

Influence of Crude Oil Contamination on the Chemical and Microbiological Aspects of Saudi Arabian Soils

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Abstract. Samples of petroleum-contaminated and uncontaminated soils were collected from Eastern region of Saudi Arabia and analyzed for soil texture, organic matter content, total soluble salts, pH, mineral content, fungal and bacterial microflora as indicators of soil pollution. The results obtained revealed that both petroleum-contaminated and uncontaminated soils were sandy, mildly alkaline, containing almost the same amounts of organic matter, soluble salts and heavy minerals. The total number of colonies of bacteria and fungi per gram of soil were higher in uncontaminated than petroleum contaminated soils. A total number of twenty fungal species were isolated from both contaminated and uncontaminated soils. The uncontaminated soil is capable of supporting the growth of all 20 species while the contaminated soil supports only 17 of these species. In both contaminated and uncontaminated soils *Aspergillus* was the dominant fungal genus (6 species) followed by *Penicillium* and *Cladosporium* (3 species each). Bacterial genera belonging to *Arthrobacter*, *Bacillus*, *Micrococcus*, *Pseudomonas* and *Staphylococcus* were also isolated.

Introduction

Most of the Eastern region of Saudi Arabia including coastal area, bordering Kuwait, became polluted with petroleum during the recent Gulf war. Microorganisms including bacteria and fungi could degrade petroleum as a carbon and energy source [1, 2] [3, p. 692], [4-7].

Aspergillus, *Penicillium*, *Trichoderma*, and *Cladosporium* species could degrade petroleum product and other high carbon compounds [8, 9, 10]. Species of bacterial genera like, *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Micrococcus*, and *Staphylococcus* could also degrade petroleum products and other high carbon organic compounds [6, 10, 11]. Edible oils, petroleum products, emulsion paints, diesel fuels etc. could be converted into useful products by biodegradation activity of these microorganisms [12, 13].

There are several problems dealing with petroleum contaminated soils which effect human health and environmental quality [14]. This study considers the influence of

spilled oil on soil chemical properties and proliferation of soil microflora in the sandy sites of Eastern Saudi Arabia.

Materials and Methods

During the Gulf war, due to spilling of petroleum oil on the soil of the war zone bordering Kuwait, several patches of petroleum oil contamination could be seen. Samples were collected from these oil patches and also from nearby non-contaminated soil. Five samples (five replicates of each) were collected from five localities of the same area (Al-Khafji area) both from contaminated and uncontaminated soil. Total number of bacteria and fungi per gram of soil were counted by using the dilution plate method for each type of sample. These samples were then mixed together and random five gram subsamples of soil were taken and homogenized into 45 ml of presterilized water. The resulting suspension was used to isolate fungi and bacteria by the dilution plate method as described earlier [15].

For fungal isolation, Czapek's Dox agar (Oxoid Ltd., London) with rose bengal (0.033 g/L) and streptomycin sulphate (0.033 g/L) was used. For bacterial isolation, nutrient agar (Oxoid Ltd., London) was used. Identification of fungi was carried out according to Ellis [6, pp. 309, 315, 465, 498, 547], Ellis [17, pp. 414, 430], Gilman [18, pp. 170, 212, 220, 225, 334, 374], Ramirez [19, pp. 300, 471], and Raper and Fennell [20, pp. 203, 301, 357, 377, 412]. The identification of bacterial flora was done according to Buchanan and Gibbons [21].

Total soluble salts, organic matter, and soil reaction (pH value) were determined according to Bower and Wilcox [22, pp. 933-951], Broadbent [23, pp. 1397-1400], and Peech [24, pp. 920-923]. Soil texture was determined by the hydrometer method [25, pp. 77-80]. Total metal content was determined according to Hashem, [26].

Table 1. Physical and chemical analysis of soil samples

Soil treatment	Soil texture	Organic matter %	Total soluble salts %	pH value	Total metal content ($\mu\text{g/g}$)					
					AL	Cd	Cu	Fe	Pb	Zn
Contaminated	Sandy	1.3	1.1	7.0	90 \pm 3	6 \pm 0.1	13 \pm 1	26 \pm 2	4 \pm 1	16 \pm 1
Uncontaminated	Sandy	1.8	1.0	7.5	85 \pm 03	4 \pm 03	11 \pm 2	24 \pm 2	3 \pm 0.1	14 \pm 1

n = 5

\pm standard deviation

Results and Discussion

The results of physical and chemical analysis of soils are shown in Table 1. Both contaminated and uncontaminated soils were similar in their texture, organic matter content, total soluble salt content, pH value and total metal content. This is consistent with the previous findings by the author in Saudi Arabian soils [27, 28]. The total number of fungal and bacterial colonies per gram of uncontaminated soil is shown in Table 2. The average number of fungal and bacterial colonies per gram soil isolated from uncontaminated and contaminated soil were 2610 fungal and 4.1×10^6 bacterial and 1280 fungal and 4.1×10^4 bacterial respectively. The contaminated soil supports a lower number of microflora than the uncontaminated one (Tables 2 and 3).

Table 2. Number of fungal and bacterial colonies per gram of uncontaminated soil

Soil sample delineation	No of colonies per gram of soil	
	Fungi	Bacteria
A	± 1.6	$\times 10^6$
B	± 14	$\times 10^6$
C	± 15	$\times 10^6$
D	± 10	$\times 10^6$
E	± 11	$\times 10^6$
Average		$\times 10^6$

Table 3. Number of fungal and bacterial colonies per gram of contaminated soil

Soil sample delineation	No of colonies per gram of soil	
	Fungi	Bacteria
A - 1	1260 ± 12	3×10^4
B - 1	1631 ± 10	6×10^4
C - 1	993 ± 9	5.5×10^4
D - 1	1350 ± 14	3.4×10^4
E - 1	1169 ± 13	2.6×10^4
Average	1280	4.1×10^4

The uncontaminated soil supports the growth of all of the 20 fungal species while the contaminated soil supports the growth of only 17 species out of the 20 species identified (Table 4). The dominant genera were *Aspergillus* (6 species) followed by *Cladosporium* and *Penicillium* (3 species each), *Ulocladium* and *Alternata* (2 species each).

Table 4. Fungal flora isolated from petroleum- contaminated and uncontaminated soil

Fungi	No of colonies per gram of soil	
	Uncontaminated	Contaminated
<i>Alternaria alternata</i>	36 ± 5	24 ± 4
<i>A. chlamyospora</i>	49 ± 6	39 ± 5
<i>Aspergillus carbonarius</i>	34 ± 7	18 ± 3
<i>A. flavus</i>	52 ± 9	29 ± 5
<i>A. funiculosus</i>	21 ± 3	14 ± 3
<i>A. niger</i>	41 ± 5	21 ± 4
<i>A. thomii</i>	16 ± 3	NF
<i>A. zonatus</i>	12 ± 3	21 ± 4
<i>Chaetomium piluliferum</i>	21 ± 4	26 ± 4
<i>Cladosporium herbarum</i>	34 ± 3	24 ± 3
<i>C. resinae</i>	26 ± 4	59 ± 6
<i>C. sphaerospermum</i>	26 ± 3	16 ± 3
<i>Eurotium</i> sp.	21 ± 5	56 ± 7
<i>Fusarium solani</i>	34 ± 4	18 ± 3
<i>Penicillium chrysogenum</i>	56 ± 3	42 ± 6
<i>P. expansum</i>	18 ± 4	NF
<i>P. notatum</i>	23 ± 4	NF
<i>Trichoderma</i> sp.	26 ± 3	46 ± 5
<i>Ulocladium atrum</i>	39 ± 5	46 ± 5
<i>U. chlamyosporum</i>	32 ± 3	39 ± 4
No. of species/genera	20/9	17/9

NF – Not found

Aspergillus thomii, *Penicillium expansum* and *P. notatum* were not isolated from contaminated soil. Generally, the number of colonies of a particular species per gram of soil was less in contaminated than in uncontaminated soil except for *Aspergillus zonatus*, *Cladosporium resinae*, *Eurotium* sp., *Trichoderma* sp., *Ulocladium atrum* and *U. chlamyosporum* where a reverse trend was observed.

The bacterial species *Arthrobacter*, *Bacillus*, *Micrococcus*, *Pseudomonas* and *Staphylococcus* were isolated from both uncontaminated and contaminated soil (Table 5) but the number of colonies per gram of soil were less in petroleum contaminated than in uncontaminated soil.

Table 5. Bacteria flora isolated from petroleum- contaminated and uncontaminated soil

Bacteria	No of colonies per gram of soil	
	Uncontaminated	Contaminated
<i>Arthrobacter</i> sp.	3539 ± 25	1696 ± 16
<i>Bacillus</i> sp.	6344 ± 26	3439 ± 15
<i>Micrococcus</i> sp.	2691 ± 21	1329 ± 14
<i>Pseudomonas</i> sp.	5648 ± 18	3126 ± 16
<i>Staphylococcus</i> sp.	3636 ± 16	1346 ± 21

Aspergillus flavus, *A. niger*, *Penicillium chrysogenum*, *Trichoderma* and *Ulocladium atrum* isolated here, were previously reported in petroleum contaminated soils in Saudi Arabia [29]. However, *Aspergillus ellipticus*, *Chaetomium bostrychodes*, *Mucor* and *Rhizopus* isolated earlier [29] were not encountered here. All fungal genera and species isolated in this work have previously been reported as biodegraders of various complex organic compounds and petroleum products [4, 6, 8, 9, 30, 31, 32].

The contamination of soil with toxic environmental contaminants such as petroleum is pervasive problem of potential human health concern particularly to those working and residing near hazardous products (e.g., polyaromatic hydrocarbons, benzene, lead, ...etc.) are compounds commonly identified as soil contaminants at various hazardous waste sites, and the toxicological profiles of several of these substances have already been drafted [33]. It is worth mentioning that more research is warranted to elucidate the oil contamination problem of soils encountered in spillage areas.

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أثر التلوث بالزيت الخام على الخواص الكيميائية والحيوية (الميكروبيولوجية) للتربة في المملكة العربية السعودية

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(سُلم في ٢١ رجب ١٤١٤هـ؛ وقُبل للنشر في ١٤ شعبان ١٤١٥هـ)

ملخص البحث . تم جمع عينات من التربة الملوثة وغير الملوثة بالبتروول من شرق المملكة العربية السعودية وحُللت لمعرفة قوام التربة والمحتوى العضوي الكلي للأملاح الذائبة وتفاعل التربة والمحتوى المعدني والفلورا الفطرية والبكتيرية.

أظهرت نتائج البحث أن التربة الملوثة وغير الملوثة رملية القوام معتدلة القلوية وتحتوي على الكمية نفسها من المادة العضوية والأملاح الكلية الذائبة والمحتوى المعدني.

كما وجد أن عدد المستعمرات البكتيرية والفطرية في الجرام الواحد من التربة أعلى في التربة غير الملوثة مقارنة بالتربة الملوثة. وقد تم عزل عشرين نوعاً من الفطريات تنتمي إلى تسعة أجناس من التربة غير الملوثة بينما تم عزل سبعة عشر نوعاً من التربة الملوثة. والأجناس الفطرية السائدة هي: اسبرجيليس (سته أنواع) يليه بنيسليام وكلا دوسبوريوم (ثلاثة أنواع). أما الأجناس البكتيرية التي تم عزلها فهي ارثروباكترو باسيليس وميكروكوكسي و بزيديموناس وستايلوكوكس.