# Application of Plant Residues and Biosurfactants: A Cost Effective Strategies for the Bioremediation of Spent Motor Oil Contaminated Soil

Eman. A. Diab

Head of Environmental Pollution Research Unit , Department of Plant Ecology and Range Management, Desert Research Center, (DRC), Cairo, Egypt

**Abstract:** A simple and cost-effective bioremediation strategy for the clean-up of a polluted Egyptian sandy soil was developed. Nonsterile bagasse with NP fertilizer and soybean meal without NP fertilizer were promising and cost-effective treatments for the development of higher counts of THB and HDM, which led to enhanced biodegradation of the spent motor oil ( $60.1\pm3.1\%$  and  $58.6\pm2.3\%$  respectively). Six bacterial isolates showed good growth in the presence of spent motor oil. They were identified as: Arthrobacter sp (EM2), Bacillus subtilis (EM6), Bacillus sp (EM10), Corynebacterium sp (EM 14), Pseudomonas aeruginosa (EM1) and Pseudomonas sp (EM19). Pseudomonas\_aeruginosa (EM1) showed higher biodegradation activity ( $68.0 \pm 2.0\%$ ), this was followed by Bacillus subtilis ( $60.3 \pm 3.4\%$ ). The mixed culture of the six bacterial strains increased the biodegradation process to  $76.3 \pm 3.2\%$  (w/w), this may represents a successful approach for the bioremediation of spent-motor oil polluted sites.

Keywords: spent-motor oil, bioremediation, oil degraders, plant residues, biosurfactants.

### 1. Introduction

Unused motor oils consisting of hydrocarbons (80-90% v/v) and additives (10-20% v/v). During use in vehicle's motor, they are altered due to the breakdown of the constituents (Ugoh and Moneke, 2011). Spent-motor oil includes aliphatic and aromatic hydrocarbons such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and heavy metals which are the main contaminants in spent-motor oil (Guerin, 2002, Jain et al , 2009). These compounds are highly toxic to plants, animals and humans when released to the environment (Objeba and Sadiq, 2002; Mandri and Lin, 2007; Wu et al, 2008).

Increasing the number of automobiles in the third world countries has led to the accumulation of hundred million gallons of spent motor oil. These huge amount of spentmotor oils in most of the developing countries are illegally disposed by dumping in landfills, drainage system, open vacant plots and farmlands (Objegba and Sadiq, 2002, Garcia-Hernandez et al, 2007; Akoachera et al, 2008).

Spent engine oils, when present in the soil cause a harmful conditions for life in soil due to poor aeration of the soil, immobilization of soil nutrients and lower the pH (Atuanya, 1987). Used (spent) motor oils including PAHs and heavy metals have been found to alter soil biochemistry includes alteration in soil microbial properties, pH,  $O_2$  and nutrient availability (Atuanya, 1987; Brookes, 1995; Objegba and Sadiq, 2002; Ugoh and Moneke, 2011).

Exposure to oil contaminated environment causes headaches, skin irritation, itchy eyes and burning sensations in internal organs. Exposure to high concentrations of spent motor oil for a long time increases the risk of liver, kidney and bone marrow damage and cancer development (Vazequez – Duhalt, 1989; Propst et al, 1999; Mishra et al, 2001; Lloyd and Cackette, 2001).

For protection of the environment it is important to develope and evaluate non-expensive remediation technologies. A popular strategy for the degradation and removal of the hydrocarbon from the polluted soil is a process known as "bioremediation" which involves the capacity of the natural microorganisms to efficiently degrade the hydrocarbons in the waste oils. This technology is environmental-friendly, wellestablished and cost effective. To achieve enhanced biodegradation, nutrients (e.g. PN fertilizer), soil amendments e.g. plant wastes, rice straw, bagasse, wheat straw, wood chips and / or biosurfactants. (Mulligan and Gibbs, 2004 ; Johnsen et al, 2005; Kang et al, 2010; Tang et al, 2010, Das and Chandran, 2011, Kumar et al, 2011, Sheppard et al, 2011, Adetute et al, 2012. Abdul Salam et al, 2012; Shahsavari et al, 2013). To the best of our knowledge no studies on the biodegradation of spent-motor oil-contaminated Egyptian soils, especially the desert, at the same time no attempt has been carried out for the isolation of hydrocarbon utilizing bacteria from Egyptian spent oil-contaminated soil.

Accordingly, the aim of the present work was to developed simple cost-effective bioremediation strategies for the clean-up of the polluted Egyptian soil under the Egyptian climate and ecological conditions.

### 2. Materials and Methods

#### 2.1 Collection of Soil Samples

Soil samples were collected from an area contaminated with spent motor oil, located at AL-katamia region. This polluted area represent a location for the illegal disposal of spent motor oils. Visual observation show that this location with the oil spillage was black in color, had hard surfaces (Fig 1) and no grasses were grown. Soil samples were collected from 0-20 cm depth by digging up the soil with a hoe. Samples were collected from 5 different locations at the same area, and

### International Journal of Science and Research (IJSR) ISSN (Online): 2319-7064 Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

directly transferred into sterile containers. The collected samples reached the laboratory, were mixed under aseptic conditions to form a composite sample. Sample also were collected from unpolluted area, to behave as control. The sample were air-dried and sieved through 2 mm mesh.



Figure 1: A spent motor oil-polluted site at Al-Katamia region from which soil samples were collected

#### 2.2. Soil Treatments

Soil microcosm test was prepared to include 7 different treatments in duplicates. Each treatment consisting of 500 ml glass beaker containing 100 g of the polluted soil sample and treated as follows :

#### a) Addition of plant residues

Bagasse steriled (Bgst), Bagasse, non-sterilized (Bg), Rice straw (R) and Cop powder (C) were used in a concentration of 5 % (w/w) each, this is in the presence and in absence of nitrogen and phosphorus (NP) fertilizers. One of the treatments (5) was left without the addition of plant residues and NP fertilizer. The NP fertilizer was added in the form of NH<sub>4</sub>NO<sub>3</sub> (100 mg / 100g soil) and K<sub>2</sub>HPO<sub>4</sub> (50 mg / 100g soil )

#### b) Addition of biosurfactants

The biosurfactants used in this study are :

- Soybean meal extract (SBE) (100mg /100g soil)
- Soybean meal powder (SBP) (1g / 100g soil)

The above biosurfactants were used also in presence and in absence of NP fertilizer. The moisture content of each treatment was adjusted at 10% (w/w) by the addition of tap water. Each of the above treatments were covered by thin aluminum foil to reduce the evaporation of water. Small glass rod was introduced to each beaker for tilling the soil. All the treatments were incubated at  $30^{\circ}$ C for a period of

90 days. The loss of water due to evaporation in each treatment was determined by weighing each beaker with its contents at the beginning of the experiment and every 2-3 days. The amount of water lost was added. From each of the above treatments, samples were taken at 0-time and at the end of 90 days for microbiological and chemical analysis.

#### 2.3 Microbiological Analysis

#### a) Counts of total heterotrophic bacteria (THB)

THB were counted using the spread technique (Ugoh and Moneke, 2011) as follows:

- Tenfold dilutions of the soil samples  $(10^{1}-10^{6})$  were prepared.
- 0.02ml from each dilution was transferred onto the surface of nutrient agar plate and then spreaded by a clean sterilized spreader.
- The inoculated plates were incubated at 30°C for a period of 3-4 days, after which the developed colonies were calculated and expressed as colony forming units (cfu)/gram soil.

#### b) Counts of oil-degrading bacteria (OD)

$K_2HPO_4$	1.0
KH <sub>2</sub> PO <sub>4</sub>	0.5
KCl	0.5
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.5
CaCl <sub>2</sub>	0.01
Trace salt solution	1 ml
Agar purified	15.0
pH	. 7.0-7.2

The trace salt solution was of the following composition (100mg per 100ml distilled water) :  $FeSO_4$ ,  $ZnSO_4$ ,  $MnSO_4$ ,  $CuSO_4$  and  $H_3BO_3$ . The inoculated 1SM agar plates were coated with thin films sterilized oil (dissolved in n-hexane). The plates were left for evaporation of the solvent, and then were put in polyethylene bags and incubated at 30°C for a period of 20-25 days, after which the developed colonies were counted and expressed as cfu/g soil. For comparison inoculated plates without oil were also incubated.

### 2.4 Extraction and determination of the residual spent motor oil in each treatment.

At the beginning of the experiment (0-time) and at the end of 90 days incubation period, two grams of the soil from each treatment were extracted with methylene chloride using the shaking method described by Chen et al (1996) as follows.

• The soil sample (2g) was introduced into 50 ml stoppered glass bottle

• 20 ml of methylene chloride was added to each sample.

• The bottles were shaken for two extraction periods of 60

minutes each, using reciprocal shaker operated at 150 rpm.The methylene chloride extract was pooled and

evaporated in a preweighed dishThe amount of residual spent oil was determined and the

loss of oil can be calculated.

## 2.5. Isolation, purification and detection of the active oil degrading bacteria.

- Dominant colonies of bacteria that appeared on ISM agar medium ( which was used for the counting of oil degraders ) were isolated and purified on nutrient agar medium.
- The purified bacteria were streaked over ISM agar plates coated with a thin layer of sterilized spent motor oil dissolved in n-hexane.
- After complete evaporation of the hexane, the plates were incubated at 30°C for a period of 15-20 days, colonies showing good growth and/or clearing zones were selected as good presumptive degraders.

## 2.6. Evaluation of the biodegradation capacity of the selected bacteria.

From each of the selected pure bacterial colonies, a bacterial suspension was made so as to give  $10^8$  bacterial cells / ml (White et al, 1998). One ml of the prepared suspension was introduced into a 250 ml conical flask containing 50 ml of the ISM liquid medium supplemental with 0.5g of sterilized spent motor oil. At least 3 flasks for

each bacterium were used. Control flasks without inoculation were prepared. The flasks were incubated at 30°C on a shaker operated at 150 rpm, for a period of 20 days after which the residual oil was recovered from the cultures using methylene chloride. The loss of the oil as result of biodegradation can be calculated (as mentioned before).

## 2.7 Extraction of the phylogenic biosurfactant found in the seeds of soybean.

50 g of soybean meal was extracted two times with hexane using the shaking method described before. The solvent was evaporated leaving behind a yellowish oily material. 100 mg of this product was suspended in distilled water (pH 8) and was mixed with 100 g of the polluted soil.

### 2.8 Identification of the bacterial strains

Bacterial isolates were identified according to Bergey's Manual of Determinative Bacteriology (Holt et al, 1994) and Bergey's Manual of Systematic Bacteriology, vol.2 (Sneath et al, 1986).

### 2.9 Statistical analysis

All values were averages of three readings, and are expressed as mean  $\pm$  SD. For determining significance of differences among the means, data were analyzed for significant differences ( p<0.05 ) between treatments.

### 3. Results and Discussion

### 3.1 Total of Heterotrophic Bacteria (THB)

Results of the counts THB (cfu/g soil) as influenced by the different soil treatment are formed in Table (1) and can be summarized in the following points:

- The amount of the residual oil in the collected polluted soil was in concentration of 3.2% (w/w). The present results show that this concentration of the a spent motor oil stimulated the development of high cfu/g soil of THB after 90 days incubation period both in presence and in absence of NP fertilizer ( $1.72-66.0x10^8$  cfu/g soil), this is in comparison to  $1.2x10^6$  cfu/g soil at the beginning of the experiment. On the other hand the presence of NP fertilizer stimulated more cfu of THB than in its absence.
- Addition of the different plant residues significantly increased the count of THB (especially in the presence of NP fertilizer) but with different values according to the type of the amendments. Under environmental stress or change in the organic carbon source, microbial community composition shifts to favor the microbial species which can utilize the new substrates. Changes in bacterial community composition followed the introduction of hydrocarbon pollutant are typically accompanies by substantial increase in total bacterial populations(Shaffnar et al, 1996). The results (Table1) show that the maximum counts of THB was  $66.0\pm2.6\times10^8$  cfu /g soil ,recorded when non sterile bagasse(Bs) was mixed with the polluted soil in presence of NP fertilizer. In this case the increased factor (F) is 6.1. On the other hand when cob powder(C) and rice straw (R) were added in presence of NP fertilizer, the counts of THB were  $18.0\pm1.0\times10^8$  and  $20.0\pm0.7\times10^8$  cfu/g soil respectively. These results confirm the effective use of the combination of plant residues and NP fertilizer.

Paper ID: SUB15700

- NP fertilizer alone failed to increase the count over  $2.45\pm0.2x10^8$  cfu/g soil. On the other hand plant residues without NP fertilizer were unable to increase the counts over  $10.8\pm0.4x10^8$ cfu/g soil. The maximum bacterial counts was recorded when non –sterile bagasse was introduced to this type of spent motor oil-polluted soil, in presence of NP (66.0±2.6 x10<sup>8</sup>).
- The higher counts of THB in presence of combination (BgNP) may be due to the presence of sugar cane residue that was left after the preparation of juices from the sugar cane plant. At the same time bagasse may add to the soil different microorganisms that are associated with it. When bagasse was sterilized and applied to the soil, the counts of THB was dramatically decreased to reach  $3.7\pm0.1$  and  $13.74\pm1.4\times10^8$  cfu/g soil in absence and in presence of NP fertilizer respectively. This finding confirms the association of microorganisms with bagasse material, that may be developed on the expense of the sugar residue in the bagasse , and may be bioaugmented to the polluted soil, causing increase in the number and the activity of THB.
- Shahsavari et al (2013) found that microorganisms in soil polluted with 1% aliphatic hydrocarbons were affected by the addition and type of plant residues, and the number of bacteria in the contaminated soil amended with plant residues such as aflafa hay, pea straw and wheat straw were greater than in the absence of these residues, The explanations of how plant residues stimulate the growth and activity of microorganisms be related to physical, chemical mav and microbiological effects. Plant residues with low density will reduce the bulk density of the soil causing increase in soil porosity and oxygen diffusion. The plant residues also may form water stable aggregates when mixed with the soil. The biodegradation of plant residues in the soil releases considerable amount of lignin products which represents precursors for soil organic matter (Zhang et al, 2008). It is known that lignin is a polymer of phenolic compounds and when it is partially decomposed by fungi and bacteria, quinones are produced and condense with amino compounds to form the organic matter of the soil (humus). This humus acts as reservoir holding water as sponge, at the same time it forms a good habitat for microorganisms.
- As for the effect of the addition of the phytogenic biosurfactants to this polluted soil (Table1), it was found that soybean meal powder containing phytogenic surfactant, represented a good amendment material in the absence of NP fertilizer. It stimulated the development of  $43.2\pm3.1\times10^8$  cfu/g soil. On the other hand soybean meal in the presence of NP fertilizer failed to increase the counts over  $23.4 \pm 1.4 \times 10^8$  cfu/g soil. It appears from these results that the microbial community of this polluted soil prefers the organic nitrogen and/or the complex nutrients found in soybean meal powder than the simple mineral NP fertilizer. On the contrary, when the biosurfactant was extracted from the soybean meal as crude product and applied to the spent motor oil-polluted soil, the counts of THB were higher in presence than in absence of NP fertilizer (30.2±1.9 and  $21.0\pm1.0\times10^8$  cfu/g respectively).
- Soybean seed meal may represent a combination of organic nitrogen, some essential nutrients and

biosurfactant, which also may act as nutrient. For this reason soybean meal powder may be used directly without NP fertilizer.

### 3.2 Hydrocarbon-Degrading Microorganisms (HDM)

The results (Table2) show that the application of plant residues increased the counts of the HDM, especially in presence of NP fertilizer.

At the end of 90 days incubation period the counts in presence of NP fertilizer were in range of  $29.1\pm1.7-268.2\pm6.4\times10^7$  cfu/g soil. This is in contrast to  $6.2\pm0.13-28.0\pm10^7$  cfu/g soil in the absence of NP.

At the beginning of the experiment (0-time)  $3.0 \times 10^3$  cfu ODM were recorded. These results indicate that spent motor oil polluting this sandy soil sample being utilized by the indigenous oil-degrading bacteria. Forsyth et al(1995) reported that when the population of the indigenous hydrocarbon-degrading bacteria is less than  $10^5$  cfu/g soil bioremediation will not occur at significant rate.

Mishra et al (2001) found that the initial counts of the indigenous oil sludge-degrading bacteria was  $10^3$ - $10^4$  cfu/g soil before adding a bacterium consortium for enhancing the biodegradation rate. In the present study HDM were  $3x10^3$ cfu/g soil at 0-time, i.e. less than  $10^5$  cfu/g soil. After the application of the different plant residue (especially in presence of NP), the counts increased to reach more than  $10^7$  cfu/g soil (Table 2). It is of importance to observe that the addition of NP alone (i.e without combination with plant residues) failed to increase the counts of HDM over  $4.7\pm0.5\times10^7$  cfu/g soil. This result indicates that the application of plant residue especially in combination with NP fertilizer for enhancing the bioremediation process may be an alternative for the bioaugmentation process with bacterial inocula, and represents more simple and cost-effective strategy as compared to the use of bacterial consortia.

The results (Table 2) clearly demonstrate that the application of the combination (non-sterile bagasse-NP) was able to stimulate the development of the higher counts and percentages of HDM (26.82±0.64X10<sup>8</sup> cfu/soil and 40.6% respectively). This was followed by rice straw-NP (5.8±0.16x10<sup>8</sup> cfu/g soil) and cob powder-NP  $(4.06\pm0.2x10^8 \text{ cfu/g soil})$ , On the other hand sterilized bagasse-NP failed to increase the counts over  $2.91\pm0.17\times10^8$  cfu/g soil. These results took the same trend of the counts of THB. Siderove et al (1998) improved the soil aeration condition for the bioremediation process by adding plant residues such as saw dust and wheat husk. Antai and Mgbomo (1989) recovered counts of  $10^8$  cfu/g soil of HDM, while Ijah and Antai (2003) reported a count of  $10^6$  cfu/g soil. Jian et al (2010) found that in a petroleum contaminated soil  $5.2\pm0.x10^6$  THB and  $4.8\pm0.6x10^6$  HDM per gram soil. The variation in counts of these organisms may be due to differences in microbial ecology of the soil or the different characteristics of the studied soil. Shahsavari et al (2013) found that the counts of oil-degrading microorganisms increased when plant residues were mixed with contaminated soil. Adding pea straw to the polluted soil led to 12 fold increase in HDM. They then suggested that monitoring hydrocarbon utilizing bacteria (HDM) than total enzyme activities is more suitable in assessing the effect of plant residues on oil-polluted soil.

As for the effect of the application of the phytogenic biosurfactant on the counts of the HDM, the results (Table 2) demonstrated the same trend of results as those recorded for THB. Addition of **soy**bean meal in the absence of NP fertilizer stimulated more cfu/g soil  $(14.4\pm0.5 \times 10^8 \text{ with a})$  percentage of 34.6 % than in presence of this fertilizer  $(6.57+0.17\times10^8, \text{ with a percentage of } (28.1\%)$ .On the other hand when the biosurfactant was extracted from the soybean meal and mixed with the polluted soil, the counts in presence of NP was more than in the absence of the fertilizer  $(6.43\pm0.15\times10^8 \text{ and } 1.63\pm0.16\times10^8 \text{cfu/g soil respectively})$ . This may indicate that the extracted biosurfactant was free from nutrients found in soy bean meal.

Biosurfactants are important biotechnological products (which are of plant origin or of microbial origin), they are characterized by lowering surface and interfacial tensions. They are non-toxic and can enhance microbial growth.

Priya and Usharani (2009) indicated that biosurfactants cause emulsification of hydrocarbon and facilitate the utilization of such compounds by microorganisms. It was reported that biosurfactant have important application in the field of environmental bioremediation of polluted sites, this is due to their efficacy as dispersant, their low toxicity and their biodegradability (Mulligan, 2005;Das et al , 2009; Kiran et al, 2010; Satpute et al , 2010).

## 3.3 Biodegradation Activity of the Indigenous Microorganisms

Results of biodegradation of the spent motor oil pollutant in this type of soil under the influence of the different amendment materials (Table 3) could be summarized in the following points:

- When the plant residue non-sterilized bagasse in combination with NP fertilizer (BgNP) were mixed with the polluted soil, highest biodegradation of  $60.1\pm3.1$  % was recorded. This was followed by the plant residue cob powder-NP ( $42.0\pm2.0\%$ ) and rice straw- NP ( $41.7\pm2.1\%$ ) combinations.
- Mixing the plant residue with the polluted soil in the absence of NP fertilizer, biodegradation of the spent motor oil was in the range of 19.4±1.2-29.3+0.3%. These biodegradation values are lower than this in the presence of NP fertilizer, but still higher as compared to treatments without plant residues in presence or in absence of NP fertilizer.
- Soybean meal, on the other hand, in absence of NP fertilizer was also a promising treatment followed by or nearly equal to BgNP treatment (60.1±3.2%-58.6±2.3%). On the other hand in presence of NP fertilizer the biodegradation of the spent motor oil dropped to 40.1±2.0%. The results (Table3) show also that no significant variation between biodegradation of the pollutant in presence of the extracted biosurfactant (SBE) and the soybean meal when NP fertilizer was present.

• The control treatment (polluted soil without the addition of plant residues or NP fertilizer) failed to degrade the spent motor oil above 12.0±0.6 %, while addition of NP fertilizer alone also failed to increase the degradation of the pollutant above 19.2±0.6%.

Shahsavari et al (2013) reported that treatment of the polluted soil by the addition of nonexpensive plant residue may be promising bioremediation method. Plant residues physically improve soil properties such as aerations, moisture, nutrition and structural properties, which finally led to the enhancement of hydrocarbon biodegradation. Plant residues have their own microbial communities which help in the biodegradation process. The same authors reported that the effect of the type of plant residues on the bioremediation has not been studied. They found that significant increase in biodegradation of the aliphatic hydrocarbons in soil amended with different plant residues. The maximum biodegradation (up to 83%) was observed when pea straw was mixed with polluted soil. This is in contrast to 57% in control soil. They found also that the addition of mixed plant residues (alfalfa hay, pea straw and wheat straw) gave less effective biodegradation (70%). Addition of plant residues into the polluted soil may increase the hydrocarbon degradation through a number of interactions :

- Plant residues contain biopolymers such as lignin, cellulose and hemicelluloses. The lignin fraction can absorb oil pollutants (Wang et al, 2007).
- The presence of lignin may cause inhibition of the movement of the oil pollutants into ground water (Zhang et al, 2008).
- Polysaccharides and other sugars released from the decaying of cellulose and hemicelluloses can stimulate the growth and activities of soil microorganisms which led to enhancement of biodegradation of the oil pollutant (Zhang et al ,2008; Axtell et al,2010).

The present results also clearly show significant effect of the phytogenic biosurfactant especially when it was in the unextracted from soybean meal on the biodegradation of spent motor oil polluting this type of soil. It is well known that petroleum hydrocarbons polluting the soil are hydrophobic compounds, and are strongly adsorbed to soil particles. Thus the biodegradation of these compounds is limited by their poor solubility and bioavailability. It may be possible to enhance the pollutants these biodegradation of by introducing biosurfactants. Mulligan and Gibbs (2004) explained that biosurfactants, are able to enhance biodegradation of hydrocarbons by increasing the bioavailability of hydrocarbon for microorganisms and by interaction of the biosurfactant with the bacterial cell surface to increase the hydrophobicity of the surface, thus allowing the hydrocarbons to easily associate to bacterial cells. Kang et al (2010) studied the effect of the biosurfactant sophorolipid on the biodegradation of aliphatic and aromatic hydrocarbons and Iranian light crude oil under laboratory conditions. They found that the addition of biosurfactant lead to increase of the biodegradation rate to reach 85-97% of the total petroleum hydrocarbons.

The present work demonstrate that the application of the plant residue non-sterilize bagasse in combination with NP fertilizer (BgNP) and soybean meal powder in absence of NP fertilizer are promising and cost-effective treatments for the development of higher counts of THB and HDM which lead to enhanced biodegradation of the spent motor oil polluting this type of Egyptian soil. These two effective treatments can be used for the treatment of polluted sites under emergency conditions.

## **3.4 Biodegradation Activity of the Selected Bacterial Strains**

Twenty pure bacterial isolates were isolated from different colonies appeared on oil-degrading agar counting medium. Six of them showed good growth and/or clear zones on ISM agar medium coated with motor oil, they were identified on the basis of Bergy's Manual of Determinative Bacteriology (Holt et al ,1994) and Bergey's Manual of Systematic Bacteriology, vol2 (Sneath et al, 1986) as:

Arthrobacter sp (EM2) Bacillus subtilis (EM6) Bacillus sp (EM10) Corynebacterum sp (EM14) Pseudomonas aeruginosa (EM1) Pseudomonas sp (EM19)

The results (Table 4) show that after 20 days incubation period the higher biodegradation (% loss) was achieved by Pseudomonas aeruginosa EM1(68±2% w/w) this was followed by *Bacillus\_subtilis* EM6 (60.3±3.4% w/w). Statistically, Arthrobacter sp EM 2, Bacillus subtilis EM6 and Bacillus sp EM10 were nearly of equal biodegradations activities. On the other hand Corynebacterium sp EM 14 and Pseudomonas sp EM 19 were of lower biodegradation activities (39.3±1.0 and 47.3±3.1% respectively) as compared to the other strains.

It's of interest to find out that the mixed culture of the 6 strains (*consortium*) was able to increase the biodegradation of the spent motor oil to  $76.3\pm3.2\%$ . These results demonstrated that this *consortium* represents successful approach for the biodegradation of the spent motor oil.

Mandri and Lin (2007) isolated three bacterial species able to utilize spent motor oil as carbon source. The three bacterial species were: Pseudomonas aeruginosa , Flavobacterium sp and Acinetobacter calcoaceticum. After 28 dayes incubation period ,the higher biodegradation activity (loss %) was achived by A .calcoaceticum (84%) as compared to Pseudomonas aerugiosa (71%) and Flavobacterium sp (60%). Ureani et al (2009) evaluated the bacterial diversity of soil environmental contamination with used engine oil, they isolated **Bacillus** stearothemophilus and Cyanobacteria from sites contaminated with used -motor oil .Makut and Ishaya (2010) isolated Pseudomonas sp , Streptococus sp and Bacillus sp from soil contaminated with spent motor oil. These three bacterial strains were able to utilize engine oil, petrol, kerosene and diesel.

**Table 1:** Colony forming units (cfu) of total heterotrophic<br/>bacteria (THB) per gram air-dried polluted soil (3.2%<br/>spent motor oil) received different amendments (plant<br/>residues treatment 1-5 and biosurfactant treatment 6-7) in<br/>presence and in absence of NP fertilizer, at the end of 90

days incubation period. Increased factors (F) also is given (data in presence of NP relative to data in the absence of NP)  $\pm =$ Standard deviation, n=3. At the beginning of the experiment (0-time), cfu/g soil was  $1.2x10^6$ , the control soil (non-polluted) had  $2.2x10^5$  cfu/g soil

	Tractmonto	No. NP	NP Present	(E) fo store
	Treatments	cfu/g soil	cfu/g soil	(r) factor
1	S	$1.72\pm0.9x10^{8}$	$2.45\pm0.2x10^8$	1.4
2	Bg st	$3.7\pm0.1x10^8$	$13.74 \pm 1.4 \times 10^{8}$	5.1
3	Bg	$10.8 \pm 0.4 \times 10^8$	$66.0 \pm 2.6 \times 10^8$	6.1
4	R	$7.1\pm0.5 \mathrm{x10}^{8}$	$20.0\pm0.7 \text{x}10^8$	2.8
5	С	$6.0\pm0.4x10^8$	$18.0 \pm 1.0 \times 10^8$	3.0
6	SBE	$21.0\pm1.0x10^8$	$30.2 \pm 1.9 \times 10^8$	2.0
7	SBP	$43.2\pm3.1x10^8$	$23.4 \pm 1.4 \times 10^8$	0.5

**Table 2:** Counts of oil-degrading bacteria (cfu/g soil), as a result of the addition of plant residues and biosurfactants, in presence and in absence of NP fertilizer. Percentage of the developed oil-degraders (OD) relative to THB are also given:  $\pm$  standard deviation, n=3. At 0-time cfu/g soil was  $3x10^3$ 

	Transformerster	No. NP	0/	NP Present	0/
	reatments cfu/g soil %		cfu/g soil	70	
1	S	$2.2\pm0.6x10^7$	12.8	$4.7 \pm 0.5 \times 10^7$	19.2
2	Bg st	$6.2\pm0.13 \text{x} 10^7$	16.8	$29.1 \pm 1.7 \times 10^7$	21.2
3	Bg	$28.0 \pm 1.0 \times 10^7$	25.9	$268.2 \pm 6.4 \text{x} 10^7$	40.6
4	R	$8.2\pm0.4x10^7$	11.5	$58 \pm 1.6 \times 10^7$	29.2
5	C	$11.4 \pm 0.6 \text{x} 10^7$	18.3	$40.6 \pm 2.0 \times 10^7$	22.6
6	SBE	$16.3 \pm 1.6 \times 10^7$	7.6	$64.3 \pm 1.5 \times 10^7$	21.3
7	SBP	$149.4 \pm 5.0 \text{x} 10^7$	34.6	$65.7 \pm 1.7 \times 10^7$	28.1

**Table 3:**Biodegradation of the spent motor oil (%loss) in the polluted soil under different treatments, in presence and in absence of NP fertilizer + standard deviation, n=3

chee of the forthizer $\pm$ standard deviation, i			
	Treatmonte	No. NP	<b>NP Present</b>
	1 reatments	Loss %	Loss %
1	S	12.0±0.6	19.2±0.6
2	Bg st	20.7±1.1	27.6±1.6
3	Bg	29.3±0.5	60.1±3.2
4	R	19.4±1.2	41.7±2.1
5	С	22.6±1.2	42.0±2.0
6	SBE	23.0±1.0	39.6±0.6
7	SBP	58.6±2.3	40.1±2.2

**Table 4:** Biodegradation (loss %) of the spent motor oil due to the activities of the selected bacterial strains  $\pm$ = standard deviation, n=3. Values within the same column followed by

1-5.	values	wittiiii	the same	conunni	Ionowcu
the sam	e letters	are no	on-signific	cantly di	ffer

	···· · ····· · · · · · · · · · · · · ·			
	Bacterial strains	Loss (%) of spent motor oil		
1	Arthrobacter sp. EM2	$58.7 \pm 3.5^{(a)}$		
2	Bacillus subtilis. EM6	$60.3 \pm 3.4^{(a)}$		
3	Bacillus sp. EM10	$57.7 \pm 2.1^{(a)}$		
4	Corynebacterum sp. EM14	$39.3 \pm 1.0$		
5	Pseudomonas aeruginosa. EMI	$68.0 \pm 2.0$		
6	Pseudomonas sp. EM19	$47.3 \pm 3.1$		
7	Mixed culture	$76.3 \pm 3.2$		

### References

- Abdulsalam, S., Adefila, S.S., Bugaje, I.M, Ibrahim, S. 2012. Bioremediation of soil contaminated with used motor oil in a closed system. J. Bioremed. Biodeg. 3 (12): 1-7
- [2] Adetutu, E.M, Ball, A.S., weber, J., Aleer, S., Dandie, C.E., Juhasz, A.L. 2012 Impact of bacterial and fungal processes on 14C- hexadecane mineralization in weathered

### Volume 4 Issue 1, January 2015

<u>www.ijsr.net</u>

hydrocarbon contaminated soil. Sci Total Environ. 414: 585-591.

- [3] Akoachere, T.K., Jane Francis, A., Theresa, N, Felicitas, Y.N. Nkwelang, G., Ndip, R.N. 2008. Lubricating oil-degrading bacteria in soils from filling stations and auto-mechanic workshops in Buea, Cameroon: Occurrence and characteristics of isolates. Afr. J. Biotech. 7:1700-1706.
- [4] Antai, S.P.and Mgbomo, E. 1989. Distribution of hydrocarbon utilizing bacteria in oil-spill areas. Microbial. Letters, 40:137-143.
- [5] Atuanya, E.J. 1987. Effect of oil pollution on physical and chemical properties of soil: a case study of waste oil contaminated delta soil in Bandel State, Nigeria. J. Applied Science, 55: 155-176.
- [6] Axtell, C., Johnson, C.G., Bumpus, J.A. 2010. Bioremediation of soil contaminated with explosives at the naval weapons station Yorktown, J. Soil. Contamination, 9: 537-548.
- [7] *Brookes, P.C. 1995.* The use of microbial parameter in monitoring soil pollution by heavy metals. Biological Fertility of Soil, 19:269-279.
- [8] Chen, C.S., Rao, P.S.C., Lee, L.S. 1996. Evaluation of extraction methods for determining polynuclear aromatic hydrocarbons from contaminated soil Chemosphere, 32: 1123-1132.
- [9] Das, N. and Chandran, P. 2011. Review Articles : microbial degradation of petroleum hydrocarbon contaminants. An Overview. Biotechnol. Res. Int. Volume 2011, Article ID 941810, 13 pages. dio: 10.4061/2011/941810.
- [10] Das, P., Mukherjee, S., Sen, R. 2009. Biosurfactant of marine origin exhibiting heavy metal remediation properties. Bioresource Technol. 100: 4807-4890.
- [11] Forsyth, J.V., Tsao, Y.M., Bleam, M. 1995. Bioremediation: When is bioremediation needed? pp: 1-14, In R.E. Hinchee, J.Fredrichson and B.C. Alleman (Eds). Bioaugmentation for site remediation. Battelle Press. Columbus, Ohio.
- [12] Garcia-Hernandez, D., Sosa-Aguirre, C.R., Sanchez-Yanez., J.M. 2007. Bioremediation of domestic sewage polluted with waste motor oil. Hydraulic Engineering in Mexico, 22:113 – 118.
- [13] *Guerin, T.F. 2002.* Heavy equipment maintenance wastes and environmental management in the mining industry. J. Environ. Manag. 66: 185-199.
- [14] Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T. 1994. Bergey's Manual of Determinative Bacteriology. 9<sup>th</sup> Ed. Williams and Wilkins, Baltimore.
- [15] *Ijah, U.J.J. and Antai*, S.P. 2003. Removal of Nigerian light crude oil in soil over 12 month period. Int. Biodeterior.Biodegrad. 51: 93-99.
- [16] Jain, P.k., Gupta, V.K., Pathak, H., Lowry, M., Jaroli, D.P. 2010. Characterization of 2T engine oil degrading indigerous bacteria isolated from high altitude (Mussoorie), India, World J. Microbial Biotechnal. 26: 1419-1426.
- [17] Jain, P.K., Gupta, V.K., Gaur, R.K. Lowry, M., Jaroli, D.P., Chauhan, U.K. 2009. Bioremediation of petroleum oil contaminated soil and water. Research J. Environmental Toxicology, 5(1): 1-26.

- [18] Johnsen, A.R., Wick, L.Y., Harms, H. 2005. Principles of microbial PAH degradation is soil. Environ. Pollut 133:71-84.
- [19] Kang, S.W., Kim, Y.B., Shin, J.D., Kin, E.X. 2010. Enhanced biodegrodation of hydrocarbons in soil by microbial biosurfactants, sophorolipid. Appl. Biochem. Biotechnol. 160: 780-790.
- [20] Kiran, S.G., Thomas, T.A., Selvin, J., Sabarathnam, B., Lipton, A.P., 2010. Optimization and characterization of a new lipopeptide biosurfactants produced by marine <u>Breviobacterium aureum</u> MSA 13 in solid state culture. Bioresource Technol. 101: 2389-2396.
- [21] Kumar, A., Bisht, B.S., Jashi, V.D., Dhewa, T. 2011. Review on bioremediation of polluted environment: A management tool. Int. J. Environ. Sci. 1 (6): 1079-1093.
- [22] *Lloyed, C.A., and Cackettle, T.A. 2001.* Diesel engines : Environmental impact and control. Air and Waste Management Association, 51:805-842.
- [23] Makut, M.D. and Ishaya, P. 2010. Bacterial species associated with soils contaminated with used petroleum products in Keffi Town, Nigeria. African. J. Microbial. Res. 4(16): 1698-1702.
- [24] Mandri, T. and Lin, J., 2007. Isolation and characterization of engine oil-degrading indigerous microorganisms in Kwazulu Natal, South Africa. African J. Biotechnol. 6: 23-27.
- [25] Mishra, S.J., Jyot, R.C., Kuchad, B.L. 2001. Evaluation of inoculum addition to stimulate in situ bioremediation of oily-sludge contaminated soil. Appl. Environ. Microbial. 67 (4): 1675-1681.
- [26] Mulligan, C. N. 2005. Environmental applications for biosurfactants. Environ. Pollut. 133: 183-198.
- [27] Mulligan, C.N. and Gibbs, B.F. 2004. Types production and application of biosurfactants. Proc. Sci. Acad. 1:31-55.
- [28] *Odjegba*, *V.J. and Sadig*, *A.O. 2002.* Effects of spent engine oil on the growth parameters, chlorophyll and protein levels of *Amaranthus hypridus* L. The Environmentalist, 22:23-28.
- [29] Priya, T. and Usharani, G. 2009. Comparatively study for biosurfactant production by using Bacillus subtilis and Pseudomonas aeruaginosa. Botany Research International. 2(4): 284-287.
- [30] Propst, T.L., Lochmiller, R.L., Qualis, C.W., McBee, K. 1999. In situ (mesocosm) assessment of immune-toxicity risks to small mammels inhabiting petrochemical waste sites, Chemosphere, 38:1049 – 1067.
- [31] Satpute, S.K., Banat, I.M., Dhakephalker, P.K., Banpurkar, A.G., Chopade, B.A. 2010. Biosurfactants bioemulsifier and exopolysaccharides from marine microorganisms. Biotechnol. Adv. 28: 436-450.
- [32] Schaffner, I.R., Wieck, T.M., Katz, M.D., Pickering, E.W. 1996. Microbiol enumeration and laboratory-Scale microcosm studies in assessing enhanced bioremediation potential of petroleum hydrocarbons. Proceesings of 11<sup>th</sup> annual conference on contaminated soils. University of Massachusetts at Amberst.
- [33] Shahsavari, E., Adetutu, E.M., Anderson, P.A., Ball, A.S. 2013. Plant residues- A low cost effective bioremediation treatment for petrogenic hyrodocarbon-contaminated soil. Science of the Total Environment 443: 766-774.
- [34] Sheppard, P.J., Adatutu, E.M., Makadia, TH, Ball, A.S. 2011. Microbial community and ecotoxicity analysis of

bioremediated, weathered hydrocarbon-contminated Soil Res. 49: 261-269.

- [35] Siderov, D.G., Borzenkov, I.A., Milekhina, E.I., Belyaev, S.S., Ivanov, M.V. 1998. Microbial destruction of fuel oil in soil induced by the biological preparation devoroil. Appl. Biochem. And Microbiol. 34: 255-260.
- [36] Sneath, P.H., Mair, N.S., Sharple, M.E., Holt, J.G. 1986 . Bergey's Manual of Systematic Bacteriology, Vol.2, Williams and Wilkins.
- [37] Tang, J., Wang, R., Niu, X., Zhou, Q. 2010. Enhanced of soil petroleum remediation by using a combination of ryegrass (Lolium perenne) and different microorganisms. Soils Tillage Res. 110:87-93
- [38] Udeani, T.K.C., Oboh, 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 J, Biotechnol. 8 (22):6301-6303.
- [39] Ugoh, S.C., Moneke, L.U. 2011: Isolation of bacteria from engine oil contaminated soils in auto mechanic workshops in Gwagwalada, Abiya, FCT-Nigeria.Academic Arena 3(5): 28-33.
- [40] Vazquez Duhall, R. and Bartha, R. 1989.
  Biodegradation of petroleum in soil environment. Cand. J. Microbiol 20: 1985-1988.
- [41] Walter, M., Boul, L., Chong, R., Ford, C. 2004. Growth substrate selection and biodegradation of PCP by New Zealand white – rot fungi. J. Environ. Management, 71: 361-369.
- [42] Wang, X., Cook, R., Tao, S., Xing, B. 2007. Sorption of organic contaminants by biopolymers: role of polarity, structure and domain spatial arrangement. Chemosphere 66:1476-1484.
- [43] White, L.G., Hawari, J., Zhoue, E., Baurbonniere, L., Inniss, W., Greer, C.W. 1998. Biodegradation of variable chain-length alkanes at low temperature by a psychrophilic *Rhodococcus* sp. Appl. Environ. Microbiol. July: 2578-2584.
- [44] Wu, Y., Luo, Y. Zou, D., Ni, J., Liu, W. Teng, Y. 2008. Bioremediation of polycylic aromatic hydrocarbons contaminated soil with *Monilinia* sp, degradation and microbial community analysis, Biodegradation, 19: 247-257.
- [45] Zhang, K., Hua, X-F, Han, H-L., Wang.J., Miao, C-C, Xu, Y-Y... 2008. Enhanced bioaugmentation of petroleum and salt-contaminated soil using wheat straw. Chemosphere, 73: 1387-1392.