Ethiopian Journal of Environmental Studies & Management 9(Suppl. 1): 920 – 927, 2016.
ISSN:1998-0507 doi: http://dx.doi.org/10.4314/ejesm.v9i1.10S
Submitted: August 29, 2016 Accepted: November 28, 2016

BACTERIOLOGICAL ANALYSIS OF SPENT ENGINE OIL CONTAMINATED SOIL PLANTED WITH COWPEA (*Vigna unguiculata*)

***ADETITUN, D.O., ADEBISI, O.O., ANIBIJUWON, I.I., KOLAWOLE, O.M., ADEOSUN, T.B. AND OLAYEMI, A.B.**
Department of Microbiology, Faculty of Life Sciences, P. M. B. 1515, University of Ilorin, Nigeria

Abstract
The bacteriological analysis of soil contaminated with spent engine oil (SEO) planted with cowpea was investigated. The aim of this study was to detect the microbial degradation of SEO in soil and how it affects the microbial activity and the effects of SEO on the growth of cowpea. SEO collected from a mechanic workshop in Ilorin was introduced into soil in varying concentrations. The experimental set up was in triplicates with six treatments of SEO. Soil samples were taken every week for the duration of six weeks for laboratory analysis. Plant growth parameters were measured every week after planting. The pH of the soil and the bacterial population of the soil were also observed. Findings revealed that the plant growth parameters were significantly reduced as the concentration of SEO increased in the soil. Bacterial counts were also determined and a total of six bacterial species were isolated from the soil samples. Bacillus subtilis, Micrococcus luteus, Pseudomonas aeruginosa and Pseudomonas putida occurred in the control and contaminated soil samples. Bacterial counts ranged from 1.89×10^5 to 4.25×10^5 cfu/ml in the soil samples with the highest occurring in the control and the least occurring in 224ml of SEO. SEO contaminated soil has adverse effect on cowpea and on bacterial flora of the soil. The results of this study revealed that Pseudomonas aeruginosa, Micrococcus luteus and Bacillus subtilis can utilize SEO in the soil. They could be harnessed for use in bioremediation of soil polluted with petroleum and petroleum products.

Key Words: *Bacteria, Spent engine oil, Soil, Cowpea*

Introduction

Panda *et al*. (2013) reported that hydrocarbons are the earth's most widely used primary energy and fuel resources, due to the energy they produce. Crude oil can be unknowingly or knowingly released into the environment leading to serious pollution problems (Atlas and Bartha, 1998). One of the features of hydrocarbon degrading bacteria is the ability of blending hydrocarbons in solution by producing surface active driving forces (Panda *et al.*, 2013). The bulk of typical engine oil consists of hydrocarbon of 18 and 34 carbon atom per molecule (Corsico *et al*., 1999). The disposal of spent engine oil (SEO) into gutters, water drains, open vacant plots

*Corresponding Author: Adetitun, D.O.
Email: adetitun.do@gmail.com

and farms is a common practice in Nigeria especially by motor mechanics. This oil, also called spent lubricant or waste engine oil, is usually obtained after servicing and subsequently draining from automobile and generator engines and much of this oil is poured into the soil (Anoliefo and Vwioko, 2001).

Hydrocarbon utilizing bacteria are important in combating the problem of oil pollution in our environment (Atlas and Bartha, 1992). Concentration of petroleum hydrocarbon determines the rate of breakdown of the hydrocarbons from the soil environment. High concentration of hydrocarbon can be inhibitory to microorganisms and concentration at which inhibition occurs varies with the compound (Riffaldi *et al*., 2006). Oil pollution whether acute or toxic has detrimental effects on agricultural lands and hence considerable influence on plant growth (Agbogidi *et al.*, 2007).

Pollution of soil with petroleum derivatives is often observed in municipal soils around industrial plants and in areas where petroleum and natural gas are obtained (Adam *et al*., 2002). No matter how small the pollution or portion of land so polluted, small pollution here and there can add up to a large portion of affected land. Cowpea was chosen for this study because it is a legume and naturally does not require fertilizer for nitrogen fixation due to its possession of nif-genes. The focus of this study is to investigate the growth performance of cowpea in the presence of SEO. It is also aimed at identifying the bacteria that are present in soil and that could thrive in SEO contaminated soil with the aim of utilising such for biodegradation studies.

Materials and Methods

Soil samples were collected from the Faculty of Science area of the University of Ilorin, Ilorin. Soil samples were collected at a depth of 5-10cm from the soil surface with a hoe and moved to improvised screen house in new and clean plastic buckets. SEO was collected in sterile container from a mechanic site situated at Tanke, Ilorin. The cowpea seeds were purchased from Tanke market, Ilorin.

Experimental Set Up

The experiment was laid out in triplicate. 1.5kg of soil sample was weighed into 18 sterile plastic vessels that have been perforated with the use of hot nails. SEO was measured and added to the soil in the containers. Six treatments of SEO (0ml, 7ml, 21ml, 56ml, 168ml and 224ml) were applied. It was thoroughly mixed with the soil and left undisturbed for 24hours to allow the volatilization of toxic components of the oil. The vessels were labeled. Three (3) healthy cowpea seeds were planted in each vessel and observation was done for six weeks. Watering was done regularly throughout the period of the experiment. Composite samples were taken for the isolation and enumeration of hydrocarbon utilizing bacteria every week for six weeks. The method of Ekpo and Thomas (2007) was used as model.

Determination of Soil pH

Two grams (2g) of soil sample was weighed and introduced into a beaker containing 20 ml of distilled water. It was continuously stirred with a glass rod for 20 minutes to reach equilibrium. At the end of 20 minutes, pH metre electrode was used to take the pH of the suspension.

Determination of plant stem height, leaf stem height, leaves length and breadth, root length and leaf area

The plant stem height, leaf stem height, leaves length and breadth and root length were all measured using a tape rule. The leaf area was calculated by multiplying the length and the breadth.

Bacteriological Analysis

Fawole and Oso (2007) was consulted for bacteriological analysis. Nutrient agar was prepared according to manufacturer's instructions. Three replicate samples from each oil-polluted soil were withdrawn every week for the enumeration of hydrocarbon utilizing bacteria (HUB). The soil bacteria were isolated by the soil dilution techniques using the pour plate method. One gram (1g) of the soil sample was weighed and added into already prepared sterile distilled water in a test tube to make a dilution factor of 10^{-1} and serially diluted to 10^{-3} using sterile syringes. One milliliter (1ml) of the 10^{-3} dilution was aseptically inoculated into sterile Petri dish. 5ml of SEO was then introduced into the already prepared nutrient agar (1L), mixed thoroughly by shaking and then poured into the Petri dishes. The plates were inoculated in triplicates. They were incubated at 37^{o}C for 24 hours. After 24 hours the plates were observed for growth; developed colonies were counted using a colony counter and recorded.

Bacterial colonies selected based on colonial morphology were sub-cultured on to sterile solidified nutrient agar using the streaking technique. This was repeated until pure culture was obtained. Bacterial isolates obtained were characterized using their colonial, cellular morphology and biochemical characteristics. Allusion was made to Holt *et al*., (1994) for the identification of the isolates.

Statistical Analyses

One way analysis of variance (ANOVA) test was used to determine whether the measured parameters differed significantly. *P* value less than 0.05 was considered to indicate statistical significance.

Results

The isolated bacteria are *Staphylococcus aureus, Micrococcus luteus, Pseudomonas putida, Pseudomonas aeruginosa, Bacillus subtilis and Proteus vulgaris*. *Bacillus subtilis, Micrococcus luteus, Pseudomonas aeruginosa* and *Pseudomonas putida* occurred in both uncontaminated and contaminated soil samples. *Staphylococcus aureus* and *Proteus vulgaris* were isolated from the control only.

Table 1 shows the pH values of soil samples at different concentrations of SEO. The pH values ranged from 5.81 to 7,89, indicating that the soil is slightly acidic to neutral. Table 2 shows the height (in cm) of the stem of the cowpea at different concentrations of SEO. The height of the stem ranged from 0.00cm to 15.33cm. Table 3 shows the breadth (in cm) of the leaves of the cowpea at different concentrations of SEO. The breadth of the stem ranged from 0.00cm to 4.90cm. Table 4 shows the length (in cm) of the leaves of the cowpea at different concentrations of SEO. The leaf length ranged from 0.00cm to 14.90cm. Table 5 shows the length (in cm) of the roots at different concentrations of SEO. The root length ranged from 0.00cm to 35.7cm. Table 6 shows the total bacterial count in the control and polluted soil. Table 7 shows the weekly distribution of bacterial isolates. Table 8 shows the

percentage occurrence of bacterial isolates from the soil samples by concentration.

Table 1: pH Values of Soil Samples at Different Concentrations of SEO

Sampling week	Polluted soil					
	0ml	7ml	21ml	56ml	168ml	224ml
1	7.35 ± 0.01^{a}	7.29± 0.01^{b}	7.32± 0.00^{b}	6.39± 0.01^{c}	5.99± 0.01^{d}	6.56± 0.01^{c}
2	7.34±0.01^{a}	7.27± 0.00^{b}	7.24± 0.01^{c}	6.30± 0.00^{d}	5.93± 0.01^{e}	5.85± 0.01^{f}
3	7.15±0.01^{a}	6.06± 0.01^{b}	6.10± 0.01^{c}	5.97± 0.01^{d}	6.08± 0.01^{e}	6.16± 0.01^{f}
4	7.24±0.01^{c}	7.45± 0.01^{d}	7.51± 0.01^{d}	6.20± 0.10^{d}	6.80±0.10^{d}	6.30± 0.10^{d}
5	7.61±0.01^{a}	6.49± 0.01^{b}	7.22± 0.0c	5.91± 0.01^{d}	5.81± 0.01^{e}	5.87± 0.00^{f}
6	7.89±0.00a	7.31± 0.00^{b}	6.64± 0.00^{c}	6.39± 0.00^{d}	6.30± 0.00^{e}	6.89± 0.10^{f}

Each value is a mean of three determinations ± SD. Values with different superscripts along the same row are significantly different (p<0.05)
Zero values indicate that there was no growth at the concentrations

Table 2: Height (in cm) of the stem of the Cowpea at Different Concentrations of SEO

Sampling week	Polluted soil (cm)					
	0ml	7ml	21ml	56ml	168ml	224ml
1	10.00±0.1^{a}	9.60±0.1^{b}	3.97±0.01^{c}	3.50±0.01^{d}	0.00±0.00^{e}	0.00±0.00^{e}
2	10.33±0.03^{a}	10.10±0.1^{b}	8.13±0.03^{c}	5.80±0.05^{d}	0.00±0.00^{e}	0.00±0.00^{e}
3	10.73±0.03^{a}	10.50±0.05^{b}	9.43±0.02^{c}	6.25±0.04^{d}	0.00±0.00^{e}	0.00±0.00^{e}
4	12.10±0.02^{a}	11.70±0.10^{b}	10.43±0.03^{c}	6.50±0.02^{d}	0.00±0.00^{e}	0.00±0.00^{e}
5	12.50±0.02^{a}	13.00±0.10^{b}	10.50±0.05^{c}	8.50±0.05^{d}	0.00±0.00^{e}	0.00±0.00^{e}
6	15.33±0.02^{a}	15.13±0.02^{b}	11.40±0.02^{c}	9.50±0.02^{d}	0.00±0.00^{e}	0.00±0.00^{e}

Each value is a mean of three determinants ± SD. Values with different superscripts along the same row are significantly different (p<0.05)
Zero values indicate that there was no growth at the concentrations

Table 3: Breadth (in cm) of the leaves of the Cowpea at Different Concentrations of SEO

Sampling week	Polluted Soil (cm)					
	0ml	7ml	21ml	56ml	168ml	224ml
1	3.10±0.03^{a}	2.90±0.03^{b}	1.80±0.01^{c}	1.10±0.01^{d}	0.00±0.00^{e}	0.00±0.00^{e}
2	3.50±0.01^{a}	2.90±0.01^{b}	2.20±0.03^{c}	1.20±0.02^{d}	0.00±0.00^{e}	0.00±0.00^{e}
3	4.10±0.02^{a}	3.90±0.02^{b}	2.60±0.01^{c}	1.40±0.01^{d}	0.00±0.00^{e}	0.00±0.00^{e}
4	4.60±0.01^{a}	4.40±0.02^{b}	3.10±0.01^{c}	1.80±0.02^{d}	0.00±0.00^{e}	0.00±0.00^{e}
5	4.90±0.03^{a}	4.80±0.01^{b}	3.00±0.01^{c}	2.20±0.02^{d}	0.00±0.00^{e}	0.00±0.00^{e}
6	4.90±0.02^{a}	4.90±0.01^{b}	3.40±0.02^{c}	2.60±0.01^{d}	0.00±0.00^{e}	0.00±0.00^{e}

Each value is a mean of three determinations ± SD. Values with different superscripts along the same row are significantly different (p<0.05)
Zero values indicate that there was no growth at the concentrations

Table 4: Length (in cm) of the leaves of the Cowpea at Different Concentrations of SEO

Sampling week	Polluted Soil (cm)					
	0ml	7ml	21ml	56ml	168ml	224ml
1	6.30±0.03^{a}	6.10±0.04^{b}	4.80±0.02^{c}	3.60±0.01^{d}	0.00±0.00^{e}	0.00±0.00^{e}
2	7.70±0.01^{a}	7.50±0.01^{b}	5.30±0.01^{c}	4.10±0.01^{d}	0.00±0.00^{e}	0.00±0.00^{e}
3	7.90±0.01^{a}	7.80±0.01^{b}	5.40±0.01^{c}	4.30±0.01^{d}	0.00±0.00^{e}	0.00±0.00^{e}
4	9.30±0.02^{a}	9.90±0.02^{b}	5.90±0.04^{c}	4.80±0.01^{d}	0.00±0.00^{e}	0.00±0.00^{e}
5	11.70±0.02^{a}	10.40±0.02^{b}	6.30±0.01^{c}	5.50±0.02^{d}	0.00±0.00^{e}	0.00±0.00^{e}
6	14.40±0.02^{a}	14.90±0.01^{b}	7.10±0.03^{c}	6.20±0.02^{d}	0.00±0.00^{e}	0.00±0.00^{e}

Each value is a mean of three determinations ± SD. Values with different superscripts along the same row are significantly different (p<0.05)
Zero values indicate that there was no growth at the concentrations

Table 5: Length (in cm) of the roots at Different Concentrations of SEO

Polluted Soil (cm)					
0ml	7ml	21ml	56ml	168ml	224ml
35.7	35.5	19.6	15.5	0	0

The root length measurement was carried out only once on the last week of sample collection (week 6). Zero values indicate that there was no growth at the concentrations

Table 6: Total Bacterial Count in the Control and Polluted Soil

Sampling week	Polluted soil					
	0ml	7ml	21ml	56ml	168ml	224ml
1	$3.56\times10^5\pm0.02^a$	$4.06\times10^5\pm0.02^b$	$1.80\times10^5\pm0.10^c$	$1.24\times10^5\pm0.01^d$	$1.41\times10^5\pm0.01^e$	$1.25\times10^5\pm0.01^d$
2	$4.12\times10^5\pm0.01^a$	$3.45\times10^5\pm0.01^b$	$2.10\times10^5\pm0.01^c$	$1.65\times10^5\pm0.01^d$	$1.89\times10^5\pm0.01^e$	$1.09\times10^5\pm0.01^f$
3	$4.25\times10^5\pm0.01^a$	$2.89\times10^5\pm0.01^b$	$1.67\times10^5\pm0.01^c$	$1.43\times10^5\pm0.01^d$	$2.10\times10^5\pm0.01^e$	$1.80\times10^5\pm0.01^f$
4	$3.89\times10^5\pm0.01^a$	$2.65\times105\pm0.01^b$	$2.08\times10^5\pm0.02^c$	$1.98\times10^5\pm0.01^d$	$1.93\times10^5\pm0.01^e$	$2.19\times10^5\pm0.01^f$
5	$4.09\times10^5\pm0.01^a$	$3.12\times10^5\pm0.02^b$	$1.95\times10^5\pm0.04^c$	$2.43\times10^5\pm0.02^d$	$2.07\times10^5\pm0.02^e$	$2.06\times10^5\pm0.02^e$
6	$4.17\times10^5\pm0.02^a$	$2.57\times10^5\pm0.02^b$	$2.45\times10^5\pm0.03^c$	$2.37\times10^5\pm0.00^d$	$2.30\times10^5\pm0.02^e$	$2.13\times10^5\pm0.03^f$

Each value is a mean of three determinations ± Standard Error of the Mean. Values with different superscripts along the same row are significantly different ($p<0.05$).

Table 7: Weekly Distribution of Bacterial Isolates

Bacterial isolates	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Micrococcus luteus	+	+	+	-	+	-
Staphylococcus aureus	+	+	-	-	-	-
Pseudomonas aeruginosa	+	+	+	+	+	+
Bacillus subtilis	+	+	+	+	+	+
Pseudomonas putida	+	+	+	+	-	-
Proteus vulgaris	+	-	+	-	-	-

(+) – Present, (-) – Absent.

Table 8: The Percentage Occurrence of Bacterial Isolates from the Soil Samples by Concentration

Bacterial isolates	0ml	7ml	21ml	56ml	168ml	224ml	Frequency of occurrence (%)
Micrococcus luteus	+	+	+	+	+	+	100
Staphylococcus aureus	+	-	-	-	-	-	20
Pseudomonas aeruginosa	+	+	+	+	+	+	100
Bacillus subtilis	+	+	+	+	+	+	100
Pseudomonas putida	+	-	+	+	+	+	80
Proteus vulgaris	+	-	-	-	-	-	20
Frequency of occurrence (%)	100	50	67	67	67	67	

(+) = Present and (-) = Absent

Discussion

The detection of *Pseudomonas* aeruginosa throughout the period of observation may be due to its possession of a strong and active hydrocarbon degrading system as reported by Teli *et al*. (2013). The results of the bacterial counts revealed that the control had the highest bacterial colony count and the least was recorded in the contaminated soil samples. This may be due to the inability of some of the organisms to survive the higher concentrations. *Proteus vulgaris* and *Staphylococcus aureus* were isolated from control samples but were not found in the

polluted samples. *Bacillus subtilis, Pseudomonas aeruginosa, Pseudomonas putida* and *Micrococcus luteus* had higher percentage occurrences. This concurs with the work of Radwan *et al.* (1995) where he related the ability of these organisms to compete well, and multiply faster. Radwan *et al.* (1995) reported that these isolates can compete well and proliferate faster. Salam (2016) reported that *P. aeruginosa* strains RM1 and SK1 demonstrated the capability to degrade aliphatic, branched alkane and aromatic components of waste engine oils. Wolińska *et al*. (2016) reported that the genera *Micrococcus* were the primary indigenous bacteria present in soil contaminated with new automobile oil, whereas species of the genera *Bacillus* were present in oil combination treated with waste oil.

The study revealed that the pollution level of 56ml significantly delayed emergence while higher SEO pollution levels, 168ml and 224ml subdued the germination of cowpea seeds. This could be attributed to the fact that SEO impaired free flow of oxygen into the soil and disrupt the activities of microorganisms that could have degraded toxic substances. The effect could also be because of formation of polar compounds dissolved in the water that could penetrate the seed coat, exerting polar necrosis (Wang *et al*., 2000; Adam and Dunca, 2002).

The reduction in measured plant parameters correlates with increased concentration of SEO in this study. This could be attributed to deficiency of availability nutrients needed to maintain physiological processes in the plants. These findings concur with the work of Ogbuehi and Ezeibekwe (2010) who reported that crude instigate deficiency of available nutrients needed to support growth especially at apical regions of the crops. The growth reductions due to high level of SEO agree with findings of Molina *et al*. (2005) who documented similar results and inferred that the negative effect could be due to impermeability effect of petroleum hydrocarbons or immobilization of nutrients mainly nitrogen or inhibitory effect of some polycyclic aromatic compounds.

The poor growth observed in the contaminated soil compared to control could be partially due to the accumulation of heavy metals which are present in high toxic level. Human and other animals that feed on the seeds and leaves of cowpea grown on engine oil polluted environment stands a risk of gradual accumulation of heavy metals in the body system. It is therefore pertinent to prevent the dumping and indiscriminate disposal of SEO in the arable land meant for agriculture. The poor growth could also be due to the inability of the plants in the polluted medium to absorb the nutrient from the soil possibly due to poor insulation and poor functioning of phloem and xylem (Edem *et al*., 2009). Agbogidi *et al*. (2007) reported that oil contamination also reduced the soil fertility by causing immobilization of nutrients by microbes. Such immobilization of nutrients leads to difficulty in the uptake of nutrients in oil contaminated soil, which will be difficult despite the presence of such nutrients in the soil.

Conclusion

It is clear from this work that the growth performance of cowpea is hindered in the presence of SEO. It is also clear that some bacteria that are present in soil could thrive in SEO contaminated soil and hence, could be used such for biodegradation studies. Findings from this work showed that some bacterial species can be employed for bioremediation and this can be exploited for oil spill clean-up campaigns. Studies of community dynamics related to petroleum degrading microbes have the potential to enhance our understanding of the roles played by microbes in the natural genesis of long term effect of petroleum products pollution and to determine new remediation strategies. Cowpea although a legume, has performed not too well under a high concentration of SEO pollution. Therefore, it has become pertinent to enact environmental law to checkmate indiscriminate disposal of SEO in our environment.

References

Adam, G.I. Duncan, H.J. (2002). Influence of diesel oil on seed germination. *Environ. Pollut.*120: 363-370.

Adam, G,I., Gamoh, K., Morris, D.G. and Duncan, H.J. (2002). Effect of alcohol addition on the movement of petroleum hydrocarbon fuels in soil. *Scitotal Environ* 6(1/3): 15-25.

Agbogidi, O.M., Emofor, P.G., Akparaobi, S.O. and Nnaji, G.U. (2007). Effect of crude oil contamination soil on the mineral element of maize (*Zea mays* L.). *Journal of Agronomy* 6(1): 188-193.

Anoliefo, G.O. and Vwioko, D.E. (2001). Tolerance of *Chromolaena odorata* (L) K. and R. Grown in Soil Contaminated with Spent Lubricating Oil. *Journal of Tropical Biosciences*, 1(1):20-24.

Atlas, R.M. and Bartha, R. (1998). Microbial Ecology: Fundamentals and Applications. 4th Edition. Benjamin Cummings Publishinmg Company Inc. Addison Wesley Longman Inc. 300-350.

Atlas, R.M. and Bartha, R. (1992). Biodegradation of Petroleum in Soil Environment at Low Temperatures. *Can J. Microbiol.,* 17:1652-1857.

Buchanan, R.E. and Gibbon, N.E. (1974). Bergey's Manual of Determinative Bacteriology (8th Edn). Williams and Wilkins Co. Baltimore, p. 1246.

Corsico, G., Mattei, L., Roselli, A. and Gommellini, C. (1999). Poly (internal olefins) Synthetic Lubricant and High Performance Functional Fluid. Marcel Dekker Inc, New York.

Edem, C.A., Dosunmu, M.L. and Bassey, F.I. (2009). Distribution of Heavy Metals in Leaves, Stems and Roots of Fluted Pumpkin (*Telferia occidentalis*). *Pakistan Journal of Nutrition* 8(3): 222-224.

Ekpo, M.A. and Thomas, N.N. (2007). An investigation of the state of microorganisms and fluted pumpkin (*Telfairia occidentalis*) in a crude oil impacted garden soil. *Nig. J. Microbiol*. 21:1571-1577.

Fawole, M.O. and Oso, B.A. (2007). Laboratory Manual of Microbiology. Spectrum Books Limited, Ibadan. pp. 6-45.

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 & Wilkins, Baltimore, MS, USA.

Molina, B.L., Vega-Loyo, L., Guerrero, R.S., Romero, I., Vega-jarquin, C. and Albones, A. (2005). Ecotoxicity Evaluation of Diesel Contaminated Soil before and after a Bioremediation Process. *Environment Science and Technology* 20: 100-109.

Ogbuehi, H.C. and Ezeibekwe, I.O. (2010). Growth Performance of Cassava Variety NR 8082 on Crude Oil Polluted Soil on Imo State. *International Science Research Journal* 2: 90-94.

Panda, S.K., Kar, R.N. and Panda, C.R. (2013). Isolation and Identification of Petroleum Hydrocarbon Degrading Microorganisms from Oil Contaminated Environment. *International Journal of Environmental Sciences*, 3(5): 1314-1319.

Radwan, S., Sorkhoh, N. and El-Nemr, I. (1995). Oil Biodegradation Around Roots. *Nature.* 376: 302

Riffaldi, R., Levi-Minzi, R., Cardelli, R., Palumbo, S. and Saviozzi, A. (2006). Soil Biological Activities in Monitoring the Bioremediation of Diesel Contaminated Soil. *Water, air and soil pollution,* 170. 3-15.

Salam, L.B. (2016). Metabolism of waste engine oil by *Pseudomonas* species. *3 Biotech*. 6(1): 98.

Teli, N., Verma D., Gavankan R. and Bhalerao, S. (2013). Isolation, Characterization Identification of Diesel Engine Oil Degrading Bacteria from Garage Soil and Comparison of their Bioremediation Potential. *International Research Journal of Environmental Sciences*, 2(2): 48-52.

Wang, J., Jiq, C.R., Wong, C.K. and Wong, P.K. (2000). Characterization of Polycyclic Aromatic Hydrocarbons Created in Lubricating Oils. *Water, Air and Soil pollution*, 120: 381-396.

Wolińska, A., Kuźniar, A., Szafranek-Nakonieczna, A., Jastrzębska, N., Roguska, E. and Stępniewska, Z. (2016). Biological Activity of Autochthonic Bacterial Community in Oil-Contaminated Soil. *Water Air Soil Pollut.* 227: 130.