### **Original Article**

# The Effect of Urban Fuel Stations on Soil Contamination with Petroleum Hydrocarbons

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## ABSTRACT

**Background:**A critical environmental impact of the petroleum industry is the contamination of soil by oil and other related products which are highly toxic and exhibit molecular recalcitrance. Therefore, this study focused on investigating the total amount of petroleum hydrocarbons (TPHs) in soil of urban fuel stations in Hamedan City, Iran.

**Methods:**Thirteen high traffic urban fuel stations were selected and random soil samples were collected from surface soils at selected fuel stations. The physical and chemical properties of the soil samples were determined in the laboratory. The concentration of TPHs in soils was determined by GC/MC.

**Results:** Results showed that concentration of TPHs in all stations was more than the standard level in soil (2000 mg kg<sup>-1</sup>). The minimum and maximum TPHs concentration observed in No. 5 and No.13 fuel station, respectively.

**Conclusion:** The results showed that spillage in urban fuel stations has clear effect on the content of TPH in soil, as concentration TPH in all of fuel stations was in the upper limit of the standard levels in soil. Soil pollution with petroleum hydrocarbons has clear effects on soil biological, chemical and physical characteristics and results in decreasedg food elements, productivity and soil plant productions.

Keywords: Fuel Stations, Soil Contamination, Total Petroleum Hydrocarbons.

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### **INTRODUCTION**

Environmental pollution with petroleum and petrochemical products has attracted much attention in recent years. The presence of various kinds of automobile and machinery vehicles has caused an increase in the use of motor oil. Spillages of used motor oils such as diesels or jet fuels contaminate our natural environment with hydrocarbon [1]. Pollution of soil with petroleum derivatives is often observed in soils around industrial plants and in areas where petroleum and natural gas are obtained [2,3], processed or distributed [4]. Processes such as oil exploration, drilling, refinement, transportation, oil processing and storage are accompanied by environmental contamination. Oil spillage occurs through tanker accidents, well blow outs, sabotage and accidental rupture of pipelines, resulting in the release of crude and refined oil into

terrestrial and aquatic environments [5]. Organic pollutants differ in charge and solubility. Nonpolar compounds such as hydrocarbons are poorly soluble in water and absorb readily into hydrophobic soil particles and soil organic matters [6]. The most common products of petroleum distillation are fuels which include: ethane and other short-chain alkanes, diesel fuel (petro diesel), fuel oils, gasoline (petrol), jet fuel, kerosene and liquefied petroleum gas (LPG). Certain types of resultant hydrocarbons may be mixed with other non-hydrocarbons, to create other endproducts such as alkenes (olefins) which can be manufactured into plastics or other compounds, lubricants which produce light machine oils, motor oils and greases, wax (used in the packaging of frozen foods), sulfur or sulfuric acid useful as industrial materials, petroleum coke used as solid fuel, and paraffin wax etc [7].

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Petroleum and its products are of major concern in pollution studies due to their structural complexity, slow biodegradability, biomagnification potential and above all, serious health hazards associated with their release into the environment [7]. The use of petroleum products lead to contamination of soil and changes in soil properties with petroleum-derived substances which can lead to water and oxygen deficit as well as to shortage of available forms of nitrogen and phosphorus [8]. Contamination of the soil environment can also limit its protective function, unfavorably affect its chemical characteristics. reduce fertility of animals and negatively influence plant production [9-11]. This threatens human health and that of the organisms that are dependent on the soil [12,13]. Crude oil in the soil makes soil condition unsatisfactory for plant growth due to the reduction in the level of available plant nutrient or a rise in toxic levels of certain elements such as iron or zinc [14]. Soil contaminated with petroleum has serious hazards and causes organic pollution of underground water, which limits its use, causes economic loss, environmental problems and decreases the agricultural productivity of the soil. The concern stems primarily from health risks, from direct contact with the contaminated soil, vapors from the contaminants and from secondary contamination of water supplies within and underlying the soil. The toxicity of petroleum hydrocarbons to microorganisms, plants, animals and humans is well established. The toxic effects of hydrocarbons on terrestrial plants and their use as weed killers have been ascribed to the oil dissolving the lipid portion of the cytoplasmic membrane, thus allowing the cell contents to escape [15]. Prolonged exposure to high oil concentration may cause the development of liver or kidney disease, possible damage to the bone marrow and an increased risk of cancer [16]. The fate and effects of spilled crude oil and its products in soils have already been the subject of several studies [5, 17-19]. The presence of inorganic ions, carcinogenic and growth inhibiting chemicals in crude oil, its effects on microorganisms and on human beings is well documented [20]. Total petroleum hydrocarbons (TPHs) are classified into different fractions: Fractions 1  $(C_6-C_{10})$  and 2  $(C_{10}-C_{16})$  are volatile or semivolatile, whereas fractions 3 (C<sub>16</sub>-C<sub>33</sub>) and 4  $(C_{34}-C_{50})$  are hydrophobic and recalcitrant.

Compounds from fractions 3 and 4 can be highly toxic and are regulated due to their mutagenicity and carcinogenicity [6]. Total petroleum hydrocarbons are one of the most common groups of persistent organic contaminants [21]. During the last decade, concerns about hydrocarbons in the environment have considerably increased. Among them, TPHs are of great interest as the accumulation of these compounds in soil might lead to significant risks to human through different exposure pathways [22]. Hydrocarbon pollution of the subsurface, especially in unsaturated soils, has become a big problem with the development of the petrochemical industry and installation of numerous petrol stations and underground pipes [23]. Therefore, with attention to the specific effects of petroleum contaminants on soils, this study was focused on investigating the effect of urban fuel stations on accumulation of TPHs in surface soil

## MATERIALS AND METHODS

#### Sample Collection and Soil Preparation

In order to investigate the effect of urban fuel stations on soil contamination and accumulation of TPHs in surface soil, thirteen urban fuel stations in Hamedan City were selected. From each station five samples were taken from 0-20 cm depth. Then the soils were mixed thoroughly and sieved through a 2 mm sieve to attain an almost homogenous fine earth mixture. All samples were collected in September, 2012. Fig 1 shows the sampling stations.

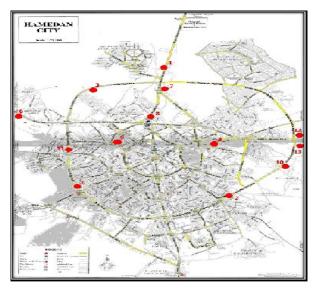


Figure 1. The map of sampled urban fuel stations in Hamedan, Iran.

### **Physicochemical Properties of Soil**

Soil samples were transferred to the laboratory for testing and measuring soil physical and chemical properties. Particle size was determined by the pipette method [24], soil pH and EC were determined in a soil-solution ratio 1:1 using a combination electrode and electrical conductivity meter. Olsen-P was determined using a soil to solution ratio 1:20 and 30 min shaking [25]. Phosphorus in the extracts was determined using the ammonium molybdate-ascorbic acid method described by Murphey and Riley (1962) [26]. Cation exchange capacity (CEC) was determined by saturation with 1 M NH<sub>4</sub>OAc pH 7. Exchangeable sodium and potassium were measured by flame photometry in ammonium acetate extracts. Calcium carbonate equivalent was determined by the acid neutralization method and total nitrogen determined by Kjeldahl digestion as described by Rowell (1994) [24].

Soil moisture content was evaluated by gravimetric method as described by Schneekloth et al(2002) [27]. In gravimetric method soil samples are dried at 105°C to a constant weight. The moisture content in dry weight basis is calculated using the following formula:

Water Content (%) = 
$$\frac{(M_W - M_d)}{M_d} \times 100$$
 (1)

where  $M_w$  and  $M_d$  are mass of wet and dry soil samples respectively.

Total C was measured as described by Allison (1965) [28].

### Total Petroleum Hydrocarbon Determination

In order to extract the oil matter from the soil, 5 g of the sampled soil was placed in a 45  $cm^3$  test tube. N-hexane (10 cm<sup>3</sup>) was poured into the test tube and the contents in the test tubes were shaken laterally in a shaker for 5 min. The oil/n-hexane extract was removed and the absorbance of the solution was measured at 400 nm with HACH DR/2000 Spectrophotometer. This process was repeated again with another 10  $cm^3$  of n-hexane in the test tube containing the contaminated soil. The extracted crude oil/n-hexane solution was also measured for absorbance. At the fourth 10 cm<sup>3</sup> extracts, the absorb-

ance reading was zero (the same as the control pure n-hexane) indicating no contaminants were present. Also, for each consecutive n-hexane extraction the absorbance reading of the extracted solutions reduced. Total n-hexane extraction was collected together in a 50 cm<sup>3</sup> standard volumetric flask, and pure n-hexane was added to the graduated mark. The sample from the 50 cm<sup>3</sup> extractions was centrifuged for about 20 min at 3000 rpm and was used for gas chromatography (GC/mass) studies [29,30].

The GC/MS study was conducted using a VG Quattro Tandem Mass Spectrometer (Waters, Manchester, England) with the following operating conditions: Electron impact ionization, electron energy 70 eV, scan range 40-500 amu at one scan 1 second. Helium at a flow rate of 1.5 cm<sup>3</sup> min<sup>-1</sup> was used as carrier gas. Samples were injected on-column onto a 30 m HP5 fused silica capillary column, 0.25 mm i.d. (Agilent, England) and the temperature was held at 55 °C for 2 min and then was increased from 55 °C to 300 °C at 5 °C min<sup>-1</sup> thereafter, was held at 300 °C for 40 min. One  $\mu$ L of the crude oil/n-hexane extract was injected into the equipment for each investigation [31].

#### Statistical Analysis

All statistical analyses were performed using the SPSS 15.0 (SPSS Inc., Chicago, IL, USA) statistical package.

#### RESULTS

Results of physical and chemical analysis of fuel station soils are presented in table 1. The content of clay ranged from 19.5-39.2%, silt from 20.2 -35.1% and sand 0.28-60.3%. Based on particle size distribution in studied soils, the texture of most soils was loam. The pH value was generally near neutral and alkaline (pH ranges from 7.01-8.5). The water content of soil varied between 5-33%, electrical conductivity from 0.1-0.63 dsm<sup>-1</sup>, organic matter from 0.83-3.53%, total carbon from 0.48-2.05%, potassium from 18.2-28.29 mg kg<sup>-1</sup>, sodium from 12.45-29.6 mgkg<sup>-1</sup>, calcium carbonate from 34.37-15.01%, total nitrogen from 0.12-1.32%, phosphorpus from 14.12-57.2 mg kg<sup>-1</sup>, cation exchange capacity from 10-29 cmol<sub>c</sub> kg<sup>-1</sup>.

Table 1. Results of soil physical and chemical analysis of fuel stations in Hamedan City.

Parameter	Unit						Fu	el station	s					
		1	2	3	4	5	6	7	8	9	10	11	12	13
EC	dS m <sup>1</sup>	0.31	0.22	0.45	0.15	0.10	0.26	0.19	0.2	0.63	0.34	0.25	0.46	0.23
pН	-	7.5	8.3	7.4	7.1	8.5	7.9	7.3	7.8	8.1	7.5	7.9	7.4	7.7
Water Content	7.	19.9	27.7	17.0	33.0	26.0	8.0	15.0	6.0	14.0	18.0	22.0	5.0	20
Organic Matter	7.	0.98	2.07	1.14	1.52	1.76	1.56	1.03	1.41	0.83	3.53	1.86	0.86	1.65
Organic Carbon	7.	0.57	1.20	0.66	0.88	1.02	0.91	0.60	0.82	0.48	2.05	1.08	0.5	0.96
Clay	7.	19.5	26.3	30.2	32.9	20.1	35.0	31.0	32.6	24.5	20.3	39.2	32.8	29.1
Silt	7.	20.2	20.0	22.3	25.0	35.1	26.1	21.1	30.5	24.2	29	32.3	27.0	25.1
Sand	7.	60.3	52.7	47.5	42.1	44.8	38.9	47.9	36.9	51.3	50.7	28.5	40.2	45.8
Soil Tex- ture	_	Sandy Loam	Sandy Clay Loam	Sandy Clay Loam	Clay Loam	Loam	Clay Loam	Clay Loam	Clay Loam	Sandy Clay Loam	Loam	Clay Loam	Clay Loam	Clay Loam
Exchange- able $K^+$	mg kg <sup>-1</sup>	26.6	18.2	28.3	23.5	19.7	25.4	18.8	21.2	19.8	20.2	27.3	24.24	22.3
Exchange- able Na <sup>+</sup>	mg kg <sup>-1</sup>	16.6	29.6	15.12	13.8	20.9	15.9	18.8	32.3	29.27	12.45	20.02	19.9	21.5
CaCO <sub>3</sub>	7.	22.1	15.01	17.18	34.37	21.2	27.2	31.2	20.8	19.1	24	25.2	33.3	22.1
Total N	7.	0.34	0.76	0.56	0.25	1.32	0.12	0.87	0.68	0.55	0.32	0.91	1.23	0.54
Olsen P	mg kg <sup>-1</sup>	14.12	28	19.2	57.2	25	20.2	48.3	15.7	35	13.5	19.9	18	40.12
CEC	Cmol <sub>c</sub> kg <sup>1</sup>	18.8	25	22	14	10	15	29	27	16	21	17	25	10.9
TPHs	$mgk^1$	7330.7	3223.1	5859.1	8391.1	2432.2	6470.9	8994.1	7227.8	4758.1	5260.2	8809.5	3886.1	11130.3

Also table 1 shows TPHs concentration in studied soils. It ranged between  $2433.20-11130.27 \text{ mg kg}^{-1}$  with a mean value of  $6444.08 \text{ mg kg}^{-1}$ . The minimum and maximum TPHs concentrations were observed in No. 5 and No. 13 fuel stations, respectively.

Table 1 shows a list of the TPHs concentration of different contaminated soils in other areas and the correlations between TPHs concentrations and selected soil properties.

## DISCUSSION

As in Table 2, the observed TPHs concentrations of urban fuel station soils are comparable with other contaminated soils. The data were compared with standard TPHs concentration in soil [32]. The TPHs concentration in all studied fuel station soils was higher than the standard TPHs concentration (2000 mg kg<sup>-1</sup>) in soil (P < 0.05).

In another study in Iran, the initial concentration of TPHs in the soil provided from Oil Refinery of Tehran was more than 50000 mg kg<sup>-1</sup> by average, which demonstrated high levels of contamination in soil [33]. Table 3 shows a positive correlation between TPHs concentrations and total nitrogen, exchangeable Na<sup>+</sup>, silt and clay percentage and pH. Regressions between TPHs concentration and total nitrogen, clay percentage and pH were significant.

Contamination might have resulted in higher pH values of the contaminated soil. The pHs of the contaminated soils were from normal toward slightly alkaline. These results are similar to the results obtained by Obire and Nwaubeta (2002), who reported alkaline pH for gasoline contaminated soils. Apart from pH, total nitrogen has been generally reported to be higher in hydrocarbon contaminated soils [34].

There was also a significant negative correlation between TPHs concentrations and EC, CEC, water content and Olsen P.

The positive correlation between TPHs concentration and clay percentage in contaminated soil suggests a reduction in the soil aeration and porosity; these conditions can reduce soil water content. Soil conductivity is a measure of the soluble salt content in the soil and is used as an overall indicator of the level of macro- and micronutrients in the soil. Negative correlation indicated that the contaminant disturbed the soil structure and modified its physicochemical properties

In a case study by Ujowundu et al (2011) [35], biochemical and physical characterization of diesel, petroleum contaminated soil was studied and it was concluded that cation exchange capacity values were lower in contaminated soils as compared to uncontaminated ones. The CEC values suggest that the contaminated soil has lower quantity of cations that can be absorbed and held by soil and therefore available for plant use. The lower CEC value also indicates reduced pH buffering capacity.

Available phosphorous in soil can decrease in hydrocarbon treated soils [34]. Correlations between TPHs concentration and other soil parameters were not significant (Table 3).

Table 2.Comparison of total petroleum hydro-
carbons (TPHs) concentration of various con-
taminated soil in Hamedan.

Pollutant	Concentration (mg kg <sup>-1</sup> )	Reference
	11000	[5]
	50000	[33]
	14569	[20]
	34358±1633	[36]
TPHs	65756	[32]
	45534±31225	[18]
	29990±1.88	[37]
	84000	[38]
	46726.8	[35]
TPH $(C_{12}-C_{23})$	2800	[39]
TPH (C <sub>23</sub> –C <sub>40</sub> )	9450	[39]

**Table 3.** Coefficient of correlation betweenTPHs concentrations and selected soil proper-<br/>ties.

Pearson correlation	TPHs
EC	-0.254*
pН	$0.542^{**}$
Water Content	-0.310*
Organic Matter	-1.00
Organic Carbon	-0.186
Clay	$0.387^{*}$
Silt	0.197
Sand	-0.283
$\mathbf{K}^+$	-0.477
$Na^+$	0.262
CaCO <sub>3</sub>	-0.292
Total N	$0.292^{*}$
Olsen P	-0.446**
CEC	-0.467*

\* Correlation is significant at the 0.05 level (2-tailed).

\*\* Correlation is significant at the 0.01 level (2-tailed).

#### CONCLUSION

Pollution of soils with petroleum hydrocarbons is one of the important environmental problems in some areas, particularly around petroleum refineries and fuel stations.

In order to identify the effect of urban fuel stations on content TPH in soil, a study was performed in fuel stations of Hamedan City, Iran. The results showed that fuel splurges have clear effects on the content of TPH in soil, as concentration TPH in all of fuel stations was in upper standard levels in soil. Soil pollution with petroleum hydrocarbons has clear effects on soil biological, chemical and physical characteristics and results in decreased food elements, productivity and soil plant productions.

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#### REFERENCES

- Husaini A, Roslan HA, Hii KSY, Ang CH. Biodegradation of aliphatic hydrocarbon by indigenous fungi isolated from used motor oil contaminated sites. World J Microbiol Biotechnol 2008; 24:2789-97.
- Adam G, Gamoh K, Morris DG, Duncan H. Effect of alcohol addition on the movement of petroleum hydrocarbon fuels in soil. Sci Total Environ 2002;286:15-25.
- Clark CJ. Field detector evaluation of organic clay soils contaminated with diesel fuel. Environ Forensics 2003;4(3):167-73.
- Ayotamuno JM, Kogbara RB, Taleat MO. Bioremediation of a petroleum-hydrocarbon polluted agricultural soil at different levels of water application in Port Harcourt. Nigeria. J Food Agri Environ2006;4(3-4):214-7.
- Ebuehi OAT, Abibo IB, Shekwolo PD, Sigismund KI, Adoki A, Okoro IC. Remediation of crude oil contaminated soil by enhanced natural attenuation technique. JApplSciEnviron Manag 2005;9(1):103-6.
- 6. Greenberg BM, Huang X, Gerhardt K, Glick BR, Gurska J, Wang W, Lampi M, Khalid A, Isherwood D, Chang P, Wang H, Wu SS, Yu XM, Dixon DG, Gerwing P. Field and laboratory tests of a multi-process phytoremediation system for decontamination of petroleum and salt impacted

soils In: Proc. 9th Int. in situ and on-site remediation symp. Batelle press; 2007.

- Kathi S, Khan AB. Phytoremediation approaches to PAH contaminated soil. Indian J Sci Technol 2011;4(1):56-63.
- Wyszkowska J, Kucharski J. Biochemical Properties of soil contaminated by petrol. *Polish J Envi*ron Stud 2000;9(6):476-85.
- 9. Gong P, Sun TH, Beudert G, Hahn HH. Ecological effects of combined organic and inorganic pollution on soil microbial activities. Water, Air & Soil Pollution 1996;96:133-43.
- Wyszkowski M, Wyszkowska J, Zoilkowska A. Effect of soil contamination with diesel oil on yellow lupine yield and macroelements contents. Plant Soil Environ 2004;50(5):218-26.
- 11. Wyszkowski M, Wyszkowska J. Effect of enzymatic activity of diesel oil contaminated soil on the chemical composition of oat (*Avena sativa* L.) and maize (*Zea maize* L.) Plant Soil Environ 2005;51(8): 360-7.
- 12. Aboribo RI. Oil politics and the Niger Delta Development Commission. The tussle for control and Domination. Afr J Environ Stud 2001; 2:168-75.
- Njoku KL, Akinola MO, Taiwo BG. Effect of gasoline diesel fuel mixture on the germination and the growth of *Vigna unguiculata* (Cowpea). Afr J Environ Sci Technol 2009;3(12):466-71.
- 14. Omosum G, Markson AA, Mbanasor O. Growth and anatomy of *Amaranthus hybridus* as affected by diferrent crude oil concentrations. AmEuras J Sci Res 2008;3(1):70-4.
- Tnapa B, Kumar KCA, Gnimire A. Areview on bioremediation of petroleum hydrocarbon contaminants in soil. Kathmandu University Journal of Science, Engineering and Technology. 2012;8(1):164-70.
- 16. Abioye PO, Abdul Aziz A, Agamuthu P. Enhanced biodegradation of used engine oil in soil amended with organic wastes. Water, Air & Soil Pollution 2010;209:173-9.
- 17. Murakami Y, Kitamura Sh, Nakayama K, Matsuoka S, Sakaguchi H. Effects of heavy oil in the developing spotted halibut. Verasper variegates. Marin Pollution Bulletin 2008;57:524-8.
- Kulakow PA, Erickson L. A nationwide field test of petroleum- contaminated soils. Proceedings of the 2000 Conference on Hazardous Waste Research. 2000. PP 283-98.
- Leahy JG, Colwell RR. Microbial degradation of hydrocarbons in the environment. American Society for Microbiology. Microbiol Rev1990;54:305-15.
- 20. Wegwu MO, Uwakwe AA, Anabi MA. Efficacy of enhanced natural attenuation (land farming) technique in the remediation of crude oil-

polluted agricultural land. Arch ApplSci Res 2010; 2(2):431-42.

- Huang XD, El-Alawi Y, Gurska J, Glick BR, Greenberg BM. A Multi-Process phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPHs) from soils. Microchem J 2005;81:139-47.
- Denys S, Rollin C, Guillot F, Baroudi H. Insituphytoremediation of PAHs contaminated soils following abioremediation treatment. Water, Air & Soil Pollution. 2006;6:299-315.
- Li G, Huang W, Lerner DN, Zhang X. Enrichment of degrading microbes and bioremediation of petrochemical contaminants in polluted soil. Water Res 2000;34(15):3845-53.
- 24. Rowell DL. Soil Science: methods and applications. Longman Scientific and Technical, 1994.
- 25. Olsen SR, Sommers LE. Phosphorus, p. 403-27. In: Page AL (Ed.). Methods of Soil Analysis, Part 2, American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin, USA. 1982.
- Murphey J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. Anal Chimica Acta 1962;27:31-6.
- 27.Schneekloth J, Bauder T, Broner Wakson R. Measurement of soil moisture. 2002. http://www.etx.colostate.edu/drought/soilmoistur e.htm.
- 28. Supaphol S, Panichsakpatana S, Trakulnaleamsai S, Tungkananuruk N, Roughjanajirapa P, Gerard O, Donnell A. The selection of mixed microbial inocula in environmental biotechnology: Example using petroleum contaminated tropical soils. J Microbiol Methods 2006;65:432-41.
- 29. Urum K, Pekdemir T, Gopur M. Optimum conditions for washing of crude oil-contaminated soilwith biosurfactant solutions. Process SafEnviron Protect 2003;81(3):203-9.
- Urum K, Pekdemir TC, Copur M. Surfactants treatment of crude oil contaminated soils. J ColloidInterface Sci 2004;276:456-64.
- Urum K, Grigson S, Pekdemir T, McMenamy S. A comparison of the efficiency of different surfactants for removal of crude oil from contaminated soils. Chemosphere 2006;62(9):1403-10.
- 32. Kang WH, Cheong JG, Kim K, Chae H, Chang CH. Restoration of Petroleum-Contaminated Soils by Field-Scale Soil Washing System. International Conference on Environmental Science and Technology IPCBEE vol. 30. 2012.
- Ravanbakhsh S, Daryabeigi Zand A, Nabi Bidhendi G, Mehrdadi N. Removal of total petroleum hydrocarbons (TPHs) from oil-polluted soil in Iran. IraJChemChemical Engin 2009;28(4):105-13.

- Obire O, Nwaubeta O. Effects of refined petroleum hydrocarbon on soil physicochemical and bacteriological characteristics. JApplSciEnviron Manag 2002;6(1): 39-44.
- 35. Ujowundu CO, Kalu FN, Nwaoguikpe RN, Kalu OI, Ihejirika CE, Nwosunjoku EC, Okechukwu RI. Biochemical and physical characterization of diesel petroleum contaminated soil in southeastern Nigeria. Res J Chem Sci 2011;1(8):57-62.
- Daryabeigi Zand A, Nabi Bidhendi G, Mehrdadi N. Phytoremediation of total petroleum hydrocarbons (TPHs) using plant species in Iran. Turkish J Agri Forestry 2010;34:429-38.
- 37. Milic JS, Beskoski VP, Ilic MV, Ali SA, Gojgic-Gvijovic GD, Vrvic MM. Bioremediation of soil

heavily contaminated with crude oil and its products: composition of the microbial consortium. J Serbian Chem Soc 2009;74(4):455-60.

- Das K, Mukherjee AK. Crude petroleum-oil biodegradation eYciency of *Bacillus subtilis* and *Pseudomonas aeruginosastrains* isolated from a petroleum-oil contaminated soil from North-East India. Bioresource Technol 2006;98(7):1339-45.
- Bento FM, Camargo FAO, Okeke BC, Frankenberger WT. Comparative bioremediation of soils contaminated with diesel oil by natural attenuation. biostimulation and bioaugmentation. Bioresource Technol 2005;96:1049-55.