IJBPAS, September, 2014, 3(9): 2119-2130

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

COMPARISON OF BIODEGRADATION POTENTIAL OF *BACILLUS SP.* AND *PSEUDOMONAS SP.* ISOLATED FROM OIL CONTAMINATED SOIL

KAUR HP*, KAUR S AND BANSAL J

Shaheed Udham Singh College of Research & Technology, Tangori, Mohali, Punjab, India

***Corresponding Author: E Mail: harjotpalkaur@gmail.com**

ABSTRACT

Today, application of microorganisms for removing engine oil pollution from contaminated sites (bioremediation) is an efficient biological eco-friendly and safer treatment to remediate used engine oil whereas conventional methods are non-economical, temporary and produces toxic compounds. This study was undertaken to assess and to compare the degradation potential of *Bacillus sp.* and *Pseudomonas sp.* isolated from oil polluted soil. These bacterial strains were isolated from oil contaminated soil collected from different mechanical workshops and filling stations by using Bushnell-Hass media containing 20% (v/v) used engine oil. The isolates were identified morphologically and biochemically using standard procedures. Bioremediation potential of bacterial isolates was measured by optical density and total cell count after every 24 hours for 384hours (16days). The percentage degradation of used engine oil was calculated after 16 days with the help of gravimetric analysis. *Pseudomonas sp.* was found to degrade better than *Bacillus sp.* and showed maximum degradation potential (maximum cfu/ml) after 240 hours of incubation whereas *Bacillus sp.* after 288 hours. An increase in degradation potential was correlated to increase in cell number indicating that the bacterial isolates were responsible for the oil degradation. *Pseudomonas sp.* degraded 84% of used engine oil and *Bacillus sp.* degraded 80% after 16 days of incubation when calculated with gravimetric analysis. In conclusion, isolated bacterial species showed biodegradation potential and can be used for the bioremediation of oil contaminated soil i.e. a cost effective, efficient technique and could be used to develop an environment friendly technology to overcome the problem of oil spills.

Keywords: Bioremediation, Degradation Potential, Gravimetric Analysis, Engine Oil, *Pseudomonas sp., Bacillus sp.*

INTRODUCTION

The pollution of soil with lubricating oil (engine oil), crude oil and petroleum products is a problem of increasing magnitude and has a serious hazard to human health, causes organic pollution of ground water which limits its use, causes economic loss, environmental problems, and decreases the agricultural productivity of the soil **[1]**. Crude oil contains toxic chemicals and hydrocarbons, which are harmful to microorganisms, plants, animals and humans and soil. Used motor oil contains more metals and heavy polycyclic aromatic hydrocarbons (PAHs) that would contribute to chronic hazards including mutagenicity and carcinogenicity. Their toxic characteristics have motivated efforts to develop bioremediation technologies to eliminate sources of its exposure. Large amount of engine oil are liberated into the environment when the motor oil is changed and disposed into gutters, water drains, open vacant plots and farmlands, a common practice by motor mechanics and generator mechanics. In addition, the oil is also released into the environment from the exhaust system during engine use and due to engine leaks. The release of oil into the environment causes environmental concern and attracts the public attention. Prolonged exposure and high oil concentration may develop liver or kidney disease, possible damage to the bone marrow and an increased risk of cancer **[2]**. Products of petroleum industry belongs to the most harmful xenobiotics, many of them are carcinogenic and mutagenous. A xenobiotic is a compound which is found in a creature but not normally produced or expected to be present in it **[3]**. Oil pollution is a major environmental concern in many countries, and this has led to a concerted effort in studying the viability of using oil-degrading microorganisms for bioremediation. The effect of oil spills on soil can lead to an enrichment of the oil-degrading microbial population **[4]**. The productive use of biodegradative processes to remove or detoxify pollutants that have found their way into the environment and threaten public health, usually as contaminants of soil, water or sediments is bioremediation **[5]**. In comparison to other methods bioremediation through microorganism is more efficient, because the degradation process is extremely related to the genetic potential of the microbe to oxidize the hydrocarbon into its intermediates **[6]**. Bioremediation can take place naturally or can be encouraged with addition of microbes and fertilizers (nutrients). Microorganisms produce enzymes in the presence of carbon sources which are responsible for attacking the

hydrocarbon molecules. It is a cost effective route requires a very less effort than the other methods used for cleaning of hazardous organic waste, uses naturally occurring diverse bacterial communities with high specificity for degradation and produces harmless end products. Bioremediation changes the harmful chemicals into water and harmless gases and the risky chemicals are completely damaged **[7]**. Bioremediation seems to be the most useful method for the removal of these toxic pollutants using microbial population as there are no side effects in comparison to other traditional methods, minimal exposure of workers to the contaminants, long time protection of public health and possible reduction in the duration of the remediation process. Bioremediation can be an alternative green promising technology for remediation of oil contaminated sites. It functions basically on biodegradation; it is the complete mineralization of organic contaminants by biological agents (microorganisms) into carbon dioxide, water, inorganic compounds, and cell protein or transformation of complex organic contaminants to other simpler organic compounds that do not affect the environment **[8]**. The purpose of this work was to evaluate the effectiveness of commonly found *Bacillus sp*. and *Pseudomonas sp*. in remediating the oil contaminated soil.

MATERIALS AND METHODS

Collection of Oil Contaminated Soil Samples

For the isolation of *Bacillus sp.* and *Pseudomonas sp.*, 22 soil samples were collected from various locations i.e. the repairing and mechanical workshops, scooter and car market, service stations and filling stations of Chandigarh, Banur, Landran, Mohali, Zirkpur and Dera Bassi (Punjab). The used engine oil was collected from the mechanical workshops of Mohali.

Isolation of Bacteria

Bushnell-Hass (BH) medium was used as the enrichment media with 20% (v/v) used engine oil (sole carbon source) to isolate oil degrading bacteria. A medium without carbon source, served as control. 5gms of oil contaminated soil (collected from various places) was added to enriched media and incubated at 30˚C at 170 rpm for one week. After one week, 1% of the inoculums were taken, added into freshly prepared enrichment media and flasks were incubated at same conditions and this procedure was repeated for 3 times. By making serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) of the third enrichment media, the bacteria were diluted and plated out on BH agar plates. 100 µl of used engine oil was spread over BH agar plates and incubated at

30˚C for 2 days. After 2 days of incubation, mixed colonies were obtained; two different types of bacterial colonies were picked, streaked onto nutrient agar plates and incubated at 30°C overnight. After incubation developed bacterial colonies, were randomly picked up, purified by sub-culturing on fresh agar plates and transferred to the nutrient agar slants, properly labelled and stored as stock cultures **[9, 10]**. The bacterial isolates were identified by morphological and biochemical tests such as colony and cell shape, Gram's reaction, catalase test, oxidase test, nitrate reduction test, indole test, methyl red (MR) and voges-Proskauer (VP) test, citrate utilization test, gelatine hydroylsis test, litmus milk test, as per standard procedure described in Bergey's Manual of Determinative Bacteriology **[11]**.

Screening of Bacterial Isolates for Degradation of Used Engine Oil

A single colony of the isolate was inoculated into 10 ml nutrient broth and kept at 30°C overnight. The overnight culture was centrifuged for 15 minutes at 3500 rpm. The cell pellet was washed twice and was resuspended with BH medium until OD600 was equivalent to 1. One ml of bacterium inoculums was transferred to the 100 ml BH broth containing 20ml (20%) used engine oil and was incubated at 120rpm in a shaking incubator at 37°C for 16 days. The growth patterns were obtained by measuring the optical density at 600 nm after every 24 hours and total viable counts/ colony forming units (cfu/ml) of the isolates were determined by the spread plate technique after the incubation of the nutrient agar plates at 30°C for 24 hours. The optical density conferred the growth of the bacterial strains in media and degradation of used engine oil by bacterial strains. As the growth of bacteria increases in the medium, the engine oil concentration decreases in the medium depicted by increased optical density.

Quantification of Oil Degradation

Two bacterial isolates one each from *Bacillus sp.* and *Pseudomonas sp.* showing maximum oil degradation potential (evidenced from growth/ optical density) were used further to check the percentage of oil degradation. The oil degradation was quantified by measuring the oil recovery after 16^{th} days of incubation using the gravimetric analysis **[12, 13]**. Width of oil and media layer in the flask was also recorded on zero day and 16^{th} day. A control devoid of the bacterial isolate was also prepared and all experiments were performed in duplicate. The percentage of engine oil remaining was calculated and compared to the control **[14]**.

RESULTS AND DISCUSSION

This study was planned to investigate and compare the degradation potential of *Bacillus sp.* and *Pseudomonas sp.* isolated from oil contaminated soil.

Isolation of Oil Degrading Bacteria

25 oil degrading bacterial isolates were obtained from 22 soil samples and further identified by morphological and biochemical testing. Oil degrading bacteria use engine oil as a sole carbon source and degrade the used engine oil. The isolates that degrade oil showed growth on BH agar plates as evidenced by degradation of engine oil around the colony **(Figure 1)**.

Morphological and Biochemical Characterization of Oil Degrading Isolates

The oil degrading isolates obtained after growth in enrichment media were identified according to their morphological and biochemical characteristics. After morphological and biochemical characterization of selected soil isolates, it was found that 56% (14) isolates belonged to *Bacillus sp.*, and 44% (11) to *Pseudomonas sp.* **(Table1 and Figure 2)**.

Bacillus sp. was found to be present in 14 soil samples whereas *Pseudomonas sp.* was found in 11soil samples out of 22 soil samples collected in this study. A number of previous studies also showed the presence of *Pseudomonas sp.* and *Bacillus sp*. in the oil contaminated soil. Ganesh and Lin (2009) isolated 59% Gram positive spore forming rods with the remaining 41% being Gram negative rods **[15]**. The hydrocarbon utilizing microorganisms isolated from the soil were species of *Bacillus, Lactobacter, Arthrobacter, Pseudomonas, Micrococcus, Zoopage,* and *Articulosporium*. *Bacillus sp.* predominated, especially in the crude oil polluted soil. This may be due to the ability of the organisms to produce spores, which may shield them from the toxic effects of the hydrocarbons **[16]**. Sathishkumar *et al.* (2008) showed that the dominant strains involved in engine oil degradation belonged to *Pseudomonas, Bacillus* and *Corynebacterium* **[17]**. Chaerun *et al.* (2004) identified *Pseudomonas aeruginosa, Bacillus cereus, Bacillus thuringiensis* and *Paracoccus seriniphilus,* actively involved in hydrocarbon utilization **[18]**. Mandri and Lin (2006), Regina *et al*. (2006) and Udeani *et al*., (2008) also observed the involvement of *Bacillus sp.,* in the degradation of crude oil **[9, 19, 20]**. Ijah and Antai, (2003) reported *Bacillus sp*. as being the predominant isolate of all the crude oil utilizing bacteria characterized from highly polluted soil samples **[21]**. A few studies have been reported on the role of *Bacillus sp*. in hydrocarbon bioremediation, although there are several reports on the

bioremediation of pollutants by the action of *Bacillus sp.* occurring in extreme environments **[22]**.

Screening of Bacterial Isolates for Degradation of Used Engine Oil and Quantitative Degradation

All the isolates belonging to *Bacillus sp.*, and *Pseudomonas sp.*, were further used to study their oil degradation abilities. As the time of incubation increases, the isolated species used the engine oil as sole carbon and energy source and grow in the medium containing engine oil, the turbidity of the medium also increased due to increase in growth of bacteria. Degradation abilities of isolated bacterial species were monitored by increase in turbidity. Due to increase in turbidity, the optical density (OD) of the medium increases, this was measured spectrophotometrically using U.V. visible spectrophotometer at 600 nm. Growth profile showed that these species have the ability to degrade used engine oil and engine oil concentration decreases as the growth of bacteria increases in the medium. Degradation potential of *Bacillus sp.* was found to be maximum after incubation of 288 hours (12 days) and *Pseudomonas sp.* after incubation of 240 hours (10 days) and it was observed that the degradation potential decreased with further incubation for both the isolated bacterial strains **(Figure 3 & Table 3)**. When relating this trend to the increase in bacterial cell count, it was observed that there was rapid increase in the cell biomass of *Pseudomonas sp.* in the first 10 days and of *Bacillus sp.* in the first 12 days of incubation and the increase in cell biomass after further incubation was decreased. This may be due to the exponential phase of cell growth but after this decrease was possible because of the cells of bacteria were near to its stationary phase of cell growth. This study also revealed that removal of oil from soil is effective within first 2 weeks of microbial growth. The initial high rate of biodegradation observed is attributed to the increase in cell biomass and nutrient availability **[23]**.

It was observed from the results that time of incubation effects the degradation potential of oil degrading isolates. *Pseudomonas* sp. was found to be best oil degrading bacteria as it showed maximum absorbance of 1.538 at 600nm after incubation of 240 hours and degrading potential decreased after 240 hours of incubation. It was observed from the total plate count that cell numbers were increased from $5.8x10^6$cfu/ml to$13.0x10^6$cfu/ml for *Pseudomonas sp*. and $5.2x10^6$cfu/ml to $11.8x10^6$cfu/ml for *Bacillus sp*. **(Table 2)**. Optical density (OD) is directly proportional to the total viable cells of bacteria and greater the cells more will be the degradation of oil. The OD and

viable cell count also showed that the isolated bacteria grew well in the media demonstrating their bioremediation potential.

Results of gravimetric analysis showed the differences in the ability of *Pseudomonas sp*. and *Bacillus sp*. to degrade used engine oil. Results also signify that *Pseudomonas sp*. had more (84.07%) biodegradation potential as compared to *Bacillus sp.* (80.25%) under similar conditions of incubation and it could be best exploited for bioremediation of oil contaminated soils **(Table 3)**. Higher oil degrading potential of *Pseudomonas sp.* may be due to its more competent and active hydrocarbon degrading enzyme system. It is known to be fast growing and capable of degrading a wide variety of organic compounds **[24]** but *Bacillus sp.* required more time to degrade used engine oil as compared to *Pseudomonas sp*. *Pseudomonas sp* although known primarily as a spoilage agent in meat and fishes, their ability to degrade hydrocarbon also was studied here. *Pseudomonas* is the most common hydrocarbon degrading bacteria reported in the literature **[25, 26, 27, 28]**. Mandri and Lin (2006) reported that the *Pseudomonas aeruginosa* had degraded 90% in 4 weeks **[9]**. Results of more degrading potential of *Pseudomonas sp*. as compared to *Bacillus sp*. coincides with the earlier studies of Raed S. Al-Wasify and Shimaa (2014) and Roostan *et al*. (2012) **[29, 30]**. Sathishkumar *et al.* (2008) reported that *Pseudomonas sp*. degraded 69% and *Bacillus sp* 64% of crude oil **[17]**. There is growing evidence that isloates belonging to the *Bacillus sp.* could be effective in clearing oil spills **[31]**.

Figure 1: Growth of Oil Degrading Isolates on BH Agar Plate

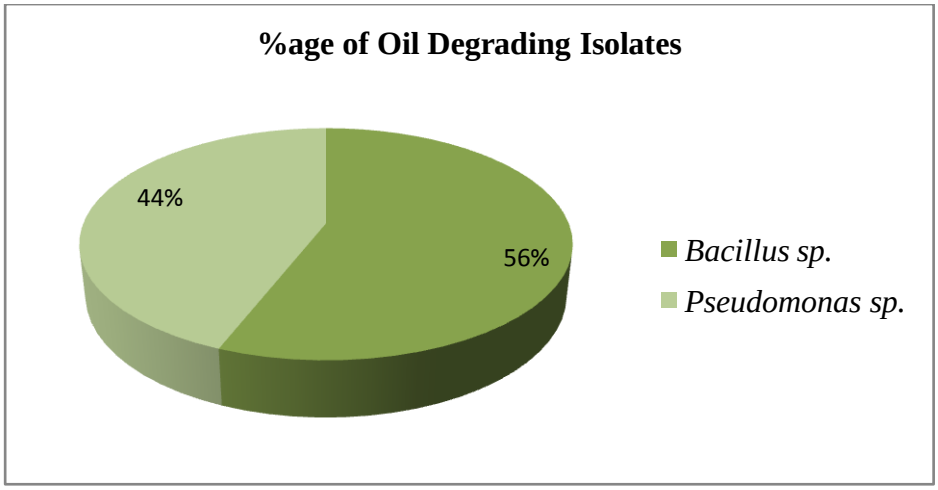

Figure 2: Prevalence of *Bacillus sp.* and *Pseudomonas sp*. in Oil Contaminated Soil

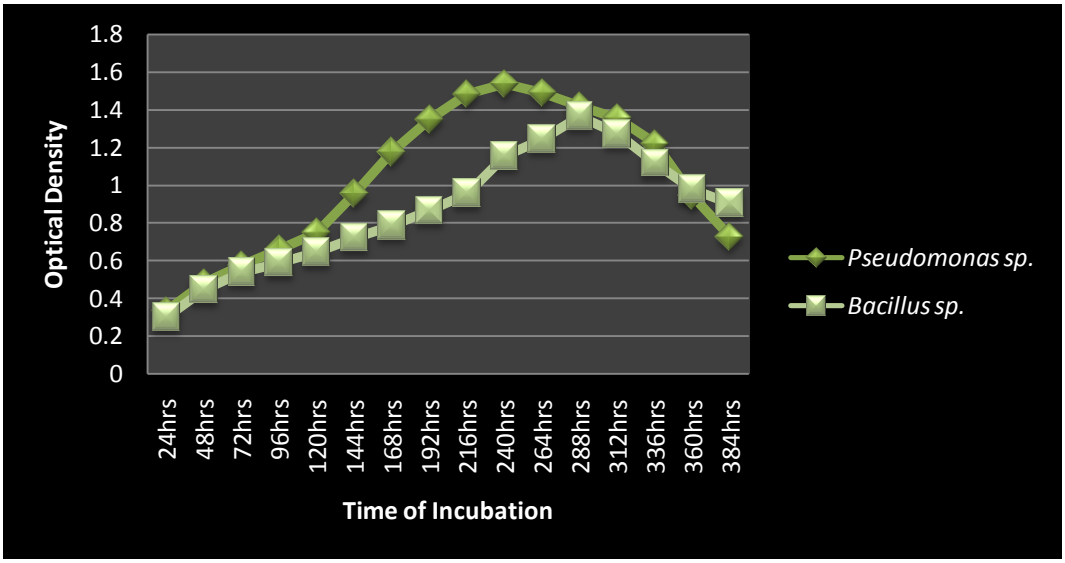

Figure 3: Comparison of Degradation Potential of Isolated Bacterial Species

Table1: Morphological and Biochemical Characteristics of Bacterial Isolates

S. No.	Character	Oil Degrading Isolate Number1,2,4,6,7,9,13,16,17,20,21, 22,23,25	Oil Degrading Isolate Number 3,5,8,10,11,12,14,15,18,19,24
1.	Colony Morphology	White, Opaque, solitary rods	Green, Thin
2.	Gram's Reaction	Positive	Negative
3.	Motility Test	Motile	Motile
4.	Catalase Test	Positive	Positive
5.	Oxidase Test	Positive	Positive
6.	Indole Test	Negative	Negative
7.	Methyl Red Test	Positive	Negative
8.	Voges-Proskauer Test	Positive	Negative
9.	Citrate Utilization Test	Positive	Positive
10.	Litmus Milk Test	Positive	Positive
11.	Gelatin Hydrolysis Test	Positive	Positive
12.	Nitrate Reduction Test	Positive	Positive
13.	Glucose Fermentation Test	No Fermentation	No Fermentation
	Organism	*Bacillus sp.*	*Pseudomonas sp.*

Table 2: Variations in Optical Density (OD) and Total Viable Count Value with Time During the Degradation of Used Engine Oil with *Bacillus sp.* and *Pseudomonas sp.*

Period of Incubation (Hours)	*Bacillus sp.*		*Pseudomonas sp.*	
	OD (600nm)	Total Viable Count ($x10^6$) cfu/ml	OD (600nm)	Total Viable Count ($x10^6$) cfu/ml
24	0.300	5.2	0.334	5.8
48	0.446	6.0	0.482	6.4
72	0.539	6.8	0.574	7.0
96	0.589	7.2	0.661	8.6
120	0.645	8.2	0.749	9.8
144	0.722	9.0	0.956	10.4
168	0.786	9.8	1.176	11.0
192	0.866	10.0	1.349	11.8
216	0.956	10.4	1.483	12.2
240	1.156	10.8	1.538	13.0
264	1.245	11.0	1.491	12.4
288	1.366	11.8	1.419	12.2
312	1.273	11.2	1.356	11.8
336	1.117	10.4	1.222	11.0
360	0.979	10.2	0.945	10.4
384	0.905	10.0	0.726	9.0

Table 3: Quanification of Oil Degradation

Bacterial sp.	Width of Oil on Zero Day (mm)	Volume of Oil on Zero Day (ml)	Width of Oil on 16^{th} Day (mm)	Volume of Oil on 16^{th} Day (ml)	%age of Oil Degradation
Bacillus sp.	06	20	1.2	4	80
Pseudomonas sp.	06	20	1.0	3.2	84
Control	06	20	06	20	0

CONCLUSION

Two bacterial species *Pseudomonas sp.* and *Bacillus sp* isolated in this study showed bioremediation potential. The results obtained revealed that *Pseudomonas sp.* demonstrated more degradation potential as compared to *Bacillus sp.* Comparison of present results and existing statistics with similar studies revealed that isolated *Pseudomonas sp.* and *Bacillus sp.* can be used for bioremediation of crude oil pollutant from the soil. Results of this study helps to use these isolated bacterial strains in different bioremediation processes based upon their efficiencies and oil degrading potential of bacterial isolates can be maximized by optimizing the process parameters. Further understanding of the mechanism of the hydrocarbon degradation process by these bacteria will help in developing strategies for removing crude oil from polluted areas.

ACKNOWLEDGMENT

The authors wish to thank SUS College of Research and Tecnology, Tangori, Punjab for providing the research facilities.

REFERENCES

[1] Wang J, Zhang ZZ, Su YM, He W, He F, Song HG, Phytoremediation of petroleum polluted soil, Petroleum Sci., 5 (2), 2008, 167-171.

[2] Lageman R, Clarke R and Pool W, Electro-reclamation, a versatile soil remediation solution, Engineering Geology, 77, 2005,191-201.

[3] Jain RK, Kapur M, Labana S, Lal B, Sharma PM, Bhattacharya D and Thakur IS, Microbial diversity: Application of microorganisms for the biodegradation of xenobiotics, Curr. Sci., 81(9), 2005, 101-112.

[4] Akoachere TK, Akenji TN, Yongabi FN, Nkwelang G and Ndip RN, Lubricating Oil Degrading Bacteria in Soil from Filling Stations and Auto Mechanic Workshops in Buea, Cameroon: Occurrence and Characteristics of Isolates, Af. J. Biotechnol., 7(11), 2008, 1700-1706.

[5] Thapa B, Ajay Kumar KC, Ghimire A, A review on bioremediation of petroleum hydrocarbon contaminants in soil Kathmandu University, J. Sci., Engg. and Technol., 8(1), 2012, 164-170.

[6] Bento FM, Camargo FAO, Okeke BC and Frankenberger WT, Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation, Biores. Technol., 96, 2005, 1049-1055.

[7] Sharma S, Bioremediation: Features, strategies and applications, Asian J. Pharm. and Life Sci., 2 (2), 2012, 6-10.

[8] Das N and Chandran P, Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview, Biotechnol. Res. Int., 2011, 1-13.

[9] Mandri T and Lin J, Isolation and Characterization of Engine oil Degrading Indigenous Microorganisms in Kwazulu-Natal, South Africa, Af. J. Biotechnol., 6 (1), 2006, 23-27.

[10] Nwachukwu SCU and Akpata TVI, Isolation of microorganisms by spread plate technique, In: Principles of Quantitative Microbiology, Lagos University Press, Nigeria, 2003, 6-10.

[11] Holt JG, Kreig NR, Sneat, PHA, Staley JT, Williams ST, Bergey's Manual of Determinative Bacteriology. 9th Ed., Lippincott Williams and Wilkins, Baltimore, USA, (Bergey, 1957), 1994.

[12] Chang R, Chemistry, 6th Ed., McGraw Hill Company, Inc., 1998, 962-963.

[13] Marquez-Rocha FJ, Hernandez-Rodriguez V, Lamela MT, Biodegradation of diesel oil in soil by a microbial consortium, Water Air Soil Pollut., 128, 2001, 313-320.

[14] Khan JA, Rizvi, SHA, Isolation and characterization of micro-organism from oil contaminated sites, Advances in Appl. Sci. Res., 2 (3), 2011, 455-460.

[15] Ganesh A and Lin J, Diesel Degradation and Biosurfactant Production by Gram-positive Isolates, Af. J. Biotechnol., 8 (21), 2009, 5847-5854.

[16] Onifade AK and Abubakar FA, Characterization of hydrocarbon degrading microorganisms isolated from crude oil contaminated soil by enhanced natural attenuation, Res. J. Biological Sci., 2, 2007, 36-40.

[17] Sathishkumar M, Binupriya AR, Baik S and Yun S, Biodegradation of crude oil by individual bacterial strains and a mixed bacterial consortium isolated from hydrocarbon contaminated areas, Clean, 36 (1), 2008, 92-96.

[18] Chaerun SK, Tazaki K, Asada R, Kogure K, Bioremediation of Coastal areas 5 years after the Nakhodka Oil Spill in the Sea of Japan: Isolation and Characterization of Hydrocarbon-Degrading Bacteria, Environ. Int., 30, 2004, 911-922.

[19] Regina OE, Emuobonuvie IF and Roseline UE, Growth responses of bacterial isolates on various concentrations of crude oil, The J. Am. Sci., 2 (2), 2006, 13-16.

[20] Udeani TKC, Obroh AA, Okwuosa CN, Achukwu PU and Azubike N, Isolation of Bacteria from mechanic workshops soil environment contaminated with used engine oil, Af. J. Biotechnol., 8 (22), 2008, 6301-6303.

[21] Ijah UJJ, Antai SP, Removal of Nigerian Light Crude Oil in Soil Over a 12–month Period, Int. Biodeterioration and Biodeg., 51, 2003, 93-99.

[22] Sepahi AA, Golpasha ID, Emami M and Nakhoda AM, Isolation and characterization of crude oil degrading *Bacillus sp*, Iranian J. Environmental Health Sci. and Engg., 5 (3), 2008, 149-154.

[23] Dibble JI and Barth R, Rehabilitation of oil polluted agricultural land: A case study, Soil Sci., 123, 1991, 56.

[24] Ijah UJJ and Okang CN, Petroleum degrading capabilities of bacteria isolated from soil, W. A. J. Biol. Appl. Chem., 38 (1-4), 1993, 9-15.

[25] Johnson K, Andersen S and Jacobsen CS, Phenotypic and genotypic characterization of phenanthrene-degrading fluorescent *Pseudomonas* biovars, Appl. Environ. Microbiol., 6 2, 1996, 3818-3825.

[26] Kiyohara H, Takizawa N and Nagao K, Natural distribution of bacteria metabolizing many kinds of polyaromatic hydrocarbons, J. Ferment. Bioengg., 74, 1992, 49-51.

[27] Bhattacharya D, Priyangshu M Sarma, Krishnan S, Mishra S and Lal B, Evaluation of genetic diversity among *Pseudomonas citronellolis* str ains isolated from oily sludge-contaminated sites, Appl. Environ. Microbiol., 69 (3), 2003, 1435-1441.

[28] Van Hamme JD, Singh A and Ward OP, Recent advances in petroleum microbiology, Microbiol. Mol. Biol. Rev., 67 (4), 2003, 503-549.

[29] Raed S al-Wasify and Shimaa RH, Bacterial biodegradation of crude oil using local isolates, Int. J. Bacteriol., 2014, 2014, 1-8.

[30] Roostan Z, Safahieh A, Mojodi F, Zolgharnein H, Ghanemi K and Abiar H, Phenanthrene biodegradatio n by *Pseudomonas aeruginosa* and *Bacillus subtilis* isolated from Persian gulf sediments, Af. J.Microbiol. Res., 6 (21), 2012, 4585-4591.

[31] Ghazali FM, Rahman RNZA, Salleh AB and Basri M, Biodegradation, Int. Biodeterior. 54, 2004, 61-67.