrence of 100% followed by *Pseudomonas* spp. and *Micrococcus* spp. each with 80% and *Serratia* spp. had the lowest percentage occurrence of 40%.

Our data show an obvious influence of waste engine oil discharge on the microbiological and physiochemical properties of soil. The relatively low heterotrophic bacterial counts observed in oil contaminated soils can be attributed to the toxic or un-favorable effect of oil contamination (Akoachere *et al.*, 2008). The ability to isolate high numbers of certain oil degrading microorganisms from oil polluted environment is commonly taken as evidence that these microorganisms are the active degraders in the environment. Although, hydrocarbon degraders may be expected to be readily isolated from an oil associated environment, the same be expected to be readily isolated from an oil associated environment, the same degree of isolates could be gotten from a totally unrelated environment such as pristine soil (Santhini *et al.*, 2009)

In motor mechanics workshops there is a constant change in the soil micro-organism as a result of deliberate spillage of used engine oil. These alter the biomass and ecology of the soil such that both microbial communities and grasses can no longer grow on the soil spots. The colour and texture of the soil are affected; this leads to different microbial flora establishment in an attempt to remedy the petroleum product spillage (Megharaj *et al.*, 2000). Although some studies have shown that, oil-polluted soils are dominated by Gram negative bacteria (McNaughton *et al*., 1999; Kaplan and Kitts, 2004), the dominant culturable hydrocarbon utilizing bacteria from the soil samples were made up of gram positive *Bacillus* and *Micrococcus* and also gram negative *Pseudomonas* and *Serratia.* The results of the present study revealed that Gwagwalada soil may harbor hydrocarbon degraders that have been exposed to hydrocarbons as a result of the indiscriminate disposure of the spent engine oil collected from the crankcase of motor vehicles, motor bikes and other machinery in Gwagwalada metropolis. It was observed that the *Bacillus* sp. played a significant role in hydrocarbon degradation having shown dominance in all the test samples. This observation is consistent with the works of Udeani *et al.*, (2009) and Makut *et al.*, (2010).The presence of *Micrococcus* and *Pseudomonas* spp. were also indicated in four out of the five samples showing an 80% occurrence. From this study, this shows that these microorganisms are also active degraders of petroleum hydrocarbon from soil. From this study, *Serratia* sp. had the lowest occurrence of 40%, showing the least degrading capabilities. Although this contradicts the works of Akoachere *et al.,(*2008) who reported that of all the isolates which were gotten, *Serratia* sp. degraded the highest amount of oil(36.2%), It is in line with the works of McNaughton *et al*., (2009).

4. Conclusions

The investigation revealed that *Pseudomonas, Bacillus, Micrococcus and Serratia* species were isolated from soils contaminated with used engine in Gwagwalada Metropolis. The result of this study indicates that indigenously it is possible to isolate bacterial micro flora capable of degrading complex hydrocarbon compounds (used engine oil).

This investigation provides information that would lead to selection of bacterial species that could be employed for bioremediation in environments polluted with used engine oil.

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

Correspondence to:
Ugoh, Sylvanus C .
Department of Biological Sciences,
University of Abuja,
PMB 117 , Abuja, Nigeria.
Cellular phone: +2348032861737
Emails: sylvaugoh@hotmail.com.

References

[1] Akoachere J. T. K., Akenji T.N., Yongabi N.F., Nkwelang G. and Ndip N.R. (2008). Lubricating oil-degrading bacteria in soils from filling stations and auto- mechanic workshops in Buea, Cameroon: occurrence and characteristics of isolates. *African Journal of Biotechnology,* 7 (11); 1700-1706.

[2] Atlas R.M. (1994). *Handbook of Microbiological Media.* Pp 175. L.C. Parks. CRC Press.

[3] Atuanya E.J. (1987). Effect of oil pollution on physical and chemical properties of soil: a case study of waste oil contaminated delta soil in Bendel State, Nigeria. *Journal of Applied Science*,55; 155-176.

[4] Bhattacharya D., Sarma P.M., Krishnan S., Mishra S. and Lal B. (2002). Evaluation of genetic diversity among *Pseudomonas citronellolis* strains isolated from oily sludge–contaminated. *Journal of* sites *Applied Environmental Microbiology*,69(3); 1435–1441.

[5] Brookes P.C. (1995). The use of microbial parameters in monitoring soil pollution by heavy metals. *Biological Fertility of Soil*, 19; 269-279.

[6] Holt, J.G., Krieg N.R., Sneath P.H.A, Staley J.T and Williams S.T (1994). *Bergey's Manual of Determinative Bacteriology*. 9th Ed. Williams and Wilkins, pp: 71-561

[7] Jain P.K., Gupta V.K., Gaur R.K., Lowry M., Jaroli D.P. and Chauhan U.K. (2009). Bioremediation of petroleum oil contaminated soil and water. *Research Journal of environmental Toxicology,* 5(1): 1-26.

[8] Kaplan C.W. and Kitts C.L. (2004). Bacterial succession in a petroleum land treatment unit. *Journal of Applied Environmental Microbiology*,70; 1777-1786.

[9] MacNaughton S.J., Stephen J.R., Venosa A.O., Davis G.A., Chang Y.J. and White D.C. (1999). Microbial population changes during bioremediation of an experimental oil spill. *Journal of Applied Environmental Microbiology*,65; 3566-3574.

[10] Makut M.D. and Ishaya P. (2010). Bacterial species associated with soils contaminated with used petroleum products in Keffi town, Nigeria. *African Journal of Microbiology Research,* 4(16); 1698-1702.

[11] Megharaj S.J., Singleton I., McClure N.C. and Naidu R. (2000). Influence of petroleum hydrocarbon contamination on microalgae and microbial activities in a long term contaminated soil. *Archives on Environmental Contamination and Toxicology,* 38: 439-445.

[12] Odjegba V.J. and Sadiq A.O. (2002). Effect of spent engine oil on the growth parameters, chlorophyll and protein levels of *Amaranthus hybridus The Environmentalist*, 22; 23-28.

Udeani T. K. C., Oboh A .A. , Okwuosa C.N. Achukwu P. U. and Azubike N. (2009). Isolation of bacteria from mechanic workshops' soil environment contaminated with used engine oil. *African Journal of Biotechnology*, 8 (22), 6301-6303.

Vazquez–Duhalt R. and Bartha R. (1989). Biodegradation of petroleum in soil environment. *Canadian Journal of Microbiology*, 20: 1985-1988.

5/4/2011