Bioremediation of diesel contaminated soil by oil degrading bacteria 
(Pseudomonas sp.) using biostimulation method

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ABSTRACT

One of the greatest problems that the people of the world are facing today is environmental pollution. It is rapidly increasing every year causing grave and irreversible consequences. Pollutants such as exhaust gases, industrial effluents, soap water, domestic wastes, medical wastes, etc. cause damage to human health and safety of other living organisms. Diesel, a derivative of Petroleum is one of the major pollutants of water and soil. In areas where oil refineries are present, air, water and soil resources have become contaminated with oil and its byproducts namely diesel. Transportation through the railways and roadways causes organic and inorganic contamination. Broken and corroded oil pipelines significantly contribute to oil pollution on a large and rapidly increasing scale. The numbers of spills that have dumped millions of gallons of crude oil into the environment have been steadily increasing over the past decade. This can lead to disastrous consequences. Therefore the pollutants need to be removed or destroyed from the environment. Bioremediation of the oil contaminated soil by biostimulation is a good option that offers the possibility of destroying the contaminants by the biological activity of indigenous microorganisms, already present in the contaminated soil. The purpose of the present study was to find out a possible ex–situ treatment method that can help clean up diesel contaminated soil sites. Several Pseudomonas sp. isolated from oil contaminated soils possess great potential to degrade oil and can be effectively used in bioremediation. The present investigation shows that Pseudomonas sp. isolated from the study samples can be effectively used to clean up oil contaminated soils.

Keywords: Oil Pollution, Diesel, ex–situ treatment method, Bioremediation, Biostimulation, Pseudomonas sp.

INTRODUCTION

The quality of life on earth is linked, inextricably, to the overall quality of the environment. The natural ecosystem encompasses all living and non-living things occurring naturally on Earth. It is an environment that interacts with of all living species [8]. The increasing pace of industrialization in public and private sectors along with urbanization explosion and green revolution are reflected in varying degree of pollution in water, soil and air [12]. Oil and oil derivatives represent a significant source of environmental pollution [19]. Long-term extraction and production of oil derivatives causes contamination of adjacent soil and a good amount of ecological hazardous oil pollutants was noted by [2]. Contamination of the soil by oil causes it to lose its useful properties such as fertility, water-holding capacity, permeability and binding capacity. The contamination of groundwater is also a potential problem, which receives a lot of untreated effluent from service stations containing oil and grease [20]. Oil hydrocarbons have been known to disrupt ecosystem functions [14]. When it is determined that the presence of such contaminants at a site creates a situation of unacceptable risk to public health and the environment, remedial action is required to reduce the respective chemical concentrations to acceptable levels or to minimize exposure to receptors [11]. The conventional techniques used for remediation have serious disadvantages. So a better approach than these traditional
methods is necessary. Bioremediation is an option that offers the possibility of destroying or rendering harmless various contaminants using natural biological activity. It is relatively of low-cost, low-technology techniques, which generally have a high public acceptance than the traditional methods [21]. Biostimulation is considered as the most appropriate bioremediation technique for diesel removal in soil [13]. The technique of biostimulation, uses microbial communities which are already present and stimulates those bacteria capable of the desired degradation [4]. Although most organisms have detoxification abilities, microorganisms, particularly bacteria, play a crucial role in bioremediation process and in sustainable development of the biosphere. They do so by producing biosurfactants. Biosurfactants are a class of biomolecules derived from microbial sources that possess surface properties akin to the chemically synthesized surfactants. Being a molecule with both a hydrophobic and a hydrophilic group, biosurfactants have a host of advantages over their chemical counterparts [17]. Some members of the genus *Pseudomonas* are able to metabolise chemical pollutants in the environment and as a result can be used for bioremediation [5].

The purpose of the present study was to investigate a possible *ex–situ* method to enhance the rate of biodegradation of diesel contaminated soil sites. The main objectives of the study were to isolate a potential strain which could be used in bioremediation of oil contaminated sites and to find out the efficiency of the isolate in preliminary screening of bioremediation. They study also aimed to investigate the use of biosurfactant in soil cleanup methods and to prove that biostimulation can be effectively employed in the remediation of crude oil polluted soil ecosystem.

**MATERIALS AND METHODS**

**Collection of Soil Samples**
The five different study sites includes Chennai Petroleum Corporation Limited (CPCL), Thiruvottiyur, Avadi, Central and Egmore EMU sheds respectively. The soil at the sites had a characteristic black color due to continuous oil spillage and the soil surfaces were hard. Samples were collected in each site by digging up the soil with a hoe and transferring directly into clean, sterile containers. Also, clean soil samples were collected from non contaminated reference areas.

**Estimation of oil using Gravimetric method**
The amount of oil in soil was estimated using the Gravimetric method. About 1 gram (g) of the soil was taken from each sample site. Petroleum ether and acetone were taken in the ratio 1:1 and was mixed with the soil sample in a separating funnel. The mixture was shaken for about 45 minutes and then was left undisturbed for about 10 minutes. The upper solvent along with oil was separated from the lower soil extract. The solvent with the oil layer was then kept in the hot air oven at about 50º C until the solvent gets evaporated. After the complete evaporation, the oil residue obtained was weighed and taken as the gravimetric value for further calculation. Analysis of soil before and after treatment was done using this Gravimetric method. The percentage of diesel oil degraded was determined from the following formula:

\[
\text{Percentage of diesel oil degraded} = \frac{\text{Weight of diesel oil degraded}}{\text{Weight of diesel oil present originally}} \times 100
\]

Where,

The weight of diesel oil degraded = original weight of diesel oil - weight of residual diesel oil obtained after evaporating the extractant.

**Isolation of Oil Degrading Bacteria**
The oil degrading bacteria was isolated by enrichment technique.

**Nutrient Broth:** The nutrient broth medium was used for the growth and maintenance of the bacterial cultures. It contained Beef extract 1.50 g/L, yeast extract 1.50 g/L, peptic digest of animal tissue 5.0 g/L, sodium chloride 5.0 g/L and dissolved in 1000 ml of distilled water. Then the media was enriched with 10 % of diesel. The pH was adjusted to 7.5-7.6 before autoclaving at 121ºC and 101.3 k Pa for 15 minutes.

**Nutrient Agar :** The nutrient agar medium contained 1.50 g/L of beef extract, 1.50 g/L of yeast extract, 5.0 g/L of peptic digest of animal tissue, 5.0 g/L of sodium chloride, 2% agar and dissolved in distilled 1000 ml water. The pH
was adjusted to 7.5-7.6. It was then sterilized by autoclaving at 121°C for 15 min. The medium was then cooled to approximately 50°C and enriched with 10% diesel prior to pouring (~20 ml) into sterile petridishes. The molten agar was left to cool and gel at room temperature.

Isolation of Bacterial Diesel Degraders
Nutrient agar plates enriched with 10% diesel were prepared. 1 g of the diesel contaminated soil sample was added to 100 ml of distilled water. The flask was placed in a rotary shaker for about 30 minutes at 30°C. Serial dilutions of the 5 samples were performed separately. Six sterilized test tubes containing 9 ml of distilled water were taken for each sample. Ten fold serial dilutions were made by transferring 1 ml from the first test tube to the next and so on. Dilutions were made from 1:10 to 1:1000000. A sterile micropipette tip was used to dispense 0.1 ml from each dilution onto duplicate nutrient agar plates. A glass spreader dipped in alcohol, flamed and cooled was used for spreading the plates. The petriplates were then incubated at 30°C for 24 hours for growth of bacteria.

Growth and maintenance of Bacterial Isolates:
A fresh single pure colony of each bacterial isolates was transferred aseptically from agar plate into Nutrient Agar broth medium using a sterile loop. The inoculated medium was then incubated at 37°C at 100 rpm in orbital shaker. All pure isolates were maintained in liquid and solid media. They were regularly sub cultured into fresh medium for short-term storage.

Screening of Biosurfactant Producing Isolates
The isolates of the microbes thus obtained was then screened for production of oil degrading biosurfactant. In our present study the screening was done by the following methods:

Drop collapse method: Biosurfactant production was screened using the qualitative drop collapse test. 2µl of diesel was added to 96 well micro-titre plates. The plates were equilibrated for 1 hour at 37°C and 5 µl of the respective culture supernatant obtained from the different soil samples was added to the surface of the oil in the well. The shape of the drop on the oil surface was observed for 1 minute. If the culture supernatant makes the drop collapse, it indicated positive result for biosurfactant presence and if the drop remained as such it indicated negative result.

Oil spread method: The petriplate base was filled with 50 ml of distilled water. On the water surface, 20 µl of diesel and 10 µl of culture was added respectively. The culture was introduced at different spots on the diesel which is coated on the water surface. The occurrence of a clear zone was an indicator of positive result

Emulsification Index (E_{24}): The emulsifying capacity was evaluated by an emulsification index. The E_{24} of the culture samples was determined by adding 2 ml of diesel and 2 ml of the inoculum in a test tube and it was shaken for 2 minutes. Then, water and diesel were added and shaken for 2 minutes to obtain maximum emulsification and allowed to stand for 24 hours. This was taken as control. The percentage of the E_{24} index is calculated by the following formula:

\[ E_{24} = \frac{\text{Height of the emulsified layer (cm.)}}{\text{Total height of the column (cm.)}} \times 100 \]

Screening for oil degrading enzyme activity
Analysis for Lipase activity
Qualitative analysis – Tributyrin Plate Assay: Media containing 0.5% peptone, 0.3% yeast extract, 1% Tributyrin and 2% agar was prepared. The PH was adjusted to 7.5 ± 0.2. The plates were then incubated with the isolates at 37°C for 24 hours. The presence of halo was observed and the diameter of the halo was recorded.

Quantitative analysis: The enzymatic assay of lipase activity was done using olive oil as the substrate. First, 1ml of Tris HCl buffer was taken along with 3ml of olive oil substrate, mixed by swirling and equilibrated at 37°C. The pH was adjusted to 7.7. To this 1 ml of the enzyme solution was added. It was mixed and incubated at 37°C for nearly 30 minutes. Then, 3ml of 95% ethanol was added to the mixture. 4 drops of 0.9% Trimorphthalein Indictor solution was added later. It is immediately titrated with standardized 50 Mm Sodium Hydroxide (NaOH) solution. Appearance of pale blue colour served as the end point. The procedure was repeated for concordant values and burette reading was noted. The blank was also titrated in the same manner and the readings were tabulated.
The quantitative activity of the enzyme can be calculated by the following formula:

\[
\text{Units / ml of enzyme} = \frac{(\text{NaOH}) \times (\text{Molarity of NaOH}) \times (1000) \times (2) \times (\text{df})}{\text{Volume (in milliliter) of enzyme used}}
\]

Where, 'df' is the Dilution Factor

**Analysis for Esterase activity**

**Qualitative and Quantitative Analysis:** Esterase activity was assayed using Tween 80. The media contained Peptone (10g), NaCl (5g), CaCl\(_2\cdot2\text{H}_2\text{O}\) (0.1g) and agar (18g). The pH was adjusted to 7.4. To the sterilized culture media, Tween 80 was added at a concentration of 1%. The medium was incubated with the isolates and the presence of halo was observed. The diameter of the halo was recorded. The Enzymatic Index was then calculated by the following formula:

\[
\text{Enzymatic Index (EI)} = \frac{\text{Halo diameter}}{\text{Colony diameter}}
\]

**Identification of the Genus of unknown bacteria**

Gram Staining and biochemical tests were done to determine the genus of unknown bacteria present in the extract. The biochemical tests such as Indole Test, Methyl Red Test, Voges-Prokauer Test, Citrate Utilization and Specific Agar Plate Test using Cetrimide agar were performed according to the Bergey’s Manual of Determination Bacteriology [7].

**Germination tests in treated and untreated soil samples**

In the present study, *Vigna mungo* (L.) Hepper was chosen for the seed germination test since it is a common fast growing leguminous plant available locally. Healthy, viable, uniformly sized seeds of Black gram (*Vigna mungo*) were taken and then surface sterilized in 1% HgCl for about 5 minutes followed by treatment in 100% alcohol for 3 minutes. The surface sterilized seeds were then sowed in trays which contained treated and untreated soil samples. After a few days of watering, the germination of the seeds was noted. The germination percentage can be calculated using the following formula:

\[
\text{Germination Percentage (%) } = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100
\]

**RESULTS AND DISCUSSION**

**Estimation of oil**

The amount of oil in contaminated soil sites before microbial treatment was estimated using the Gravimetric method. The readings were tabulated in Table: I. The amount of oil obtained from the EMU workshop at Central station was higher than the rest of the other 4 samples. The sample obtained at CPCL has been observed to contain the lowest amount of oil (Table: I). The same effect was observed by other researchers in their studies on oil contaminated soil sample [10].

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Source</th>
<th>Soil (g)</th>
<th>Amount of Oil in the Sample (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Central</td>
<td>1</td>
<td>0.7773</td>
</tr>
<tr>
<td>2</td>
<td>Egmore</td>
<td>1</td>
<td>0.5118</td>
</tr>
<tr>
<td>3</td>
<td>Avadi</td>
<td>1</td>
<td>0.3927</td>
</tr>
<tr>
<td>4</td>
<td>Thiruvottiyur</td>
<td>1</td>
<td>0.375</td>
</tr>
<tr>
<td>5</td>
<td>CPCL</td>
<td>1</td>
<td>0.091</td>
</tr>
</tbody>
</table>

Table I shows the amount oil present in each of the soil sample. It was measured by Gravimetric method.
Screening of Biosurfactant producing isolates

Drop collapse method: The distinct bacterial colonies obtained out of enrichment technique were used for screening purpose. The five samples were screened for biosurfactant producing activity and when compared to the other biosurfactants, the extract obtained from the EMU shed at Thiruvottiyur showed the most positive result (Table: II). Similarly, significant biosurfactant activities were reported when Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans and Phanerochaete chrysosporium were used for the analysis [18].

Table II: Evaluation of Biosurfactant Activity by Drop Collapse Method and Oil Spread Method

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample Sites</th>
<th>Biosurfactant Activity by Drop Collapse Method</th>
<th>Biosurfactant Activity by Oil Spread Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CPCL</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>2</td>
<td>Thiruvottiyur</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>Avadi</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>Central</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>Egmore</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

++ indicates faster activity of microbial culture

Table II shows the results obtained during the evaluation of Biosurfactant activity of the samples by Drop collapse method and Oil spread method. The sample obtained from Thiruvottiyur showed the best activity.

Oil spread method: The present study revealed a very high surfactant activity in EMU shed. The results obtained were tabulated in Table: II. The samples collected from Avadi and Central were closely behind, with both showing readings of ++ respectively. The increase in biosurfactant activity was also analysed by other researchers. The assay was performed using Pennzoil and mineral oil instead of diesel [1].

Emulsification Index: The emulsifying capacity of the five samples was evaluated by an emulsification index ($E_{24}$). There was a considerable increase in the emulsifying activity in the extract from Thiruvottiyur (61.538 % EA) when compared with the extract from Avadi which showed the next enhanced effect of about 56.25 % EA (Table: III). This method was also used previously by other scientists in their investigation of polluted soil samples. Similar results were reported [6].

Table III: Emulsification Index ($E_{24}$) of Different Soil Samples

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Soil Sample</th>
<th>Height Of Solution (H1)</th>
<th>Height Of Emulsion (H2)</th>
<th>Emulsification Index (EA %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Avadi</td>
<td>1.6</td>
<td>0.9</td>
<td>56.25</td>
</tr>
<tr>
<td>2</td>
<td>Thiruvottiyur</td>
<td>1.3</td>
<td>0.8</td>
<td>61.538</td>
</tr>
<tr>
<td>3</td>
<td>CPCL</td>
<td>1.5</td>
<td>0.8</td>
<td>53.333</td>
</tr>
<tr>
<td>4</td>
<td>Central</td>
<td>1.7</td>
<td>0.9</td>
<td>52.941</td>
</tr>
<tr>
<td>5</td>
<td>Egmore</td>
<td>1.7</td>
<td>0.9</td>
<td>52.041</td>
</tr>
</tbody>
</table>

The values are the mean of 3 replicates.

Table III shows results obtained from the evaluation of Emulsification Index of the various samples. Thiruvottiyur sample emerged as the best one with an Emulsification Index of 61.538 EA %. The sample obtained from Avadi was closely behind with a result of 56.25 EA%.

Analysis for Lipase activity

Qualitative Analysis - Tributyrin plate assay: After screening for biosurfactant activity, the best two samples (Thiruvottiyur and Avadi) were used for oil degrading enzyme analysis using Tributyrin plate assay. The experimental results showed the maximum oil degrading capacity of microbes isolated from Avadi site was more than that of the Thiruvottiyur sample (Table: IV) (Figure: I).

Other researchers also observed slight improvements in their study samples when they were analysed for Lipase activity using tributyrin plate assay [16].

Quantitative Analysis: The Qualitative analysis for Lipase activity carried out by titration method showed significant change in the extract from Thiruvottiyur site of about 60 EU/ml when compared to the sample from Avadi site which showed a value of 30 EU /ml (Table: V) (Figure: II) respectively. Similar investigation by others showed that a lipase enzyme from Candida rugosa exhibited the highest hydrolytic activity of 88 U mg⁻¹enzyme [22].

Available online at www.scholarsresearchlibrary.com
Analysis for Esterase activity

Qualitative analysis and Quantitative analysis: Qualitative analysis for Esterase activity was performed. It can be observed that the extract from Thiruvottiyur showed significant activity with a zone of clearance of 20 mm. The extract which was extracted from Avadi approximately produced a difference of about 6 mm. (Table: IV) (Figure: I). Increased esterase enzyme activity was observed in the Thiruvottiyur sample during quantitative analysis. An enzymatic index of 2.5 E was reported to be high when compared to the other sample. (Table: V) (Figure: II). Similar results were obtained by other investigators [15].

Table IV: Qualitative Analysis for Enzyme Activity

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>Lipase Enzyme Zone of Clearance in mm</th>
<th>Esterase Enzyme Zone of Clearance in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thiruvottiyur</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Avadi</td>
<td>19</td>
<td>14</td>
</tr>
</tbody>
</table>

Table IV shows the results obtained from the qualitative analysis for enzyme activity.

Figure I: Qualitative Analysis for Enzyme Activity

Identification of the Genus of unknown bacteria

The bacteria extracted from Thiruvottiyur study sample was identified in this present study. Based on the morphological characteristics and on the biochemical tests (Gram Staining, Indole Test, Methyl Red Test, Voges-Prokauer Test and Citrate Utilization), the bacteria was identified to be *Pseudomonas* sp. Further, confirmatory test was done using Cetrimide agar Test which produced beautiful yellow-green colonies and exhibited fluorescence under Ultra-Violet light. (Table: VI).

Other researchers also reported the occurrence of the same yellow-green colonies when microbes were isolated from contaminated soil of their study area [3].
Table VI: Biochemical Tests for Identifying the Bacteria

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gram staining</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Indole</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Methyl Red</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Voges proskauer</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Citrate</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Cetrimide agar</td>
<td>+</td>
</tr>
</tbody>
</table>

Where - indicates negative result while + indicates positive result.

Table VI shows the results of various biochemical tests which were performed. The bacterial isolate showed positive results for Citrate Utilization and Specific Agar Plate Test.

Analysis of oil content after treatment

The *Pseudomonas sp.* extracted from the soil sample obtained from Thiruvottiyur was used for treatment oil contaminated soil. The treatment was carried out for a period of 15 days. The amount oil left in the soil after treating with *Pseudomonas sp.* was obtained using the Gravimetric method (Figure: III). The percentage of degradation of oil after treatment was calculated. The readings have been tabulated in Table: VII.

Similar results were obtained by other investigators when the rate of oil degradation was estimated by gravimetric analysis using 5ml of n-hexane in separating funnel [10].

Table VII: Estimation of Oil Degradation in Percentage

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>Oil Content Before Treatment (%)</th>
<th>Oil Content After Treatment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CPCL</td>
<td>9.1</td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>Thiruvottiyur</td>
<td>39.27</td>
<td>7.6</td>
</tr>
<tr>
<td>3</td>
<td>Avadi</td>
<td>37.5</td>
<td>12.1</td>
</tr>
<tr>
<td>4</td>
<td>Central</td>
<td>77.73</td>
<td>21.3</td>
</tr>
<tr>
<td>5</td>
<td>Egmore</td>
<td>51.18</td>
<td>14.1</td>
</tr>
</tbody>
</table>

The values are the mean of 3 replicates.

Table VII shows the percentage of oil degradation. The best result was obtained in the sample obtained from Central EMU shed which showed a result of 21.3%.
Germination tests in treated and untreated soils

The soil site with the best biosurfactant activity was chosen for the germination study. The present investigation led to the preliminary assessment and comparison of the biosurfactant effect in treated and untreated samples by germination test. After a period of time the germination percentage was calculated (Table: VIII). The germination percentage in the treated soil (75%), was clearly greater than that of the untreated soil. To conclude, the results confirmed that the bioremediation mediated by the *Pseudomonas sp.* extracted from Thiruvottiyur sample has been very effective.

Similar results were obtained by other scientists when the experiment was performed in selected plants such as winter wheat, soybean and sunflower. Growth parameters such as germination percentage, plant height and fresh weight were observed [23].

From the results obtained above, it can be understood that biostimulation can effectively be used to combat pollution. The findings of other researchers also support the fact that biostimulation could indeed be a solution for degrading environmental pollutants [9].

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>Germination Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Untreated Soil</td>
<td>50%</td>
</tr>
<tr>
<td>2</td>
<td>Treated Soil</td>
<td>75%</td>
</tr>
</tbody>
</table>

*The values are the mean of 3 sets*

Table VIII shows germination percentage obtained as a result of the germination tests. The germination percentage of treated soil sample was significantly higher (75%) than that of the untreated soil sample (50%).

CONCLUSION

Humans have often been involved in activities which have led to the destruction of the environment. Industrialization has led to a tremendous increase in the accumulation of waste products and causes damage to the environment. Oil pollution is one of the worst types of pollution. Wastes emanating from industries and oil refineries, which contain petroleum hydrocarbons, metals, radioactive materials and salts, have the potential to cause soil pollution preventing the growth of plants. The conventional techniques of remediation have some major
disadvantages. The recent trend in environmental remediation is by the use of ‘Bioresmediation’ by ‘Biotreatment’. Since bioremediation is based on the process of natural attenuation it is considered to be more acceptable than other technologies. The present study has shown that the indigenous *Pseudomonas sp*. bacteria isolated from the polluted study sample (Thiruvottiyur) possessed the capacity to produce suitable biosurfactants. The biosurfactant isolated from the bacteria showed increased lipase and esterase enzyme activity. Therefore it was used for the degradation of oil contaminated soil. The fertility and toxicity of the soil before and after the treatment was assessed. The effectiveness of the bioremediation mediated by the biosurfactant extract was studied by finding out the germination percentage of a fast growing leguminous plant (*Vigna mungo* (L.)). It was observed that the germination percentage in the treated soil was notably greater. Therefore the findings of the study revealed that the *Pseudomonas sp*. isolated from the EMU workshop at Thiruvottiyur possesses remarkable oil degrading properties and can be effectively employed in the bioremediation of oil contaminated soils. The results also prove that biostimulation is an effective method of reducing environmental pollution. It can be considered as one the effective clean-up technologies of the future. By implementing this method, pollution can be effectively mitigated and the balance in the ecosystem can be restored.

Acknowledgements
The authors would like to thank Dr. B. Janarthanam and Dr. E. Sumathi, Poonga Biotech for providing lab facilities.

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