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ORIGINAL ARTICLE

Biochemical and Physical Characterization of Petroleum Hydrocarbon Contaminated Soils in Tehran

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KEYWORDS

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ABSTRACT: Contamination of soil was investigated in this study from the Tehran Oil refining Co. of Iran. Fifteen soil samples were collected at several points in the Azimabad, 15 km south of Tehran City, Iran. Samples were collected at depths of 0–30 cm. Control sampleswere prepared to determinebackgroundlevels ofsoil contaminationwithpetroleumhydrocarbonsfor comparison with contaminatedsites. Total petroleum hydrocarbon (TPH) and poly-aromatic hydrocarbons (PAH) concentrations varied from 101334.0–101367.1 and 25321.1–25876.6 mg kg^{-1} respectively. The results elevated levels of TPH and PAH contents when compared with the control sample. Soil acidity (low pH of 5.3–5.9) and low electrical conductivity provided evidence of reduced metabolic activities on the affected site.Microbialgrowthrates for bacteria and fungi expressed as colony forming units were 2.62×10^9 and 4.14×10^6CFU/g soil, respectively for the contaminated and 5.76×10^9 and 6.83×10^6CFU/g soil, for the control treatments respectively. These drastic changes can have impact on the nutrient cycle and prevents the absorption of nutrients by plant root sand lead to a reduction in yield.

INTRODUCTION

All over the world, scientists and environmental challengers have encountered overcoming problems of soil, airandwatercontamination. Large-scale leakage of crude oil in the soil, excessive leakage and careless

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disposa land waste management problems of oil leakage from underground pipe line sand fuel tanks constitute the major sources ofoil pollutioninour environment. Carcinogenic, mutagenicandtoxic effects of this kind of pollutantshave causedto attracted more attention and become a favorite topic for research [1, 2].

Large amounts of hydrocarbons in contaminated sites can bea serious threat to the health of humans, plants and animals lives.

By early1900, there were relatively fewstudieson theinteractionbetweenhydrocarbons andsoilenvironments [3]. Carr was one of the earliest researchers studied the pollution caused by leaking pipe fractures [4]. Other researchers have investigated the effects of crude hydrocarbons as potential agricultural amendments to stimulate agricultural production through increased nitrification [5]. Furthermore, the inter action between oil and hydrocarbon gases was investigated, again as these cases affected crop response through the motivation or appeasement of microbial nitrogen and phosphorous mineralization [6, 7]. Lately, there lease of hazardous and toxic materials in to the air, water, soil and sediment has become awide spread problem [8-10].

Oil exploration sites, pipelines or leakages from underground surface tanks, inappropriate disposal of petroleum wastes, caused environment contamination by petroleum hydrocarbons. Itisan environmental problembecause the presence of hydrocarbons insoil has changed it properties.Petroleum and its derivatives are extremely toxic to organisms, particularly humans. They also contain smidgen amounts of nitrogen and sulphur compounds, which are hazardous by themselves and can react with surroundings.

A hydrocarbon is an organic compound.Total petroleum hydrocarbon (TPH) is a phrase used for mixture of hydrocarbons such as hexane, benzene, toluene, xylenes, naphthalene, fluorine etc.; constituents found in crude oil which are polluting the environment. Because of the complexity of different chemicals in crude oil and other petroleum products, it is not feasible to measure each one individually. Howsoever, it is effective to measure the total amount of these compounds at contaminated sites.

Polycyclic aromatic hydrocarbons (PAHs), recognized as poly-aromatic hydrocarbons or polynuclear aromatic hydrocarbons. PAHs in the environment mostly emanate from anthropogenic source like mineral oil spill or gas factories, or from biological production in Oxygen-deficient sediments. Manifold aerobic bacteria have been isolated that can dissociation aromatic hydrocarbons to carbon and energy. The degradation passage has formerly been explicit [11, 12]. PAHs identified as natural constituents in fossils fuels. They are part of the thousands of components in petroleum products and formed because of incomplete combustion of organic materials through pyrolysis and pyrosyn thesis [13-16]. Transport activities and petroleum refining are main participants to localized ladings to PAHs into the environment. Such ladings may happen via depletion of industrial effluents and via accidental distribution of naive and refined products. Howsoever, PAH distributed into the environment may emanate from many sources containing gasoline and diesel fuel combustion [17] and tobacco smoke [18]. PAHs are fined in air [19, 20], sediment and soil [21-24], surface and groundwater, and runoff [25, 26] are interspersed from the atmosphere to vegetation [27] and pollute foods [28]. Oil release affects the physical and chemical properties of soils [29].

The contaminated soil with petroleum is unsuitable for crop growth for several months or years. To evaluate quality and fertility of soil, awareness about physical, chemical and biological properties of the soil are necessary. Microorganisms being in cordial contact with soil environment in many ways and their population are one of the best indicators to monitor soil pollution.

The aim of this study was to provide information on the effect of petroleum hydrocarbons on soil physic-chemical properties and on microbial community of the soil.

MATERIALS AND METHODS

Sample Collection

The sampling site is located at Azimabad, 15 km south of the Tehran, Iran, named Tehran Oil Refining Co. (within longitude 51°25'22"E and latitude 35°32'31"N).
A primary check was carried out on the site before sample collection in order to convince that problems during sampling would be minimized. Fifteen soil samples from surface (0-30 cm depth) were selected from around the waste spillage pond to represent areas of contamination. Control sample was obtained to distinguish the backward levels of petroleum hydrocarbons in the freestanding soil for comparison with the contaminated site.

Physic-chemical analysis of soil

Soil samples were crashed and air- dried for four days and soil particles were sieved through a 2 mm mesh to obtain a uniform size [30]. Mass of soil was mixed up well until pollution in its different points get homogeneous; subsequently before of extraction and analyze was kept in 4°C temperature in aluminum foils. Soil properties were analyzed by standard methods after transport the samples into laboratory. Total nitrogen was measured by Kjeldahl digestion [31]. Electrical conductivity (EC) and pH of the soils were measured in a 1:1 (W/V) soil/water mixture [32].
Soil texture and sand, silt and clay percentage were analyzed using standard methods according to Gee and Bauder [33]. Total organic carbon is an alternative analytical method for measuring petroleum hydrocarbons using the wet oxidation technique [34]. Heavy metals include Cd, Cu, Fe, Pb and Zn extracted with DTPA was determined by ICP-AES. Calcium and Mg were determined by titration and Na and K were measured by flame photometry in extracts [35].

Microbial analysis of soil

Hydrocarbon-degrading bacteria’s were enumerated using a Most Probable Number (MPN) method and heterotrophic bacteria’s were enumerated using colony enumeration on cultivation environment. Bushnell Haas medium (composition: magnesium sulfate 0.2 gL^{-1}, calcium chloride 0.2 gL^{-1}, monophosphate potassium 1 gL^{-1}, ammonium phosphate dibasic 1 gL^{-1}, potassium nitrate 1 gL^{-1} and ferric chloride 0.5 gL^{-1})that supplemented with 2% (w/w) sodium chloride with pH= 7.2, sterile with autoclave at temperature 120 °C for 20 min. After of preparation, cultivation environment was used from soil samples. Three g of each soil samples was located in a small container containing 10 ml of Bushnell Haas medium supplemented with 2 percent (w/w) Nail and blended to form slurry for 30 min, then 1 ml of this slurry was set into a small container containing 9 ml of Bushnell Haas medium supplemented with 2 percent (w/w) NaCl. A dilution rows was ready from this sample, from $10^{-1}$to 10^{-12}, and used to inseminate plates and each scarcity has three replication, in a form that for each soil sample 36 experiment tube should be prepared, after diluting Risasorin identifier in a rate of 90 μl added to tubes and then sterilized crude oil in a rate of 0.2 ml should be added to each tube and plates for numeration of hydrocarbon-degrading bacteria were incubated at room temperature (26-27 °C) for 2 weeks. After two weeks, while color variation was observed, tubes extracted from incubation, and all plates were read using a MPN chart to specify bacteria, this require that all needed appliances were sterilized [36, 37]. Fungi were enumerated on sabouraud dextrose agar [38, 39].

TPH and PAH analysis

Total petroleum hydrocarbons and PAH from soil samples have been studied by Soxhlet extraction procedure with same proportion from n-hexan and di-chloromethan [40]. For PAH analysis, high pressure liquid chromatography (HPLC) and for TPH analysis, gas chromatography (GC) was used [41].

RESULTS AND DISCUSSION

The result of physicochemical properties of contaminated and uncontaminated (control) soils with petroleum waste spillage as presented in Table 1.

Table 1. Some physical and chemical characteristics of the contaminated (15 samples) and uncontaminated (control) soils.

		Clay	Sand	silt	Total C	Total N	pH	EC	TPH	PAH	Na^{+}	K^{+}	Ca^{2+}	Mg^{2+}	Cd^{2+}	Cu^{2+}	Fe^{2+}	Pb^{2+}	Zn^{2+}
		%						dSm^{-1}	$Mg\ kg^{-1}$		$Mg\ kg^{-1}$ (extracted with NH_4AOC)				$Mg\ kg^{-1}$ (extracted with DTPA)				
Contaminated soils	**Min**	12.0	48.0	40.0	17.2	0.2	5.3	7.0	101334.0	25321.1	24.2	119.4	11.8	11.4	6.4	79.5	58.7	23.4	12.0
	Max	16.0	47.0	37.0	18.6	0.9	5.9	7.9	101367.1	25876.6	37.5	126.2	19.3	19.0	6.9	92.5	64.2	23.9	12.9
	Aver	12.0	48.0	40.0	17.9	0.5	5.6	7.4	101350.7	25595.4	29.1	122.3	15.5	16.3	6.5	87.3	61.9	23.2	12.3
Control soil		9.0	52.0	39.0	2.3	0.8	7.4	8.2	2.3	0.002	33.6	145.1	20.4	24.7	0.004	86.2	14.1	18.3	12.4

Soil reaction (pH)

Table 1 shows that pH of the control soil was 7.4 whereas the range of pH in contaminated soils was 5.3 to 5.9. The pH of the contaminated soils was significantly lower than the control soil. Petroleum waste and oil must have dispirited the leaching of fundamental salts, which are increases, the pH in soil. The binding of the oil with soil matrix in the affected area perhaps posed a major insistence to the removal of such basic ions. While the oilmay have hadsome clear impact in pH decreasing, it is more likely that production of organic acids by microbial metabolism cause for different reasons. The lower pH in the contaminated soils could be because of the impact of the petroleum hydrocarbons, which could lead to disorder of gaseous exchange and keeping of carbon dioxide in soil. The results of these conditions are decreasing acidity and porosity of the soil.

The low pH, the important factor, may have affected on the microbial growth specially bacteria and fungi in the contaminated soils, was observed to be low. Furthermore, soil pH might have affected nutrient accessibility. The pH is not only required for specifying the accessibility of many nutrients in soil but also in determining the destination of soil pollutants, their decomposition and leachability through the soil. Therefore, acidic pH might have implications on nutrient availability in the contaminated soils. Such pH ranges, for instance, might have affected the solubility of minerals [42]. These results are similar to the results, reported the decreasing the soil pH due to oil discharge on soil [43]. Moreover, other researchers reported the soil acidic pH in presence of kerosene, diesel oil, and gasoline and oil spillage [42, 44].

Electrical conductivity (EC)

Electrical conductivity (EC) represents the ions concentration in soil solution and is therefore describes the condition of dissolve solutes. Electrical conductivity was significantly lower in the contaminated soils than in the control soil (Table 1). Maybe the released oil was not the only factor that directly responsible for the changes in EC, whereas organic compounds such as crude oil cannot conduct electrical current well. In the absence of oxygen, direct dehydrogenation due to presence of anoxic biodegradation mechanism responsible for anaerobic metabolism of hydrocarbons. This reaction occurs in the presence of an electron acceptor like nitrate ion, which may be relatively responsible for the noticed changes in EC [42].

Lower EC also indicates low salinity in the contaminated samples. Results revealed that the mean inorganic element concentrations decreased in the contaminated soils in the contrary with the control soil (Table 1). This may be due to interference with the extraction analysis due to free hydrocarbon in the soil but more likely to utilization/complexation of the nutrients by resident micro flora. It is unlikely that the oil release is directly responsible for loss of macronutrients. However, the intense infusion of degradable hydrocarbon likely stimulated aerobic and anaerobic microbial metabolism.

Total organic carbon and total nitrogen

The organic carbon content and total nitrogen of the contaminated soils show the mean value of 17.9 and 0.5% respectively, as compared with 2.3 and 0.8% of the control soil (Table 1).

The organic carbon amount in all the polluted soils was great. This is attributed to the steady input of petroleum hydrocarbons.

The reduction in the concentration of total nitrogen in the contaminated sites indicates that the incidence of oil spillage affect and reduced the process of nitrification.

Oil degradation or hydrocarbon employing microorganisms like *Azobacter* spp. Normally become more numerous while nitrifying bacteria like *Nitrosomonas* spp. become reduced in abundance [45]. This probablyexplains the relatively lower values of total nitrogen obtained for the contaminated soils. Enrichments capable of the degradation of hydrocarbon fractions like toluene under anoxic denitrifying conditions have also been reported in agricultural soils, compost, aquifer material, and contaminated soils from various geographic regions of the world [46, 47].

The optimum C/N ratio reported to 12.5:1 [48]. Partly more PAH degradation in soil sample has been reported with a C/N ratio near 12.5:1 [49]. In this study, analysis of the soil samples revealed mean C: N ratio of 17.9:0.5 and 2.3:0.8 in contaminated and control soils, respectively.

Total petroleum hydrocarbon (TPH) and poly-aromatic hydrocarbons (PAH)

The mean concentrations of TPH and PAH of samples are shown in Table 1. The overall content of TPH and PAH recorded in the petroleum-contaminated soils ranges from 101334.0 to 101367.1 and 25321.1 to 25876.6 mg kg^{-1} respectively. However, no significant level of TPH and PAH was recorded for the control soil sample derived from same geographical non-spilled areas.

The results showed increase in TPH concentrations when compared with control sample. The high levels of TPH contamination obtained in this study for the spilled soils are similar to concentrations reported earlier [10, 50]. In other studies, the soil TPH concentration was reported between 100-500 $mgkg^{-1}$ [51, 52]. Furthermore, some studies reported the TPH content in soil to vary extremely by a few thousand $mgkg^{-1}$ [9, 53, and 54].

High levels of the TPH and PAH can make soil situations undesirable for plants and microbial growth [55]. Poly-aromatic hydrocarbons are hydrophobic mixture with low ability to dissolve in water, which have a more propensity to bind with organic matter or soil, restricting their access to microorganisms [56, 57]. PAH having the potential to cause cancer and/or mutagen in some circumstances and have been arranged as important pollutants. This content of contamination will cause increase the toxic substances such as cresol, phenols, chlorine which may prevent the growth of the hydrocarbon oxidizers [39]. Besides heavy metal concentration was found higher in all contaminated soil samples as compared to control soil. Between studied heavy metals, iron and lead content was exceptionally higher in spilled soils. The presence of trace metals in the environment and specially, in soils, industrial and anthropogenic wastes have a major potential threat to living organisms. While it gets into food chain, via plants, animals and water sources leads to bio magnifications and bioaccumulation in living cells and tissues.

Physical properties

Soil physical properties are very important because several factors such as movement of nutrients through soil pores, soil aeration and water holding capacity are direct or indirect affect from soil physical properties [49]. In the present investigation, the range of clay percentage in contaminated soils was 12% to 16% as compared to 9% in control soil, indicating probability of a higher amount of hydrocarbon degradation in control soil. The mean higher percentage silt and clay in the contaminated soils (Table 1) in the contrary with the control soil suggest a decrease in the soil porosity and aeration. Decreased ventilation has a direct effect on microbial growth, which can raise the biodegradation of petroleum compounds [12].

From mentioned results, it could be concluded that hydrocarbon contamination negative changes on characteristics of soil. Crude oil applied in soil had little effect on the measured physical parameters and water infiltration rates (hydraulic conductivity generally) in these soils reduced [3].

Microbial population

Table 2 shows the bacteria and fungi population in contaminated and control soils. The high concentration of the oil especially the PAH cause losses in the microbial population in contaminated soil could. The results on the bacterial growth rate declared in bacterial colony forming units were 2.62×10^{9}CFU/g soil, in the contaminated and 5.76×10^{9}CFU/g soil, in the control treatments. The fungal growth rate declared in colony forming units were 4.14×10^{6}CFU/g soil, in the contaminated and 6.83×10^{6}CFU/g soil, in the control treatments. The lower microbial population in the contaminated soils could be attributed to the effect of the petroleum waste and oil spillage, which could lead to disorder of gaseous exchange and retention of soil carbon dioxide. This situation caused pH increases and porosity decreases in soil. The quick effect of oil contamination in the soil is the degradation of the microbial population due to the presence of additives substance in the refined oil.The additives and other modifying chemicals could impede rapid microbial application of the hydrocarbon in the petroleum waste and oil. The lack of these microorganisms lessens the biological activity in the contaminated soil.

Table 2. Bacteria and Fungi population in the contaminated (15 samples) and uncontaminated (control) soils.

Contaminated soils	Population (CFU/g soil)		
	Min	Max	Aver
Bacteria (Exploitation)	2.21×10^9	2.28×10^9	2.13×10^9
Bacteria (Heterotrophic)	3.10×10^9	3.19×10^9	3.12×10^9
Fungi	4.10×10^6	4.19×10^6	4.14×10^6
Control Soil			
Bacteria (Exploitation)			5.22×10^9
Bacteria (Heterotrophic)			6.30×10^9
Fungi			6.83×10^6

Content, chemical mixture and toxicity of contaminants are also among the critical factors for microbial variety in contaminated soils. Comparatively, higher level of TPH was recorded in contaminated soil samples. This suggests the probability of reduced microbial population in these soil samples [49].

CONCLUSIONS

Petroleum wastes changed the physical, chemical and microbial characteristics of the soil. It altered soils' structural class and changed soil acidity, which can affect plant roots and subsequently impede nutrient availability and uptake from the soil. This finally may lead to decrease in crop yield.

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The authors declare that there is no conflict of interests.

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