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BIODEGRADATION OF OIL USING SOIL ISOLATES

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ABSTRACT

Biodegradation of hydrocarbons by natural populations of microorganisms is the main process acting in the depuration of hydrocarbon polluted environments. Unregulated use of hydrocarbons causes high pollution to the environment which causes catastrophic consequences for the biotic and abiotic components of the ecosystem. The present work was to isolate and identify the hydrocarbon degrading bacteria. In this direction samples were collected from various oil contaminated sites. The isolated strains were further studied to determine their biodegradation activity on hydrocarbons. Microbial growth and protein profile was determined using calorimeter. In this study we observed that, the culture 7 has maximum potential for the degradation the oil at pH 11, and 37 C. The culture 7 degrades 71% kerosene as well as crude oil.

Key words: Biodegradation, Hydrocarbons, Bioremediation, Bioattenuation, Bioaugmentation.

INTRODUCTION

Biodegradation is the usage of bioremediation and biotransformation approaches to exploit the natural capacity of the bacterial xenobiotic metabolism to remove or accumulate contaminants in the atmosphere, particularly hydrocarbons **[1].** Episodic and chronic oil pollution causes major disruption of the local ecological environment **[2].** Light hydrocarbons (gas, gasoline , diesel), synthetic hydrocarbons (lubricants, synthetic oil , palm oil), halogenated solvents, and other large molecules (polycyclic aromatic hydrocarbons, PAHs, etc.) are the oil polluting compounds **[3].** Organic compounds are implicated in almost 75 per cent of these contaminated sites' pollution **[4].** Many substances are destroyed quickly, some are resistant to degradation and some are non-biodegradable. Biodegradation of various petroleum compounds occurs concurrently but at different rates because different species of microbes target different compounds preferentially **[5].** Which contributes to the gradual disappearance over time of individual petroleum components. Microorganisms generate enzymes which are responsible for attacking the hydrocarbon molecules in the presence of carbon sources. Biodegradation principles are based upon bioattenuation, bioaugmentation, and biostimulation **[6].** Crude oil (petroleum) a liquid blended with a variety of hydrocarbon compounds obtained from ancient remains of algae and plants and found in reservoirs below the surface of the Earth **[7].** Some crude oils have specific constituents of nitrogen and sulphur containing molecules ("resins"). Particular content of crude oil: Crude oil is a complex mixture of hydrocarbons such as Paraffin (15-60%), Naphthalene (30-60%), Aromatics (3-30%), Asphaltics (remainder) and chemicals **[8].** Depending on where and how the petroleum was produced the composition varies widely. A chemical analysis can in reality be used to fingerprint the petroleum source. Yet the characteristic properties and composition of raw petroleum or crude oil were defined **[9]**. Shukor, M.Y., et al., 2009 identified diesel degrading strain isolated from a contaminated site with hydrocarbons.

Based on partial 16S rDNA molecular phylogeny and biologic GP microplate panels and micro log database, the isolate was tentatively classified as Staphylococcus aureus strain DRY11. Isolate 11 showed an almost linear increase in cellylar growth as regards concentration of diesel.Optimization studies using different nitrogen sources showed that the best nitrogen source was potassium nitrite. An almost complete removal was seen from the reduction in hydrocarbon peaks observed using Solid Phase Micro Extraction Gas Chromatography analysis after 5 days of incubation **[10]**. Yussoff, N.A., *et al.*, 2010, isolate four species of bacteria, *Acinetobacter lwoffi, Aeromonas hydrophila, Pseudomonas aeuroginosa* and *Pseudomonas putida* from soil contaminated hydrocarbons and selected for the determination of growth requirements and the ability to degrade petroleum hydrocarbon. The bacteria were grown in mineral salt medium (MSM) supplemented with two types of crude oil, either Sumandak or South Angsi at 1% (v/v) concentration. Gas chromatography analysis showed that the South Angsi crude oil components of C12 to C25 were more extensively degraded by *A. lwoffi* after 24 hr of incubation compared to the other bacteria over the same period **[11]**. Bijay Thapa, Ajay Kumar KC, *et al.*, 2012, illustrated the pros and cons of using bioremediation process for the remediation of petroleum contaminants in soil. They included land farming, composting, anaerobic degradation, bioaugmentation, biostimulation etc. and they thus developed most efficient and less time consuming technologies **[12]**. KV Sajna., 2015, had worked on the isolation of the bacterial strain. In this direction samples were collected from the New York. In this study, by applying the various isolation techniques almost twenty potent bacterial strains were isolated. These bacterial strains were allowed to grow by providing suitable nitrogen and carbon sources so that it was found that *Acinetobacter baumanni, Serratia marcescens and Pseudomonas sp.* were able to degrade the hydrocarbons at much higher rates. An optimum pH and temperature was also provided for growth of bacteria. The bacterial strains were incubated for certain period to reach optimum growth kinetics **[13]**.

MATERIALS AND METHODS

Sample collections:

Samples were taken from 2-2.5 inches below ground level in sterile polybags and were brought to laboratory. Sample from garage and service workshop were black in color while sample from petrol pump was sandy and off white brownish in color. Two month old used engine oil was collected from Shakeel Mechanic's shop, Gomti Nagar, Lucknow. Oil sample was brownish black in color **[14]**.

Isolation of oil degrading bacteria:

The collected soil samples were serially diluted in sterilized 0.85% saline and spread over the nutrient agar plates. The culture plates were incubated at 37°C for 24 hours, once the growth observed then these cultures were selected on the behalf of morphological parameters and streaked over nutrient agar plates **[15]**. Further the screening of oil degrading bacteria was performed by screening the protein profile by lowrys assay **[16]** on the minimal salt media supplemented with oil and observing the clear zones on minimal salt agar media with oil.

Media selection:

The best media was selected on the basis of maximum growth of culture and maximum protein production by protein profile analysis. Additional effect of pH and temperature was performed **[17, 18].**

Bacterial Growth Curve:

The culture was inoculated in sterilized optimized media and incubated at 37°C for few weeks. Then OD was taken at 620 nm, in spectrophotometer, after 24 hours of time interval **[19]**.

Oil degradation percentage:

Culture 7 was inoculated in minimal salt media supplemented with 1% oils and incubated for observing the oil degradation and calculating its percentage **[20]**.

Oil Degradation% =

$$\frac{\mathbf{V\ of\ oil\ on\ 0\ day - V\ of\ oil\ on\ 5th\ day}}{\mathbf{V\ of\ oil\ on\ 0\ day}} \boldsymbol{x100}$$

RESULTS

Collection of sample:

The soil samples were collected from four different areas, such as:

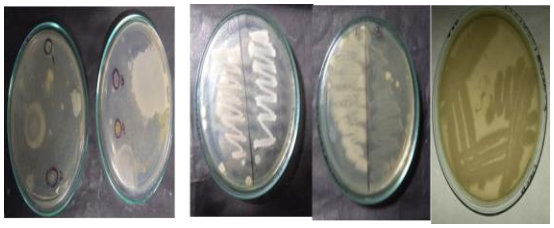
i. Shakil Garage, Gomti Nagar, Lucknow.
ii. Rakesh Service Workshop, Transport Nagar, Agra.
iii. Indian Oil Petrol pump, Mathura Road, Agra.
iv. Petrol pump, near Bank Of India, Gomti Nagar, Lucknow

S1 **S2** **S 3** **S 4**

Figure 1: soil samples collected from oil contaminated sites.

Isolation of oil degrading bacteria:

Bacteria from oil were isolated by serial dilution method and spread plate method. These colonies were further selected on the basis of morphology and then pure cultures were prepared by streak plate method.



a: spread plates **b: streak plates**

Figure 2: Grown bacterial cultures on spread plates and streak plates.

Screening of purified cultures for oil degradation potential:

The cultures were inoculated to the minimal salt media supplemented with 1% oil and then the OD was taken after 24 hours for the analysis of bacterial growth and protein profile. Where culture 3,7,11 shown maximum production further the screening of these three cultures were carried out by the clear zone appearance on the minimal agar media with oil where culture 7 shown maximum zones and selected for oil degradation.

Table 1: Determination of growth and protein profile of cultures at 620 nm and 680 nm and its concentration.

Isolates	Days	OD (620 nm)	OD (680 nm)	Conc. (mg/ml)
C 1.	1.	0.00	0.01	0.002
	3.	0.00	0.00	0.00
	5.	0.01	0.01	0.002
C 2.	1.	0.00	0.00	0.00
	3.	0.02	0.02	0.01
	5.	0.01	0.01	0.002

C 3.	1.	0.00	0.00	0.00
	3.	0.02	0.03	0.014
	5.	0.00	0.01	0.002
C 4.	1.	0.00	0.02	0.01
	3.	0.00	0.01	0.002
	5.	0.00	0.00	0.00
C 5.	1.	0.00	0.00	0.00
	3.	0.10	0.01	0.002
	5.	0.08	0.01	0.002
C 6.	1.	0.00	0.00	0.00
	3.	0.02	0.02	0.01
	5.	0.05	0.01	0.002
C 7.	1.	0.00	0.00	0.00
	3.	0.03	0.03	0.014
	5.	0.01	0.01	0.002
	1.	0.02	0.02	0.01

C 8.	3.	0.01	0.01	0.002
	5.	0.00	0.00	0.00
C 9.	1.	0.00	0.01	0.002
	3.	0.10	0.00	0.00
	5.	0.01	0.01	0.00
C 10.	1.	0.00	0.00	0.00
	3.	0.00	0.02	0.01
	5.	0.04	0.01	0.002
C 11.	1.	0.00	0.00	0.00
	3.	0.02	0.03	0.014
	5.	0.03	0.01	0.002
C 10.	1.	0.00	0.02	0.01
	3.	0.08	0.01	0.002
	5.	0.02	0.00	0.00

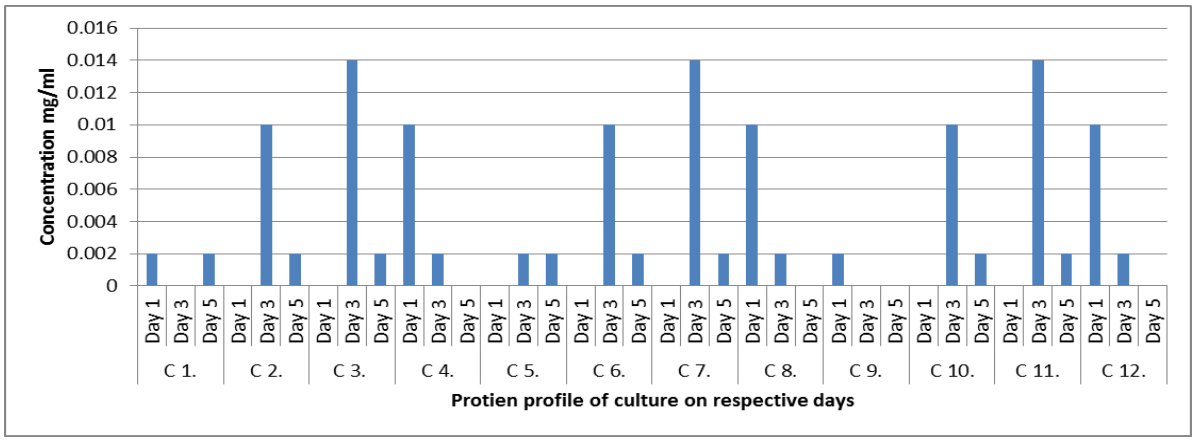


Figure 3: Graphical representation of the protein profile analysis of cultures on day 1, 3,5

Table2. Study of colony morphology

Cultures	Shape	Surface	Elevation	Margin	Texture
C 1.	Circular	Rough	Flat	Entire	Hard
C 2.	Circular	Rough	Raised	Entire	Hard
C 3.	Circular	Smooth	Flat	Entire	Gummy
C 4.	Circular	Smooth	Convex	Entire	Gummy
C 5.	Irregular	Smooth	Convex	Discrete	Gummy
C 6.	Circular	Smooth	Convex	Discrete	Gummy
C 7.	Circular	Smooth	Convex	Discrete	Gummy
C 8.	Circular	Smooth	Convex	Discrete	Gummy
C 9.	Circular	Smooth	Convex	Discrete	Hard
C 10.	Circular	Smooth	Convex	Entire	Gummy
C 11.	Circular	Rough	Flat	Entire	Gummy
C 12.	Circular	Rough	Flat	Entire	Gummy

Figure 4: clear zone shown by culture 7 on minimal agar media and oil.

Identification of culture by biochemical tests:

Table 3: The selected culture 7 was identified by various biochemical tests.

Tests	Results
Grams staining	Positive (Bacillus)
Endospore test	Positive
Catalase test	Positive
Mannitol test	Negative.

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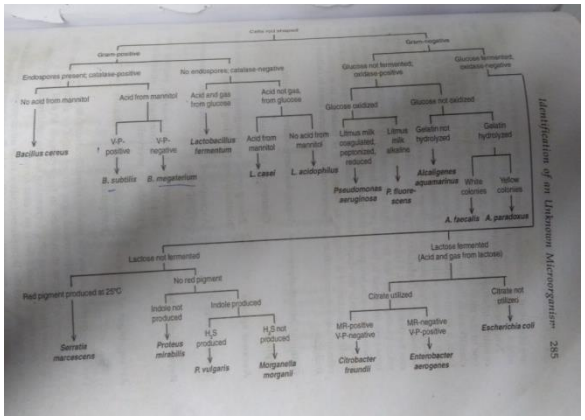

Figure 5: flow chart for the characterization of the culture 7.

Selection of production media:

The media optimization was carried out by applying the one factor at a time method.

A: Selection of media:

Selection was carried out on the basis of bacterial growth and protein profile analysis. Where MSM 1 media was selected as best media for oil degradation.

Table 4: Bacterial growth of culture 7 on media at 620 nm.

Media	OD (620 nm)		
	1 day	3 day	5 day
NB	0.57	1.16	1.01
LB	0.18	1.11	0.45
MSM-1	0.18	0.45	1.10
MSM-2	0.11	0.13	0.11

Table 5: Protein profile of the culture 7 on media at 680 nm.

Media	OD (680nm)	Conc. (mg/ml)
1 day		
NB	0.00	0.00
LB	0.03	0.014
MSM1	0.16	0.064
MSM2	0.11	0.02
2 day		
NB	0.09	0.02
LB	0.06	0.012
MSM1	0.30	0.26
MSM2	0.13	0.04
3 day		
NB	0.03	0.014
LB	0.06	0.012
MSM1	0.56	0.30
MSM2	0.11	0.02

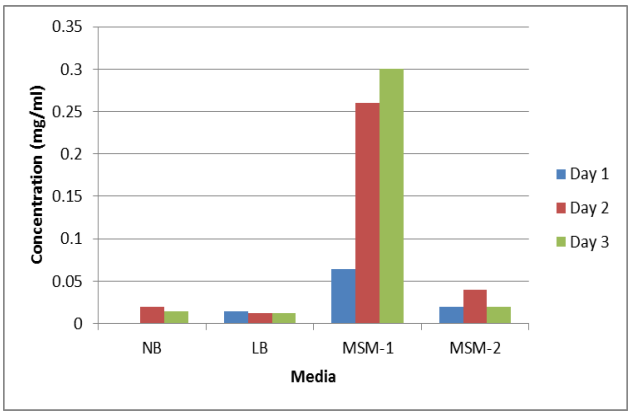

Figure 6: Graphical representation of the protein profile of culture7

Temperature	Growth	Remarks
22^{O}C	No Growth	-
28^{o}C	Growth	++
37^{O}C	Growth	+++
50^{O}C	Growth	+

B: Effect of pH and temperature on bacterial growth:

pH 11 and 37°C temperature shows maximum production of culture 7

Table 6: Effects of pH on the growth of culture 7

pH	OD (620nm)		
	1 day	3 day	5 day
5	0.08	0.09	0.03
7	0.03	0.06	0.06
9	0.05	0.03	0.00
11	0.10	0.27	0.99

Table 7: Effects of temperature on the growth of culture 7

Calculation of oil degradation percentage of culture 7

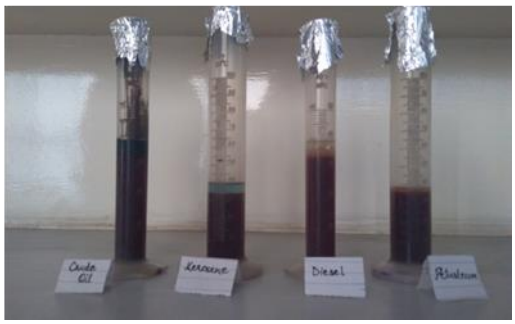

Figure 7: representation of modified media with crude oil, petrol, diesel and kerosene with culture 7

Table 8: Determination of oil degradation percentage of culture7

Oil	Percentage of degradation of oil
Crude oil	71 %
Petrol	21 %
Diesel	46 %
Kerosene	71 %

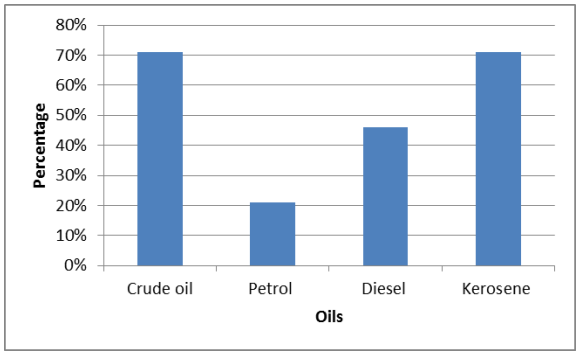

Figure 8: Graphical representation of oil degradation percentage of culture 7

DISCUSSION AND CONCLUSION

In this study the soil sample was collected from various oil contaminated sites because the aptitude of native bacterial residents to petrify crude oil contaminated hydrocarbons in crude oil contaminated site were confirmed by many researchers **[21].** Further microorganism was isolated by serial dilution method. Firstly the culture was screened on the basis of growth and the protein profiling in the presence of MSM-1 hence the culture 1 from the sample 4 was selected for the oil degradation potential hence termed as culture 7.

After the selection of the culture the media was selected for the enhancement of the growth of the culture the four media LB, NB,MSM-1, MSM-2 was selected for this purpose, NB is composed of yeast, beef, peptone, NaCl, and LB composed of yeast, peptone, NaCl. MSM-1 and MSM-2 composed of various salts, supplemented with engine oil was determined by growth kinetics throughout the incubation period that reflect the ability of isolated *Bacillus sp.,* to degrade and utilize crude oil as carbon source. This technique has been used several studies to determine the oil degradation potential of bacteria in crude oil. On the basis of the growth kinetics and protein

profile we select one carbon source,

In the current study purified culture was characterized for various staining and biochemical tests according to Bergey's manual **[22].** The isolated strains were characterized in the basis of morphological analysis, gram staining, endospore staining and by some biochemical tests including catalase test, mannitol test, NaCl concentration test and protease test. Results were obtained by comparing the results with Bergey's manual also done earlier by **[23].**

The isolate showing maximum oil degradation abilities was found to be gram positive, *bacillus sp.,* endospore positive, catalase positive and mannitol negative. Thus, specie was identified as *Bacillus cerus.*

REFERENCES

[1] Joutey, N. T., Bahafid, W., Sayel, H., & El Ghachtouli, N. (2013). Biodegradation: involved microorganisms and genetically engineered microorganisms. *Biodegradation-life of science*, 289-320.

[2] Kassotis, C. D., Tillitt, D. E., Lin, C. H., McElroy, J. A., & Nagel, S. C. (2016). Endocrine-disrupting chemicals and oil and natural gas operations: potential environmental contamination and recommendations to assess complex environmental mixtures. *Environmental health perspectives*, *124*(3), 256-264.

[3] Onuska, F. I. (2017). Analysis of polycyclic aromatic hydrocarbons in environmental samples. In *Analysis of trace organics in the aquatic environment* (pp. 211-248). CRC Press.

[4] Top, E. M., & Springael, D. (2003). The role of mobile genetic elements in bacterial adaptation to xenobiotic organic compounds. *Current Opinion in Biotechnology*, *14*(3), 262-269.

[5] Thapa, B., Kc, A. K., & Ghimire, A. (2012). A review on bioremediation of petroleum hydrocarbon contaminants in soil. *Kathmandu university journal of science, engineering and technology*, *8*(1), 164-170.

[6] Lebeau, T. (2011). Bioaugmentation for in situ soil remediation: how to ensure the success of such a process. In *Bioaugmentation, biostimulation and biocontrol* (pp. 129-186). Springer, Berlin, Heidelberg.

[7] Yen, T. F. (1975). Genesis and degradation of petroleum hydrocarbons in marine environments.

[8] Chaurasia, P. K., Bharati, S. L., & Mani, A. (2018). Enzymatic Treatment of Petroleum-Based Hydrocarbons. In *Handbook of Research on Microbial Tools for Environmental Waste Management* (pp. 396-408). IGI Global.

[9] Rostler, F. S., & Sternberg, H. W. (1949). Compounding rubber with petroleum products-correlation of chemical characteristics with compounding properties and analysis of petroleum products used as compounding ingredients in rubber. *Industrial & Engineering Chemistry*, *41*(3), 598-608.

[10] Shukor, M. Y., Hassan, N. A. A., Jusoh, A. Z., Perumal, N., Shamaan, N. A., MacCormack, W. P., & Syed, M. A. (2009). Isolation and characterization of a Pseudomonas diesel-degrading strain from Antarctica. *J. Environ. Biol*, *30*(1), 1-6.

[11] Hamzah, A., Rabu, A., Azmy, R. F. H. R., & Yussoff, N. A. (2010). Isolation and characterization of bacteria degrading Sumandak and South Angsi oils. *Sains Malaysiana*, *39*(2), 161-168.

[12] Thapa, B., Kc, A. K., & Ghimire, A. (2012). A review on bioremediation of petroleum hydrocarbon contaminants in soil. *Kathmandu university journal of science, engineering and technology*, *8*(1), 164-170.

[13] Sajna, K. V., Sukumaran, R. K., Gottumukkala, L. D., & Pandey, A. (2015). Crude oil biodegradation aided by biosurfactants from Pseudozyma sp. NII 08165 or its culture broth. *Bioresource technology*, *191*, 133-139.

[14] Roy, A. S., Baruah, R., Borah, M., Singh, A. K., Boruah, H. P. D., Saikia, N., ... & Bora, T. C. (2014). Bioremediation potential of native hydrocarbon degrading bacterial strains in crude oil contaminated soil under microcosm study. *International Biodeterioration & Biodegradation*, *94*, 79-89.

[15] Rahman, K. S. M., Rahman, T., Lakshmanaperumalsamy, P., & Banat, I. M. (2002). Occurrence of crude oil degrading bacteria in gasoline and diesel station soils. *Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms*, *42*(4), 284-291.

[16] Bhatnagar, T., Boutaiba, S., Hacene, H., Cayol, J. L., Fardeau, M. L., Ollivier, B., & Baratti, J. C. (2005). Lipolytic activity from Halobacteria: screening and hydrolase production. *FEMS Microbiology Letters*, *248*(2), 133-140.

[17] Wang, L., Wang, W., Lai, Q., & Shao, Z. (2010). Gene diversity of CYP153A and AlkB alkane hydroxylases in oil-degrading bacteria isolated from the Atlantic Ocean. *Environmental microbiology*, *12*(5), 1230-1242.

[18] Ibrahim, M. L., Ijah, U. J. J., Manga, S. B., Bilbis, L. S., & Umar, S. (2013). Production and partial characterization of biosurfactant produced by crude oil degrading bacteria. *International Biodeterioration & Biodegradation*, *81*, 28-34.

[19] Liu, H., Yao, J., Yuan, Z., Shang, Y., Chen, H., Wang, F., ... & Choi, M. M. (2014). Isolation and characterization of crude-oil-degrading bacteria from oil-water mixture in Dagang oilfield, China. *International Biodeterioration & Biodegradation*, *87*, 52-59.

[20] Lin, Q., & Mendelssohn, I. A. (1998). The combined effects of phytoremediation and biostimulation in enhancing habitat restoration and oil degradation of petroleum contaminated wetlands. *Ecological Engineering*, *10*(3), 263-274.

[21] AKHAVAN, S. A., DEZHBAN, G. P. I., Emami, M., & Nakhoda, A. M. (2008). Isolation and characterization of Crude oil degrading Bacillus spp.

[22] Srivastava, J., Naraian, R., Kalra, S. J. S., & Chandra, H. (2014). Advances in microbial bioremediation and the factors influencing the process. *International Journal of environmental science and technology*, *11*(6), 1787-1800.

[23] Udeani, T. K. C., Obroh, A. A., Okwuosa, C. N., Achukwu, P. U., & Azubike, N. (2009). Isolation of bacteria from mechanic workshops' soil environment contaminated with used engine oil. *African journal of Biotechnology*, *8*(22).