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APPLICATION OF OIL DEGRADING BACTERIAL ISOLATES FOR REMEDIATION OF OIL CONTAMINATED SOIL

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Abstract: Five Bacterial isolates MJU1101, MJU1102, MJU1103, MJU1104 and MJU1105 were isolated from oil contaminated soil and were screened for oil degradation potential in MSM (minimal salt media) supplemented with 5% used engine oil by studying the growth and protein profile. Out of the five three isolates MJU1101, MJU1102 and MJU1105 showed good oil degradation potential, and were identified based on Bergey's manual. All the three isolates individually and in combination were used to remediate the oil contaminated soil, and the effect of remediation on soil fertility was checked by sowing seeds of chickpea (***Cicer arietinu*m) in treated and non treated soil. It was seen that no germination occurred in untreated soil but chickpea germinated and grew well in soil treated with isolate MJU1101 and MJU1102. Germination was good and faster in MJU1101.

***Keywords*:** Bioremediation, Oil spills, Oil degradation potential, *Cicer arietinu*m, soil fertility.

Introduction:

Oil spill is defined as release of liquid petroleum hydrocarbons into environment. Cause of the oil spill can be the releases of crude oil from tankers, offshore platforms, drilling rigs and wells as well as spills of refined petroleum products (such as gasoline and diesel) and their by-products, heavier fuels used by large ships such as bunker fuel, or the spill of any oily refuse or waste oil, automobiles also use engine oils and after a specific period of time they are thrown into the soil or water body leading to pollution of the environment. Another significant route by which oil enters the marine environment is through natural oil seeps[1]

Oil spills are causing a major threat to the soil as well as water body. [2] reported that diesel oil spills on agricultural land generally reduces plant growth in diesel oil contaminated soils range from direct toxic effect on plants [3] and reduced germination [4] to unsatisfactory soil condition due to insufficient aeration of the soil because of the displacement of air from the space between the soil particles by diesel oil. Contamination of the soil by oil also causes it to lose its useful properties such as fertility, water-holding capacity, permeability and binding capacity. Contamination of groundwater is also a potential problem. The other significant impact is on surface water, mostly the nearby streams, which receive a lot of untreated effluent from service stations containing oil and grease as well as non-biodegradable detergents [5].

Spillage of oil to the water body is also a major threat to the aquatic life. Oil contains numerous toxic compounds that are released into the water as it breaks down. The most toxic to marine species are polycyclic aromatic hydrocarbons (PAHs) – chemicals such as benzene and toluene that are known carcinogens and neurotoxins to humans and animals, and in addition, cause other health problems[6].

Traditional methods (Booms, Skimmers, Sorbents, Vacuums, Shovels, incineration) and methods such as phyto-remediation, used for remediating oil pollution are costly and some of them are time taking. Bioremediation method used in the present study is cost effective as well as without any side effects leading to complete mineralization of oil.

Looking at the importance of Bioremediation over traditional methods and the previous studies [7; 8; 9; 10; 11] on bioremediation and its significance the present study was carried out to search oil degrading bacterial isolates and to check their oil degradation potential on soil fertility.

Materials and methods:

Collection of Soil Sample

Oil contaminated soil sample was collected from **Shakeel Motors, Vibhuti Khand, Gomti Nagar, Lucknow** from 2-2.5 inches below the ground level in sterile polybags and transferred to the laboratory. Soil sample was black in colour.

Collection of Oil sample

Used engine oil was collected from **Shakeel Motors, Vibhuti Khand, Gomti Nagar, Lucknow.** Oil sample was brownish black in colour.

Isolation of Bacteria from Soil

Bacterial flora was isolated from the oil contaminated sites by serial dilution agar plating method, in which soil sample was diluted upto 10^{-5} dilution and spread on respective sterile nutrient agar plates. Plates were incubated at 37 °C for 24 hours and observed after completion of the incubation period. Five different bacterial isolates were selected for further studies based on their colony morphology studies. They were tentatively named as MJU1101, MJU1102, MJU1103, MJU1104 and

MJU1105. All the five were sub cultured on sterile NA plates by quadrant streaking.

Screening of Isolates for Oil Degradation

All the five isolates were subjected to oil degradation studies wherein the oil degradation potential was judged based on their growth and protein profile in minimal salt media (0.8 g/l NaCl, 0.8 g/l KCl, 0.1 g/l $CaCl_2$, 2.0 g/l Na_2HPo_4, 0.2 g/l $MgSO_4$, 0.1 g/l $FeSO_4$, 8.0 g/l Glucose, 2.0 g/l NH_4Cl pH 7.2) supplemented with 5 % used engine oil. Six flasks each containing 100 ml of MSM supplemented with 5% used engine oil were prepared, autoclaved and cooled to room temperature. Five flasks were inoculated with 24 hour old grown broth culture of MJU1101, MJU1102, MJU1103, MJU1104 and MJU1105 respectively and the sixth flask was stored as blank. Growth profile was studied by tracking the growth of bacterial isolates on mineral salt agar plates and also by reading the absorbance of the inoculated media against the uninoculated blank for 10 days. Protein profile was also studied for 10 days by Lowry's method [12] in which a small aliquot of inoculated media was reacted with Lowry's reagents and concentration of protein was calculated by comparing the absorbance reading to the standard graph. As the minimal media contained limited amount of carbon source, the only source of carbon left after some time would be used engine oil. Thus the isolate or flask showing good growth and protein profile suggests good oil degradation potential of the isolate.

Identification of the Isolates having good oil degradation potential

Three of the five isolates namely MJU1101, MJU1102 and MJU1105 showing good oil degradation potential were selected for further studies and identified by performing various staining and biochemical activities based on Bergey's manual [13].

Application of Oil degrading Bacterial Isolates to Oil Contaminated Soil

In order to apply the isolates positive in screening to oil contaminated soil for remediating the same, 400 ml nutrient broth was prepared, autoclaved and cooled to room temperature. First three flasks were inoculated with 1 ml of 24 hour old grown broth culture of the isolates MJU1101, MJU1102 and MJU1105. Fourth flask was inoculated with 500 µl of all the three bacterial isolates positive in screening. All the flasks were incubated at 37 °C/ 120 rpm for 24 hours. After proper growth of the isolates the culture broth of MJU1101 was mixed with approximately 50 g of dried, autoclaved cow dung and incubated at 37 °C for 5 days. Similarly the other culture broths were also mixed with cow dung and incubated at 37 °C for 5 days. After that 50 g of oil contaminated soil was added to the cow dung culture mixture and again incubated at 37 °C for 10 days. In between the cow dung culture mixture was moistened with autoclaved and cooled MSM.

Effect of Remediation on Soil Fertility

In order to see the effect of remediation on soil fertility germination of seeds of chickpea was taken as a model and five pots were used, out of the five pots 1st, 2nd and 3rd were filled with the soil treated with isolate MJU1101, MJU1102, MJU1105 respectively and the 4th pot was filled with the soil treated with all the three isolates in combination. 5th pot was filled with untreated oil contaminated soil. All the pots were sown with seeds of chickpea **(*Cicer arietinu*m)** and the germination of seeds was tracked.

Results:

Isolation of Bacteria from Soil

Five different bacterial strains namely MJU1101, MU1102, MJU1103, MJU1104 and MJU1105 were selected from mixed culture plate based on colony morphology were sub cultured and used for further studies.

Screening of Isolate for Oil Degradation

All the five bacterial isolates were screened for oil degradation potential by inoculating the isolates in MSM supplemented with 5 % used engine oil and tracking the **growth and protein profile** of the isolates for 10 days. Growth of bacteria in the flask indicates the oil degrading potential as MSM has a limited carbon source that too for a limited period of time the only source of carbon left is oil. Similarly the concentration of protein in the flask also indicates the oil degradation potential.

Growth Profile

Growth of isolates in the flask was tracked by two methods and the results of the same are given below:

a) Enumeration on MSM Plates

In the first method number of cells in the flask was enumerated by spreading 10 µl of the culture from flask on sterile MSM agar plates. ***Table 1*** below shows number of colonies on 1st, 5th and 10th day of incubation.

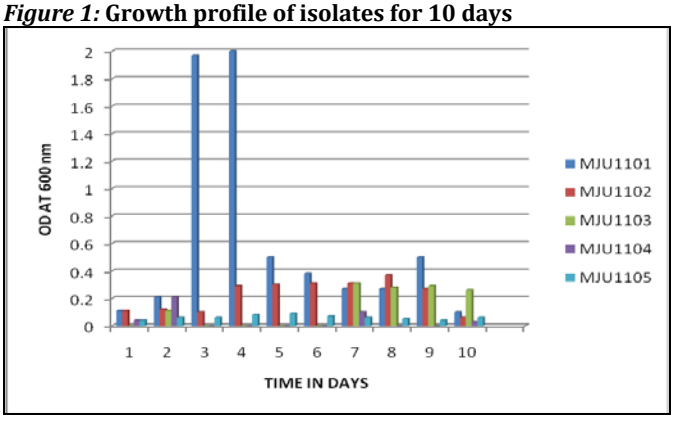
***Table 1*: Enumeration on MSM Plates**

S. No.	ISOLATE	No. OF COLONIES ON DAY ONE	No. OF COLONIES ON DAY FIVE	No. OF COLONIES ON DAY TEN
1.	MJU1101	LAWN	300	300
2.	MJU1102	80	250	240
3.	MJU1103	22	200	120
4.	MJU1104	25	50	30
5.	MJU1105	10	40	50

b) Absorbance at 600 nm

In the second method the growth of bacterial isolates in the flask was tracked by reading the absorbance of the inoculated media against uninoculated blank for 10 days at 600 nm. Results of the same can be seen below in ***Figure 1.***

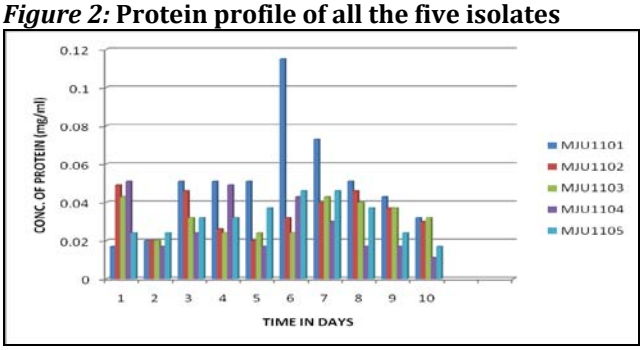
***Figure 1:* Growth profile of isolates for 10 days**



Protein Profile

Concentration of protein in the inoculated flasks was determined by Lowry's method of protein estimation and protein profile of all the five isolates can be seen below in **Figure 2.**

Figure 2: **Protein profile of all the five isolates**



Selection of the Best Oil Degrading Isolates

Based on the growth and protein profile of all the five isolates three isolates MJU1101, MJU1102 and MJU1105 were selected for further studies i.e their identification and application to oil contaminated soil, and benefit to soil fertility.

Identification of Isolates Showing Oil Degradation

All the three isolates selected for further studies were identified by comparing the staining and biochemical characteristics with Bergey's manual **(Aneja K.R., 2003)**

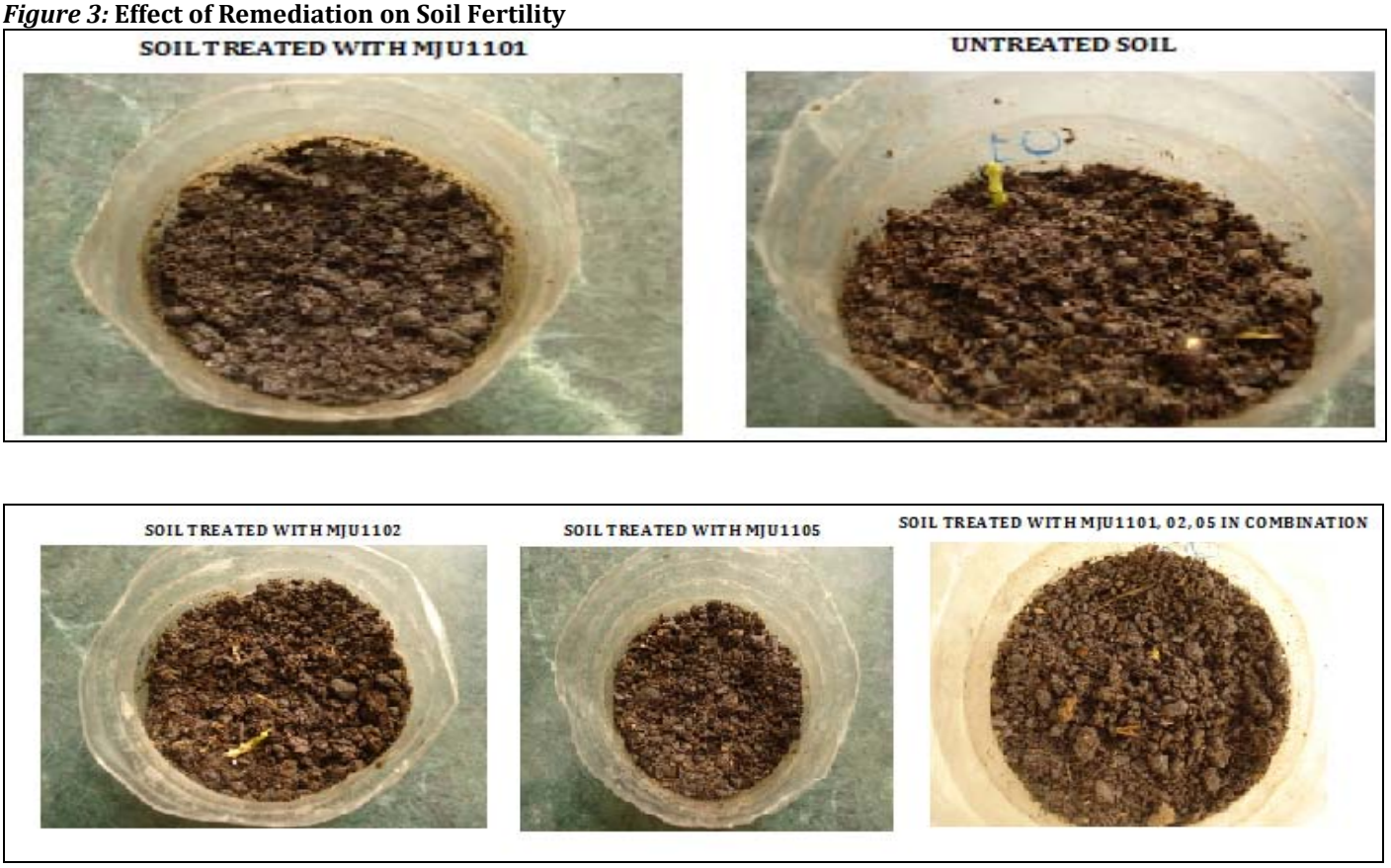
***Table 2*: Identification of Isolates**

S. No.	STAINING/ BIOCHEMICAL TESTS	RESULTS FOR MJU1101	RESULTS FOR MJU1102	RESULTS FOR MJU1105
1.	Gram staining	+ ve Rods	+ ve Coccus	+ ve Rods
2.	Endospore staining	+ ve	+ ve	+ ve
3.	Catalase test	+ ve	+ ve	+ ve
4.	Mannitol Fermentation test	- ve	+ ve	+ ve
5.	Voges Proskeurs test	NA	NA	- ve
6.	Glucose fermentation test	NA	- ve	NA
ISOALTE IDENTIFIED AS		*Bacillus cereus*	*Staphylococcus aureus*	*Bacillus megaterium*

Effect of Remediation on Soil Fertility

Out of the four treated pots, the pots treated with isolates MJU1101 and MJU1102 individually showed germination of seeds but it was faster and better in the pot containing soil treated with MJU1101 and thus it can be called as the best out of the four treatments. **Figure 3** below shows the treated as well as untreated pots after 8 days incubation.

Figure 3: **Effect of Remediation on Soil Fertility**



Discussion:

Oil degrading microorganisms were isolated from soil contaminated with engine oil using serial dilution agar plating method similar methods have been used by [2] for isolation of oil degrading microorganisms.
Microorganisms isolated from oil contaminated soil were screened for oil degradation potential by studying the growth and protein profile of the isolates when they were inoculated in minimal salt media similar methods of screening have been used by **[14].**

Oil degrading bacterial isolates were identified by comparing the staining and biochemical characteristics with Bergey's manual.

For degradation of oil attached to soil bacterial isolates were first seeded to cow dung and then the oil contaminated soil was mixed to it and incubated at 37 °C for 10 days this a new method of treatment and after the results it can be said to be an effective method also as the results are just on 10 days treatment and are expected to be better when the treatment time is increased.
Chickpea **(*Cicer arietinu*m)** was taken as a model plant system in order to reduce the experiment duration because it has been known for its quick germination period.

Conclusion:

Based on the above study it can be said that the isolates MJU1101 and MJU1102 can be very good source for onsite oil degradation that is they can be used directly on the oil contaminated plots in order to remediate the same. It can be seen from the results of soil remediation that however isolate MJU1105 was effective during flask level studies it was not able to work directly on oil attached to the soil. Lots of factors may be working behind that but it would be a premature statement to say it was ineffective as the incubation for germination is just eight days it might be possible that the seed germinates after some more time.

Future prospects of the current research work includes increase in the contaminated oil treatment time so that the culture gets enough time to act on the oil attached to the soil. Restricted time period of the present study did not allow us to give much time for soil treatment in the incubator. Future work also includes the oil degradation studies in small water bodies and its effect on aquatic life.

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