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EFFICIENCY OF SOME NUTRIENT SUPPLEMENTS ON BIODEGRADATION OF SPENT ENGINE OIL IN POLLUTED SOIL

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ABSTRACT

The aim of this study was to evaluate the effect of some nutrient samples–chicken manure (CM), Nitrogen, phosphorus, potassium compound (NPK), Nitroben (*Azotobacter*/*Azospirillum*) (NT) and Azolla (AZ) as amendments for the bioremediation of oil polluted control soil (PCS) during 120 days. The organisms isolated from oil free soil (OFS) and oil samples were species of: *Bacillus, Psuedomonas, Klebsiella, Escherechia , Staphylococcus, Micrococcus, Streptococcus, Burkholderia, Nitrosococcus, Aspergillus, Penicillium, Fusarium, Rhizopus* and *Mucor*. The results of soil analysis showed that CM, NPK, NT and AZ considerably increased the phosphorus andpH of the soil to slightly alkaline condition which favors the biodegradation of the spent engine oil polluted soil. It was also concluded that nitrogen is a necessary nutrient for bacterial biodegradation activities. The highest percentage loss of spent oil was 79.07 by NPK followed by CM, NT and AZ with percentages 77.13, 73.37 and 70.43 respectively. These findings demonstrate the potential of chicken manure, Nitroben, Azolla as well as NPK to considerably increase the biodegradation of the spent lubricating oil polluted soil. In conclusion, the present work suggests the use of chicken manure as potential alternative agents to rationalize spent engine polluted soil as remediation alternative to the expensive chemical and physical methods.

KEYWORDS: Biodegradation; spent engine oil; Chicken manure; NPK; *Azotobacter Azospirillum; Azolla*,

INTRODUCTION

Soil and surface water pollution by used lubricating oil is a common occurrence in most developing countries. This has been shown to have large harmful effects on the environment and human beings. Large amounts of spent engine oil are liberated into the environment when the motor oil is changed and disposed into gutters, water drains, open vacant plots and farmlands, a common practice by motor mechanics and generator mechanics. Spent engine oil is a mixture of several different chemicals, including low and high molecular weight (C15-C20) aliphatic hydrocarbons, aromatic hydrocarbons, polychlorinated biphenyls, chlorodibenzofurans, lubricative additives, decomposition products, heavy metal contaminants such as aluminum, chromium, tin, lead, manganese, nickel, and silicon that come from engine parts as they were down (Umar *et al*., 2015; Iren and Ediene, 2017).

Hydrocarbon contaminations are hazardous to the health of plants and are also carcinogenic, mutagenic and potent immune - toxicants posing a serious threat to human and animal health (Salam *et al.* 2014 and Wagner *et al*.2015). Improperly discarded spent engine oil, renders impacted environment unfit for purpose, alters soil microbial properties, oxygen content and nutrient availability.

Oil spills are frequent due to river-navigation accidents. Recent Accidents occurred on the Nile River in July (www.medanelakhbar.com/egypt/news153000.html) and January 2017 (http://alwafd.org/article/1445717),.November.(http://www.vetogate.com/2458536),March (http://onaeg.com/?p=2526129), and January 2016 (http://www.dostor.org/958629), November (http://www.albawabhnews.com/1616025), September (http://www.vetogate.com/1804361) and March 2015 (http://mo-og.net/sahifa/?p=44401), and many other accidents of oil contamination from unknown source discharged a huge quantity of hydrocarbons to the River. Unfortunately, this is the case, where unknown source of nearly tons of oil, hydrocarbon discharged into the upstream of the Nile River within the Egyptian Territory.

Bioremediation can be an alternative green technology for remediation of such hydrocarbon-contaminated soil. Bioaugmentation involves the introduction of microorganisms into contaminated media to promote the degradation of contaminants. It has become popular for increasing the rate and extent of reductive polycyclic aromatic hydrocarbons in soil (Coulon *et al*., 2012 and Ojonoma *et al*.2017).

Organic material can be degraded by microorganisms, especially bacteria aerobically or anaerobically. These organisms may transform or mineralize organic contaminants into less harmful, non-hazardous substances, which are then integrated into natural biogeochemical cycles (Roy *et al*. 2015 and de la Cueva *et al*., 2016).

Ugochukwu *et al*. (2016) and Benchouk and Chibani (2017) reported that addition of organic materials such as poultry and green manure singly or in combination to improve the chemical properties (pH, OC, total nitrogen, available P, Ca, K, and Mg) of the oil polluted soil will enhance the solubility and removal of these contaminants, improving rates of oil biodegradation.

Bacillus subtilis, Klebsiella aerogenes, Staphylococcus epidermidis, Klebsiella pneumoniae, Actinomyces bovis, Aeromonas hydrophilia, Pseudomonas fluorescens, Pseudomonas aeruginosa, Corynebacterium spp., Escherichia coli, Acinetobacter calcoaccticus, Chryseomonas luteola, Bacillus cereus, Streptococcus faecalis, Proteus vulgaris and *Serratia marcescens* were isolated from oil contaminated soil by several authors. NPK served as a good supplement for the growth of the petroleum utilizing bacteria in oil – polluted soils (Kandil *et al.*, 2013 and Kamble and Kathmale, 2014).

It has been reported that Azolla alone or in mixed culture with other aquatic plants has a high capacity to accumulate toxic elements such as mercury, cadmium, chromium, copper, nickel and zinc (Zayed *et al*., 2000 and Rai and Tripathi, 2009).

The aim of this study is to enhance biodegradation of used engine oil polluted soil, using nutrient supplementation: NPK, chicken manure, Azolla and a commercial bacterial fertilizer, Nitroben (*Azotobacter\Azosprillium*). This is indeed an interesting topic that will undoubtedly shed light on the issue of biodegradation and acclimation.

MATERIAL AND METHODS

I. Material

Collection of Samples

The soil sample used was collected from Cairo University Experimental farm. Soil sample was collected with a soil uger of surface depth (0-15 cm), having no pollution history and devoid of hydrocarbon contamination. Soil sample collected were air-dried, allowed to pass

through a 2mm mesh sieve and weighed out into perforated containers for physicochemical analysis.

Chicken manure was collected from Cairo University Farm.
Processing of Chicken manure used for this study was sun-dried for 5days. The wastes were milled into semi fine particles (using Corn Mill dx-2200, China) and sieved using 2mm mesh.

Used lubricating oil

Was collected from the Mobil Car Service Centre, Cairo, Egypt.

Nitroben, (commercial fertilizer) (Azotobacter ⁄ Azospirillum) and NPK (20:10:10) were purchased from the Agricultural Economics Research Institute (AERI), Agriculture Research Center (ARC) Giza, Egypt.

Azolla, was kindly provided by Soil, Water and Environment Research Institute (SWERI), Agriculture Research Center, Giza, Egypt.

Culture Media

Isolation of indigenous bacteria from spent engine oil: (Takahashi *et al*., 1963).
Nutrient agar medium (Difco).
Potato Dextrose agar (PDA) medium.
Citrimide agar base (Difco), selective media for *Pseudomonas* sp.

Isolation of indigenous fungi from spent engine oil

(Nwachukwu, 2000). Mineral salt medium (MSM) supplemented with 1% crude oil was used for isolation, and preliminary identification of petroleum utilizing fungi of soil samples.

II. METHODS

Microbiological Analysis

Bacterial soil samples were isolated by a streak plate method using 0.1 ml aliquots of appropriate dilution onto nutrient agar slants. Individual cultures were identified by morphological and biochemical techniques using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

For fungal isolation three-fold serial dilution was performed on the above soil samples. An aliquot (0.2 mL) of each diluent were plated on sterile potato dextrose agar (PDA) using the

pour plate method. Incubation was done at 28 ± 2 °C for three days. The fungi were then identified and characterized according to the method of Carpenter (1977).

Characterization and identification of spent engine oil utilizing isolates

All organisms isolated were tested for their ability to grow on and utilize engine oil as the sole carbon source.

Identification of bacteria isolates inhabiting oil

PCR amplification of general 16S rDNA gene (Kisand *et al*. 2002).

16S rDNAs were amplified by using bacterial universal primers Forward-27f (5′-AGAGTTTGATCATGGCTCAG-3′) and Reverse-1492r (5′ TACGGYTACCTTGTTACG ACTT-3′) were used for amplification.

Identification of fungal species inhabiting oil

John (1979); Domsch *et al*. (1993) and Samson *et al*. (2000).

Identification was based on current universal keys. The Data Base Identification Program of the Regional Center for Mycology and Biotechnology (RCMB) For *Aspergilli*.

Soil characterization

The soil was characterized before and after pollution and treatment. Organic carbon was determined by Loss- on-ignition method (Allen 1989). Particle size and texture of soil samples were determined using the method of Bouyoucous (1951), soil pH was measured electronically using a glass electrode pH meter in Water and in KCl2 using a soil: liquid suspension ratio of 1: 2.5 as modified by Jones (2001). Total nitrogen was determined by Kjeldahl method (Bremner, 1965). Concentrations of heavy metals (Cu, Zn Pb, Cr and Ni) were determined by atomic absorption Spectronic 21 spectrophotometer. Available phosphorus was determined using Bray II method as described by Bray and Kurtz (1945). Electrical Conductivity was carried out as described by Chopra and Kanzer (1988). Triplicate determinations were made.

Estimation of total petroleum hydrocarbon (TPH)

Oil content was determined spectrophotometrically according to the toluene extraction method (Odu *et al*. 1980). TPH in soil was estimated with reference to a standard curve derived from used lubricating oil diluted with toluene.

TPH (mg/kg) was determined according to (Romanus *et al*., 2015).

TPH= Instrument reading (Conc. obtained from calibration) x Volume of extract (mL) x DF
Weight of sample (kg).

TPH = Total petroleum hydrocarbon, DF = Dilution factor and Conc. = Concentration.

Calculation of percentage oil degradation: (Ijah and Ukpe, 1992).

% of oil degraded = Original oil concentration - Final oil concentration X 100
Original oil concentration.

The original oil concentration is the initial concentration of oil (weight of extracted oil in g/g) obtained before applying anything on the contaminated soil. While the final concentration of oil is the concentration of oil extracted on day 30, 60, 90 and 120 of treated soil.

Statistical Analysis

Data were analyzed by using analysis of variance (ANOVA) from SPSS-12 statistical software package for personal computer multiple comparisons.of.mean.were.made.with.Duncan's>tests at 95%.

III. Experimental design

Eighteen planting pots were filled with the topsoil from the location earlier described. The pots with a uniform weight of 2 kg (sieved with 2 mm mesh size) .Each were arranged in a nursery garden at April (2015) with prevailing temperature 28 ± 2°C.

10% (w/w) used lubricating oil was added separately to each pot, thoroughly mixed, and left undisturbed for 48 hours to allow the volatilization of toxic components of the oil. Pots were divided into 6 groups. Each group consisted of three replicates arranged in rows. The groups were OFS: Oil free soil, PCS: (soil + 10 g % spent oil) Treatment with only soil and used lubricating oil served as controls. The pots provided with the nutrients had the symbols, (NT): soil +10 g % oil +35 g.kg-1soil Nitroben (*Azotobacter* / *Azosprillium*); (CM): soil + 10 g % oil +10% w/w chicken manure, (NPK): soil +10 g % oil +12g.kg-1 soil NPK and (AZ): soil + 10 g % oil + 60 g of dried Azolla kg-1 soil. The contents of each pot were tilled twice a week for aeration in order to remove the effect of the lack of oxygen and preparing aerobic soil conditions, and the moisture maintained at soil field capacity by the addition of water. Soil

treatment, lasted for 30 days. After 30 days of the experiment, soil samples were collected from each pot and transferred directly into clean containers for physicochemical analysis at the 30th, 60th, 90th and 120th days. Control soil (OFS. & PCS), and treated soil were all characterized for pH, electrical conductivity, total organic carbon , total nitrogen and phosphorus, texture and heavy metals (Cu, Pb, Ni, Zn and Cr) using standard analytical methods to determine the effect of oil pollution on these properties. Total petroleum hydrocarbon (TPH) was also determined.

The experimental design was fully randomized. The results are average of 3 biological replicates per treatment or sample.

RESULTS AND DISCUSSION

The bacterial and fungal isolates obtained in our study, based on their capability to grow well on spent engine oil as carbon and energy source, were identified according to current universal keys. The bacterial isolates identified using 16s r DNA were: *Burkholderia cepacia (*KU860094*), Pseudomonas putida* (KU860101) and *Nitrosococcus watsoni* (KU860098). Fungal isolates were *Penicillium citrinum* and *Aspergillus terreus var. aureus.*

On the other hand, bacteria and fungi successfully isolated and identified from the soil sample under investigation were : two *different Pseudomonas spp., Staphylococcus sp., Escherichia coli, Klebsiella* sp., *Corynebacterium sp. , Micrococcus sp., Streptococcus sp.* two different *Bacillus spp., Aspergilllus niger, Aspergillus ochraceus, Mucor* sp., *Fusarium sp. and Rhizopus* sp.

Microorganisms play a major role in the removal of contaminants taking advantage of their versatile catabolic activity to degrade or convert such compound to harmless substances. Different bacteria have been reported that have promising abilities of breaking down the complex hydrocarbon chain and utilize their carbon energy sources (Roy *et al.* 2014 and Nkem *et al*. 2016). Raza *et al*. (2011), isolated *Micrococcus spp., Corynebacterium spp.,* and *Bacillus spp.* from crude oil-contaminated soil. Similar results have been reported by other researchers (Jaboro *et al*., 2013 and Gayathiri *et al*.2017), who reported *that Streptococcus spp., Arthrobacter spp., Staphylococcus spp., Micrococcus spp., and Bacillus sp*. can grow and degrade crude oil.

According to Atlas (1999) and Salam *et al*. (2014), more rapid rates of degradation occur when there is a mixed microbial community than can be accomplished by a single species. Apparently the genetic information in more than one organism is required to produce the enzymes needed for extensive petroleum biodegradation. *Pseudomonas* is reputed to possess broad substrate affinity for different classes of hydrocarbons such as alicyclics, heterocyclics, and aromatics. The findings by Moneke and Nwangwu, (2011), support the view that *Pseudomonas* and *Klebsiella* species are among the efficient hydrocarbon degrading bacteria prevalent in Nigerian environment. According to Widnyana and Javandira (2016). *Pseudomonas* spp. and *Bacillus* spp. belongs to the largest groups of rhizosphere bacteria. *Penicillium sp, Rhizopus sp , Aspergillus niger* and *A. terreus* showed its ability for bioremediation of petroleum hydrocarbons (Obire *et al*. 2009; Azubuike *et al*. 2016 and Salihu and Ja'afaru 2017 and many others).

To support the biological activity, and hence bioremediation, nutrients are required. For the degradation of hydrocarbons, microorganisms commonly require carbon, nitrogen and phosphorous. The amount of different nutrients and its ratio particularly, C, N and P is quite possible regarding the success of the bioremediation process (Silva-Castro *et al.* 2013, 2015). Based on the USDA textural class (2002), classification of the soil in Table 1, shows that the soil is clay loamy. Particle size analysis shows that the clay (36.9%), silt (38.6%), coarse sand (7.3%), and fine sand fractions (17.2%) were all in the same range for the OFS, PCS and bio-remediated soils (the chicken manure used, is tabulated as an example). Spent engine oil did not negatively affect these soil properties as seen from the results.

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In the present study, there was a highly significant drop in soil pH after oil pollution compared to oil free soil (OFS) during the 120 days of experiment (Fig1).This is in line with the findings of Shukry *et al.* (2013) and Iren and Ediene (2017) who reported that soil pH was reduced due to the presence of hydrocarbon that produce organic acids when acted upon by microorganisms.

At the same time, results recorded at the 30th day of nutrients addition (Fig1) showed a decrease in pH for all treatments compared with the OFS (oil free soil). An observation was reported by Chen *et al*., (2017) that the decrease in pH during remediation treatment may have resulted from the production of acid radicals through the process of nitrification of the applied fertilizer.

Furthermore, a significant increase in pH (neutral to weakly alkaline) in the amended polluted soil compared with the OFS (Oil free soil) after the 30th day till end of the experiment. However, pH ranges were between 7.18 and 7.79.

Electrical conductivity (EC) was significantly lower in the oil-affected soil than in the treated soil (Fig 2). The values of EC detected at the 30th day of OFS; NT; CM; NPK and AZ were 0.68; 0.57; 0.69; 0.67; and 0.50 ds/m respectively as against 0.43ds/m in the polluted soil. This confirms the previous work of Sedat and Sahriye (2011); Azeez and Van Averbeke (2012). Poultry manure and NPK represented higher values in conductivity and reached its maximum at the 120th day (0.85 and 0.83 ds/m respectively).

Reduced conductivity was due to the nonpolar nature of the oil bringing about reduced ionic movement in the soil. Reduced phosphorus level was due to possible oxidation of free phosphorus in the soil to phosphates because hydrocarbons act as electron acceptors or oxidizing agents due to the presence of oxygen in them thereby producing a reducing environment (Oyedele *et al.*2015).

From the results obtained at the 30th day of the experiment (Fig.3), a significant increase in soil organic carbon (OC) for NT, CM, NPK and AZ in g% are: 9.5, 9.5, 9.4, 8.1 and 8.5 respectively, relative to OFS (4.7 g %). On the other hand at the 60th day to the end of the experiment , organic carbon content decreased in the different amended soils except for chicken manure treatment at the 90th day, which showed no significance compared to the PCS (polluted control soil).This may be as explained by Asuquo *et al*. (2001) who stated that microorganisms in the amended soils utilizing the carbon for their cell carbon, thus the high organic carbon content of the PCS soil (0% amendment) is explained by the effect of oil contamination which results in initial immobilization of the available C in the soil by surviving microorganism. However, the organic carbon content dropped during the remediation treatment.

The results of soil properties (Fig.4) indicated a significant decrease in percentage nitrogen in oil polluted soils treated with Nitroben (0.15%), poultry manure (0.17%), NPK (0.19%) and Azolla (0.15%) when compared with 0.26% nitrogen before oil contamination during the first 30 days of the experiment. There was a highly significant decrease in % nitrogen (0.08%) in the soil treated with only spent oil. An increase in percentage nitrogen was observed for nitrogen values at the 60th day of bioremediation for all the nutrients used. A slight decrease

was observed during the 90th and continued to the end of the experiment. NPK addition represented the highest nitrogen content over the all other additives during experiment time. The correlation analysis, between the total nitrogen content and remediation period, showed a negative relationship.

A decrease in Phosphorus was recorded in the soil treated with only spent oil compared with the unpolluted soil (Fig.5), this decrease is obvious from the beginning of the experiment, and continued to the end. Our findings go with those of Ndukwu *et al.*, (2015) and Chikere *et al*. (2017).

Fig.5 also shows that, available phosphorus increased at the 30th day of the soil amended with NT, CM, NPK and AZ; the increase in available phosphorus relative to PCS (polluted control soil) varied depending on the amendment type.

Amendment of polluted media with nitrogenous-based fertilizers containing nitrogen and phosphorus could increase the carbon, nitrogen and phosphorus ratio in media for resident hydrocarbon-degraders to use (Chizoruo *et al.*2016). Nitrifying bacteria belonging to the genera, *Archromobacter, Burkholderia, Azotobacter, Arthrobacter* and *Alcanivorax* are found in the soil and contain the enzyme, nitrogenase, which is responsible for nitrogen fixation (Ibiene and Okpokwasili, 2011 and Ogugbuea *et al*.2017). Hence, in this study, the biodegradation of petroleum hydrocarbons in polluted soil augmented with nitrogen fixing bacteria (*Azotobacter* and *Azospirillum*) was investigated to determine their bioremediation enhancement potentials (Figs.4 and 5).

Table 2, represents an increase in the values of Cr, Zn, Pb, Ni and Cu in the soil after oil pollution at the 30th day relative to unpolluted soil (OFS), followed by a decrease in all amendments added till the end of the experiment. Addition of chicken manure resulted in a highly significant percentage removal of Cr, Zn, Ni and Cu at the 120th day (Fig.6). On the other hand, the removal efficiency shown by *Azolla pinnata* was comparatively high for Zn, Cr, Ni and Cu and lower for Pb. Arora *et al*. (2004) reported that, *A. pinnata* have been shown to absorb Cr, Pb, Cd, Zn and other heavy metals and showed tolerance when present in low concentrations. The biosorption of metal ions was depended on the experimental conditions particularly pH and concentration of metal ions in the medium. Many metal cations are more soluble and available in the soil solution at low pH (below 5.5) including Cd, Cu, Hg, Ni, Pb, and Zn (Ndukwu *et al*., 2015 and Barakat *et al*.2016). In case of NPK

fertilizer, the source of N is urea, therefore, this is also one of the reasons for reducing the soil pH and increasing the availability of metals (Sarwar *et al.*, 2010).

Treatment of engine oil polluted soil with different nutrient amendments positively enhanced the percentage biodegradation during 60–90 days (Fig.7). This result may be due to differences in the used nutrients, particularly N and P, in the amendments that stimulate indigenous microorganisms (Silva-Castro *et al.* 2015 and Wu *et al.*, 2016).

In this study, a significant increase in TPH removal percentage of used engine oil treatment with NPK, followed by chicken manure, Nitroben and then *Azolla* with percentages 79.06%, 77.1%, 73.3 % and 70.4% respectively, as compared to Oil free soil (OFS) (Fig.7). The different nutrients used showed no more effect after 90 days.

A similar decrease in total petroleum hydrocarbon on biostimulating crude oil polluted soil in the University of port-harcout by Chikere *et. al.*, (2009) was observed. TPH decreased from 3666mg/kg on pollution to 135.01mg/kg (with poultry manure) and 89.68mg/kg (with NPK fertilizer) after treatment for 157 days (Isitekhale *et al*. 2013)

The results of this study have shown the negative impact of spent engine oil on physicochemical properties on soil such as pH, total phosphorus and conductivity which were significantly reduced. However, the low solubility and adsorption capacity of high molecular weight hydrocarbons limit their availability to microorganisms. Santisi *et al.* (2015) and Fegenyie *et al.* (2017) indicate that biological treatments are more efficient and cheaper than chemical and physical methods.

Our findings showed that 10% chicken manure supported high spent oil remediation (77.1%) in the polluted soil**.** Poultry droppings is a potential source of nutrients for microbial activity and it promotes microorganisms capable of utilizing hydrocarbons as source of carbon potentially useful in soil hydrocarbon pollution response action.

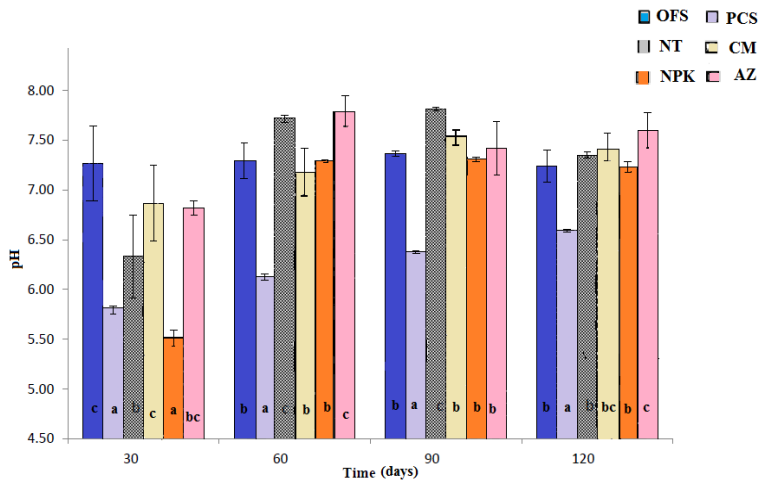

Fig 1: Effect of nutrients on hydrogen ion concentration of spent oil polluted soil,during time intervals.

Key : OFS = Oil free soil, PCS = Soil + 10% spent engine oil , NT = Nitroben (Azotobacter/Azosprillium) + Soil + 10% spent engine oil CM = Chicken manure + Soil + 10% spent engine oil , NPK= NPK+ Soil + 10% spent engine oil , AZ = Azolla+ Soil + 10% spent engine oil Means and SE of 3 replicates are presented. Different letters indicate significant difference between treatments within each time interval

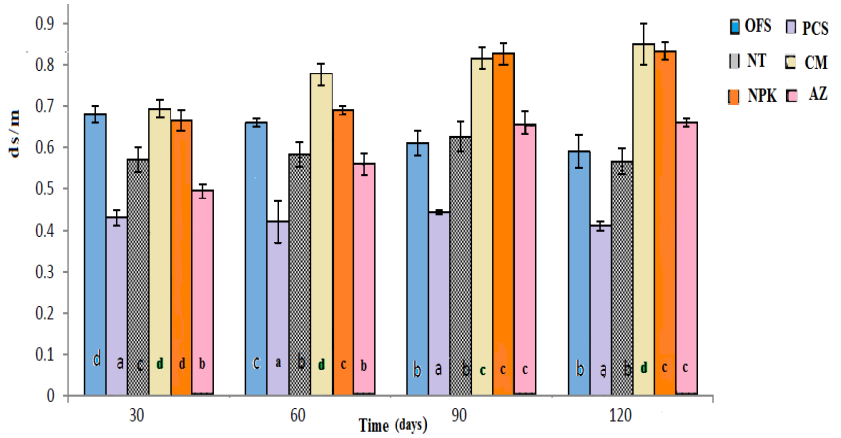

Fig 2: Effect of nutrients on electric conductivity of spent oil polluted soil, during time intervals.

Key : OFS = Oil free soil, PCS = Soil + 10% spent engine oil , NT = Nitroben (Azotobacter/Azosprillium) + Soil + 10% spent engine oil CM = Chicken manure + Soil + 10% spent engine oil , NPK= NPK+ Soil + 10% spent engine oil , AZ = Azolla+ Soil + 10% spent engine oil Means and SE of 3 replicates are presented. Different letters indicate significant difference between treatments within each time interval

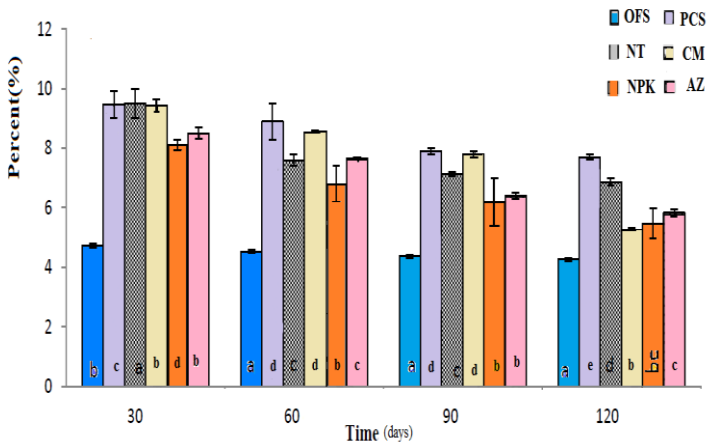

Fig 3: Effect of nutrients on Organic carbon content of spent oil polluted soil, during time intervals.

Key : OFS = Oil free soil, PCS = Soil + 10% spent engine oil , NT = Nitroben (Azotobacter/Azosprillium) + Soil + 10% spent engine oil CM = Chicken manure + Soil + 10% spent engine oil , NPK= NPK+ Soil + 10% spent engine oil , AZ = Azolla+ Soil + 10% spent engine oil Means and SE of 3 replicates are presented. Different letters indicate significant difference between treatments within each time interval

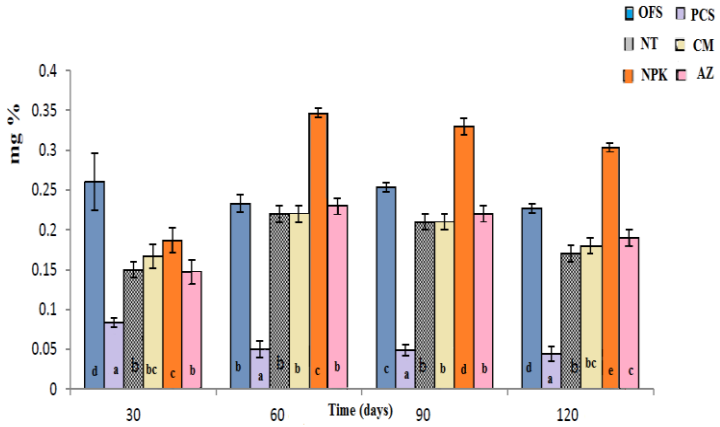

Fig 4: Effect of nutrients on Nitrogen content of spent oil polluted soil, during time intervals.

Key : OFS = Oil free soil, PCS = Soil + 10% spent engine oil , NT = Nitroben (Azotobacter/Azosprillium) + Soil + 10% spent engine oil CM = Chicken manure + Soil + 10% spent engine oil , NPK= NPK+ Soil + 10% spent engine oil , AZ = Azolla+ Soil + 10% spent engine oil Means and SE of 3 replicates are presented. Different letters indicate significant difference between treatments within each time interval

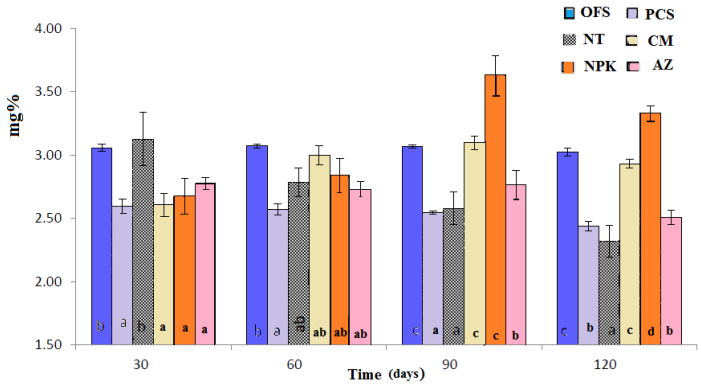

Fig 5: Effect of nutrients on Phosphurus content of spent oil polluted soil, during time intervals.

Key : OFS = Oil free soil, PCS = Soil + 10% spent engine oil , NT = Nitroben (Azotobacter/Azosprillium) + Soil + 10% spent engine oil CM = Chicken manure + Soil + 10% spent engine oil , NPK= NPK+ Soil + 10% spent engine oil , AZ = Azolla+ Soil + 10% spent engine oil Means and SE of 3 replicates are presented. Different letters indicate significant difference between treatments within each time interval

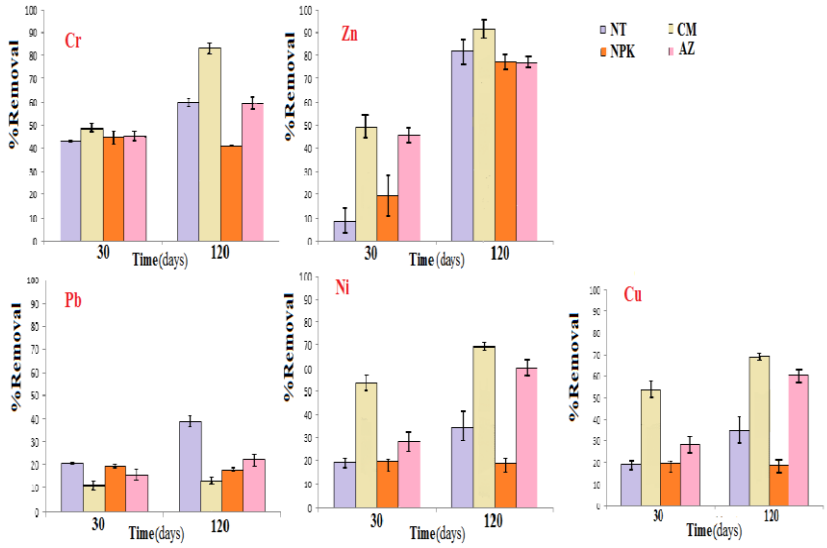

Fig 6: Effect of nutrients on Heavy metals content of spent engine polluted soil, during timeintervals.

Key : NT = Nitroben (Azotobacter/Azosprillium) + Soil + 10% spent engine oil , CM = Chicken manure + Soil + 10% spent engine oil NPK = NPK+ Soil + 10% spent engine oil , AZ = Azolla+ Soil + 10% spent engine oil

Means and SE of 3 replicates are presented. Different letters indicate significant difference between treatments within each time interval

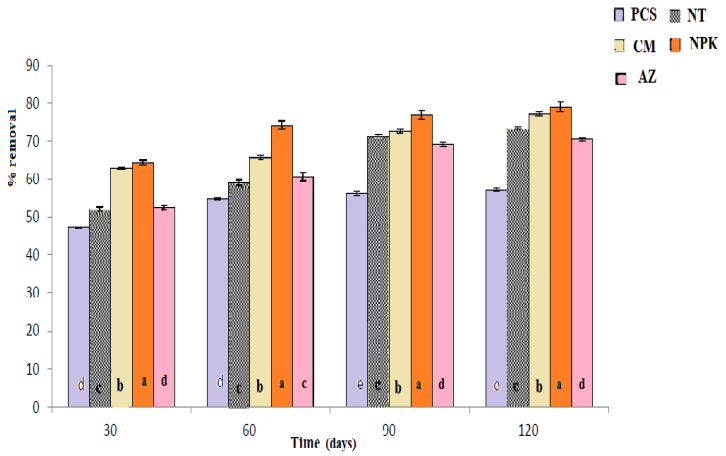

Fig 7: Oil degradation percentage of amended polluted soil during 120 days.

Key : PCS = Soil + 10% spent engine oil, NT = Nitroben (Azotobacter/Azosprillium) + Soil + 10% spent engine oil
CM = Chicken manure + Soil + 10% spent engine oil, NPK = NPK+ Soil + 10% spent engine oil, AZ = Azolla+ Soil + 10% spent engine oil
Means and SE of 3 replicates are presented. Different letters indicate significant difference between treatments within each time interval

Table 1: Results of nutrient analysis; soil physicochemical properties before, one week after soil pollution and after a remediation process (chicken manure treatment).

Soil Properties	**Soil (Value)**	**Soil + spent engine oil**	**Remediated soil** (Soil+ spent engine oil+chickenmanure)after 30 days
% Clay	36.92	36.71	36.55
% Silt	38.61	38.73	38.81
% Fine sand	17.20	17.30	17.23
% Coarse sand	7.33	7.18	7.28
Textural class	Clay-loamy		
pH	7.10	5.2	6.7
EC (ds/m)	0.63	0.40	0.65
Total N (%)	0.26	0.090	0.13
Organic C (%)	4.1	5.40	8.6
Phosphorus (mg %)	3.14	2.5	2.43
Conc.Of heavy metals (mg/ kg)			
Cr	0.16	90.5	46
Zn	15.5	40.0	20.5
Pb	13.5	75.0	66.5
Ni	Zero	51.0	24
Cu	19.4	63.0	21

Table 2: Analysis of Heavy Metals in: unpolluted, polluted and amended soil during time intervals.

Conc. Of heavy metals (g / kg) during time intervals (days)										
treatments	Cr		Zn		Pb		Ni		Cu	
	30	120	30	120	30	120	30	120	30	120
OFS	0.18	0.18	12.3	4	12	12	0.06	0.03	22.3	16.3
PCS	84.3	59.06	36.6	30	72	62.6	50.5	47.4	57.3	45.6
NT	51.36	36	36.6	7.3	59.6	45.6	42.3	33.8	56.3	40
CM	46.3	15.3	20.3	3.3	66.7	65	24.1	16	21.3	7.1
NPK	50	53.1	32.3	9	60.1	61.3	41.9	42.3	52.6	36.6
AZ	49.3	36.3	21.8	9.1	63	58.3	37.4	20.6	34.2	12.2

OFS: Oil free soil control, PCS: soil+10% Spent engine oil, NT: Nitroben(Azotobacter/ Azosprillium) CM: Chicken manure, NPK : NPK 20:10:10, AZ: Azolla.

REFERENCES

1. Allen, E., (1989). Chemical Analysis of Ecological Materials. Second ed. Blackwell Science, Oxford.
2. Asuquo FE, Ibanga IJ, Hungafa N, Effects of Quaiboe crude oil contamination on germination and growth of Okra (*Abelmoschus esculentus. L*) and fluted pumpkin (*Telfairia occidentalis L*) Proc. Of the 27th Annual Conf. of the soil science of Nig. Nov 5-9. University of Calabar., 2001.
3. Arora, A., Sood, A. and Singh, P. Hyperaccumulation of Cadmium and Nickel by *Azolla* Species. Indian J. Plant Physi., 2004; 9: 302-304.
4. Atlas RM, Biodegradation of environmental pollutants. In "Principles of Microbiology," McGraw-Hill, New York, 1999; 805-811.
5. Azeez, J. and Van Averbeke W. Dynamics of Soil pH and Electrical Conductivity with the Application of Three Animal Manures. Communications in Soil Science and Plant Analysis, 2012; 43: 865-874.
6. Azubuike C., Chikere C., and Okpokwasili G. Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World J Microbiol Biotechnol., 2016; 32(1): 18.
7. Barakat, M., Ismail, S. and Ehsan, M. Immobilization of Ni and Zn in Soil by Cow and Chicken Manure. International Journal of Waste Resources, 2016; 6(3).
8. Benchouk, A. and Chibani, A. PETROLEUM-HYDROCARBONS Biodegradation by Y *Pseudomonas* strains isolated from Hydrocarbon-contaminated soil. Journal of Fundamental and Applied Sciences., 2017.

9. Bouyoucous, J. A recalibration of the hydrometer method for making mechanical analysis of soils. Agronomy Journal., 1951; 43: 435-438.
10. Bray, R., and Kurtz, L. Determination of total, organic, and available forms of phosphorus in soil. Soil Sci., 1945; 59: 39-45.
11. Bremner, JM. In: Black, C A (ed). Methods in soil analysis, Part 2. Chemical and Microbiological Properties.Amer.Soc.Agron.Inc.Madison, Winsonsin, 1965; 1149-1179.
12. Carpenter, P. Microbiology. 4th Edn. W. B. Saunders Company, Philadelphia, 1977; 401-402.
13. Chen, J. , Lü, S., Zhang, Z., Zhao, X., Li, X., Ning, P. and Liu, M. Environmentally friendly fertilizers: A review of materials used and their effects on the environment.Sci Total Environ, 2017; 613-614, 829-839.
14. Chikere, C., Okpokwasili G. and Chikere B. Bacterial diversity in a tropical crude oil - polluted soil undergoing bioremediationAfrican Journal of Biotechnology., 2009; 8(11): 2535-2540.
15. Chikere, C., Azubuike, C., and Fubara, E. Shift in microbial group during remediation by enhanced natural attenuation (RENA) of a crude oil-impacted soil: a case study of Ikarama Community, Bayelsa, Nigeria. 3 Biotech., 5, 2017; 7(3): 228.
16. Chizoruo, I., Onyekachi, I., Chinaka, C. and Chinyelue, E. Remediation of mild crude oil polluted fresh water wet land with organic and inorganic fertilizer. International Letters of Natural Sciences., 2016; 2300-9675(54): 75-84.
17. Chopra G. and Kanzar C. **(**1988**).** Analytical Agricultural chemistry, 2nd edition, Prentice-Hall, India Coulon, F., Brassington, K., Bazin, R., Linnet, P., Thomas, K., Mitchell, T., Lethbridge, G., Smith, J. and Pollard, S. Effect of fertilizer formulation and bioaugmentation on biodegradation and leaching of crude oils and refined products in soils. Environ. Technol., 2012; 33: 1879-1893.
18. De la Cueva, S., Rodríguez, C., Cruz, N., Contreras, J. and Miranda, J. Changes in Bacterial Populations During Bioremediation of Soil Contaminated with Petroleum Hydrocarbons. Water, Air, & Soil Pollution, 2016; 227(3): 1-12.
19. Domsch, K., Gams, W. and Anderson, T. Compendium of Soil Fungi., 1993.
20. Fegenyie, B., Kinikaa, R., Uzorb, C., Okahb, A. and Solomonb, L. A Conspectus Review Efficacy of Locally Sourced Organic Biostimulants on Enhanced Biodegradation of Hydrocarbon-Contaminated Soil. *Rep Opinion*, 2017; 9(4): 62-69.
21. Gayathiri, E., Bharathi, B., Selvadhas, S. and Kalaikandhan, R. Isolation, Identification and Molecular characterization of Hydrocarbon degrading bacteria and its associated

genes - A review. International Journal of Pharma and Bio Sciences, 2017; 8(2): (B): 1010-1019.

22. Holt, J., Krieg, N., Sneath, P., Staley, J. and Williams, S. Bergey's Manual of Determinative Bacteriology, 9th edn. Baltimore: Williams & Wilkins., 1994.
23. Ibiene, A. and Okpokwasili, G. Comparative toxicities of three agro-insecticide formulations on nitrifying bacteria. *Report and Opinion*, 2011; 3(12): 14-17.
24. Ijah, U. and Ukpe, L. Biodegradation of crude oil by Bacillus strains 28A and 61B isolated from oil spilled soil. Waste Manag, 1992; 12: 55–60.
25. Iren, O. and Ediene, V. Effect of Spent Engine Oil Discharge on Soil Properties in Selected Automobile Workshop in Calabar, Cross River State, Nigeria. International Journal of Innovative Science, Engineering & Technology, 2017; 4(2): 5-264.
26. Isitekhale, H., Aboh, S., Edion, R. and Abhanzioya, M. Remediation of Crude Oil Contaminated Soil with Inorganic and Organic Fertilizer Using Sweet Potato as a Test Crop. Journal of Environment and Earth., 2013; 3(7).
27. Jaboro, A., Akortha, E., Obayagbona, O. Susceptibility to heavy metals and hydrocarb on clastic attributes of soil microbiota. International Journal of Agriculture Biosciences, 2013; 2(5): 206.
28. John, I. The Genus Penicillium & its Teleomorphic States., 1979.
29. Jones, B. Laboratory guide for conducting soil tests and plant analysis. Boca Raton, Fl. CRC Press., 2001.
30. Kamble, B. and Kathmale, D. Effect of different levels of customized fertilizer on soil nutrient availability, yield and economics of onion. *J. Appl. and Natural Sci.*, 2015; 7(2): 817–821.
31. Kandil, A., Ali, S. and Fathalla, F. Effect of organic and mineral fertilizers on vegetative growth, bulb yield and quality of onion cultivars. *E Sci. J. Crop Prod.*, 2013; 2(3): 91-100.
32. Kisand, V., Cuadros, R., and Wikner, J. "Phylogeny of culturable estuarine bacteria catabolizing riverine organic matter in the northern Baltic Sea," Applied and Environmental Microbiology, 2002; 68(1): 379–388.
33. Moneke, A. and Nwangwu, V. Studies on the Bio utilization of some Petroleum Hydrocarbons by Single and Mixed Cultures of some Bacterial Species. *African Journal of Microbiology Research*, 2011; 5(12): 1457-1466.

34. Ndukwu, B., Onweremadu, E., Ahukaemere, C., Ihem E., Nkwopara, U. and Osujieke, D. Distribution and concentration of cadmium in automobile polluted soils in Owerri, South Eastern Nigeria. *Nigerian Journal of Soil Science*, 2015; 25: 135-145.
35. Nkem, B., Halimoon, N., Yusoff, F., Johari, W., Zakaria, M., Medipally, S. and Kannan, N. Isolation, identification and diesel-oil biodegradation capacities of indigenous hydrocarbon-degrading strains of *Cellulosimicrobium cellulans* and *Acinetobacter baumannii* from tarball at Terengganu beach, Malaysia. Marine Pollut Bull., 2016; 107: 261–268.
36. Nwachukwu, S. Journal of Environmental Biology., 2000; 21: 241-250.
37. Obire, O. and Anyanwu, E. Impact of various concentrations of crude oil on fungal populations of soil. *Int. J. Environ. Sci. Technol*., 2009; 6: 211-218.
38. Odu, C., Nwoboshi, L., Fagado, S. and Awani, P. Post-impact study of SPDC'S Nun River 8" delivery line oil spillage. Final report. SPDC, Nig, 1980; 95.
39. Ogugbuea, C., Solomonb, L. and Olalic, I. Enhanced Biodegradation of Petroleum Hydrocarbons in Polluted Soil Augmented with Nitrogen-Fixing Bacteria. Life Science Journal, 2017; 14(1).
40. Ojonoma, O., Samuel, O. and Johnson, G. Comparative Studies on the Biodegradation of Crude Oil-polluted Soil by *Pseudomonas aeruginosa* and *Alternaria* Species Isolated from Unpolluted Soil. Microbiology Research Journal International., 2017; 19(1): 1-10.
41. Oyedele, A., Oloye, M., Daramola, B. and Ayoko, O. Evaluation of Organic Manures Effects on Crude Oil Polluted Soil. Canadian Open Soil Science and Environmental Management Journal, 2015; 1(1): 1-13.
42. Rai, P. and Tripathi, B. Comparative assessment of *Azolla pinnata* and *Vallisneria spiralis* in Hg removal from G.B. Pant Sagar of Singrauli Industrial region, India. *Environmental Monitoring and Assessment*, 2009; 148: 75–84.
43. Raza, C., Liaqat, S., Bashir, S., Naseer, M. and Ishrat, N. Characterization of crude oil contaminated soil bacteria and laboratory-scale biodegradation experiments. Biologia (Pakistan), 2011; 57(1,2): 47.
44. Romanus, A., Ikechukwu, E., Patrick, A., Goddey, U. and Helen, O. Efficiency of Plantain Peels and Guinea Corn Shaft for Bioremediation of Crude Oil Polluted Soil, Journal of Microbiology Research, 2015; 5(1): 31-40.
45. Roy, S, Baruah, R., Borah, M., Singh, K., Boruah, D., Saikia, N., Deka, M.and Dutta, N.and Bora, C. Bioremediation potential of native hydrocarbon degrading bacterial

strains in crude oil contaminated soil under microcosm study. International Biodeterioration & Biodegradation, 2014**;** 94: 79.

46. Roy M, Giri A., Dutta S and Mukherjee P. Integrated phytobial remediation for sustainable management of arsenic in soil and water. Environ Int., 2015; 75: 180–198.
47. Salihu, A. and Ja'afaru, M. Potential of using *Aspergillus niger* in the biodegradation of lubricating oil. Basic Research Journal of Microbiology, 2017; 4(3): 25-32.
48. Salam, L, Ilori, M, Amund, O, Numata, M, Horisaki, T and Nojiri, H. Carbazole angular dioxygenation and mineralization by bacteria isolated from hydrocarbon-contaminated tropical African soil. Environ Sci Pollut Res.; doi: 10.1007/s11356-014-2855-2., 2014; 21: 9311–9324.
49. Samson, R., Hoekstra, E. and Frisvad, J. (2000): Introduction To Food- And Airborne Fungi. Santisi, S., Cappello, S., Catalfamo, M., Mancini, G., Shahian, H., Genovese, L., Giuliano, L. and Yakimov, M. Biodegradation of crude oil by individual bacterial strains and a mixed bacterial consortium. Brazilian Journal of Microbiology, 2015; 46(2): 377-387.
50. Sarwar, N., Saifullah, S., Zia, M., Naeem, A., Bibi, S. and Farida, G. Role of Mineral Nutrition in Minimizing Cadmium Accumulation by Plants. *J. Sci. Food Agric.*, 2010; 90: 925–937.
51. Sedat, C., Sahriye, S. Effects of chemical fertilizers and different organic manures application on soil pH, EC and organic matter content. Journal of Food, Agriculture and Environment (JFAE), 2011; 9(3&4): 739-741.
52. Shukry, W., Al-Hawas, G., Al-Moaikal, R. and El-Bendary, M. Effect of petroleum crude oil on mineral nutrient elements, soil properties and bacterial biomass of the rhizosphere of jojoba. British Journal of Environment and Climate Change; 2013; 3: 103–118.
53. Silva-Castro, G., Rodelas, B., Perucha, C., Laguna, J., Gonzalez-Lopez, J., Calvo, C. Bioremediation of diesel-polluted soil using biostimulation as post-treatment after oxidation with fenton-like reagents: assays in a pilot plant. Sci Total Environ., 2013; 15(445–446): 347–355.
54. Silva-Castro, G., Uad, I., Rodrı´guez-Calvo, A., Gonza´lez –Lopez, J. and Calvo, C. Response of autochthonous microbiota of diesel polluted soils to land-farming treatments. Environ Res., 2015; 137: 49–58.
55. Takahashi, J., Kobayashi, K., Karwabata, Y. and Koichi, Y. Studies on the utilization of hydrocarobons by microorganisms.Part III.Production of bacterial cell from hydrocarobons .Agr.Biol.Chem., 1963; 27(12): 836.

56. Ugochukwu, O., Michael, U., Amechi, S., Chikaodili, G., Samuel, C., Etim, J., Ifeanyi, E.and Kingsley, C. Effect of Chicken droppings amendment on bioremediation of crude oil-polluted soil.*European Journal of Experimental Biology*, 2016; 6(4): 62-68.
57. Umar, M., Victoria, R. and Fadimatu, M. "Metal & Ions Eating Microbes Induced Corrosion of Refinery Industrial Materials." International Journal of Humanities & Social Science (IJHSS), 2015; 4(4): 136-148.
58. USDA (United State Department for Agriculture). National Resources Conservation Service, 2-29, http://websoilsurvey.nrcs.usda.gov. 2002.
59. Wagner, M., Bolm-Audorff, U., Hegewald, J., Fishta, A., Schlattmann, P., Schmitt, J. and Seidler, A. Occupational polycyclic aromatic hydrocarbon exposure and risk of larynx cancer: a systematic review and meta-analysis. Occup Environ Med., 2015; 72(3): 226-33.
60. Widnyana, K. and Javandira, C. Activities *Pseudomonas* spp. and *Bacillus* sp. to Stimulate Germination and Seedling Growth of Tomato Plants. Agriculture and Agricultural Science Procedia., 2016; 9: 419 – 423.
61. Wu, M., Dick, W., Li, W., Wang, X., Yang, Q., Wang, T., Xu, L., Zhang, M. and Chen, L. Bioaugmentation and biostimulation of hydrocarbon degradation and the microbial community in a petroleum-contaminated soil., 2016., 107: 158-164.
62. Zayed, A., Smits, E., DeSouza, M.., Lin, Z. and Terry, N. Remediation of selenium polluted soils Bañuelos, G. (Eds.), Phytoremediation of Contaminated Soil and Water. Lewis, Boca Raton, 2000; 61–83.