Soil fungal population study related to oil pollution along different distances from kawrgosk oil refinery of erbil-iraq

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Abstract

Soil fungal population along different distances from Kawrgosk Oil Refinery was analyzed in relation to physicochemical characteristics and residual oil contents. Fourteen species belonging to twelve genera were isolated. The total number of fungi from sixteen studied sites was 243×10^3 cfu g^{-1} dry soil. Maximum fungal population 41×10^3 cfu g^{-1} dry soil was in site 6 and minimum population 2×10^3 cfu g^{-1} dry soil was in the refinery center. The most frequently isolated fungi were yeasts 96×10^3 cfu.g^{-1} dry soil with a percentage of occurrence 41.03 %, followed by *Aspergillus ochraceous* 43×10^3 cfu.g^{-1} dry soil (18.38 %), *Rhodotorula* sp. 24×10^3 cfu.g^{-1} dry soil (10.26 %), *Penicillium* spp. 23×10^3 cfu g^{-1} dry soil (9.83 %) and *A. niger* 12×10^3 cfu.g^{-1} dry soil (5.13 %) and the least frequently isolated species was *A. terreus* 1×10^3 cfu.g^{-1} dry soil (0.43 %). The correlation between total cfu of fungi with oil residue was negative by r value of -0.092 and its correlation with silt and clay contents, pH, total P, K, and S were also negative by r values of -0.005, -0.135, -0.290, -0.090, -0.255 and -0.227 respectively ($p<0.05$). The correlation between total cfu of fungi with moisture, sand, EC, total organic C and total N were positive by r-values of 0.005, 0.143, 0.355, 0.161 and 0.152 respectively.

دراسة عن المجتمعات الفطرية نسبة الى التلوث النفطي عبر مسافات مختلفة عن مصفى الكوركوسك – أربيل – العراق

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الخلاصة

تم تحليل المجتمعات الفطرية عبر مسافات مختلفة عن مصفى الكوركوسك نسبة الى الصفات الفيزيائية والكيمياوية ومتبقي النفط في التربة. تم عزل أربعة عشرة صنفاً لاثني عشر نوعاً من الفطريات. كان العدد الكلّي للفطريات 243×10^3 وحدة مكونة للمجتمع غم$^{-1}$ للتربة الجافة في ستة عشرة موقعا قيد الدراسة. كان أعلى مجتمع فطري 41×10^3 وحدة مكونة للمجتمع غم$^{-1}$ للتربة الجافة في الموقع رقم 16 وأدنى مجتمع فطري 2×10^3 وحدة مكونة للمجتمع غم$^{-1}$ للتربة الجافة في مركز المصفى. كان الفطر المعزول الأكثر تكراراً الخمائر 96×10^3 وحدة مكونة للمجتمع غم$^{-1}$ للتربة الجافة بنسبة مئوية للتواجد 41.03%، يتبعه Aspergillus ochraceous 43×10^3 وحدة مكونة للمجتمع غم$^{-1}$ للتربة الجافة (18.38%) Rhodotorula sp. 24×10^3

وحدة مكونة للمجتمع. غم−1 للتربة الجافة (10.26%) Penicillium spp. 23×10^3 وحدة مكونة للمجتمع. غم−1 للتربة الجافة 9.83% و A. niger 12×10^3 وحدة مكونة للمجتمع. غم−1 للتربة الجافة 5.13% وكان الصنف المعزول الأقل تكراراً A. terreus 1×10^3 وحدة مكونة للمجتمع.غم−1 للتربة الجافة 0.43%. كان معامل الارتباط بين الوحدات الكلّية المكونة للمجتمعات الفطرية ومتبقي النفط سالبة بقيمة−0.092 وكذلك كان معامل الارتباط بين الوحدات الكلّية المكونة للمجتمعات الفطرية مع الغرين والطين والأس الهدروجيني والمحتوى الكلّي للفسفور والبوتاسيوم والكبريت سالبة بقيم−0.005، −0.135، −0.290، −0.255 و−0.227 على التوالي (p<0.05). وكان معامل الارتباط موجبة بين الوحدات الكلّية المكونة للمجتمعات الفطرية ومحتوى الرطوبة والرمل والتوصيل الكهربائي والمحتوى الكلّي للكربون العضوي والنتروجين الكلّي للتربة بقيم 0.005، 0.143، 0.355، 0.161 و0.152 على التوالي.

Introduction

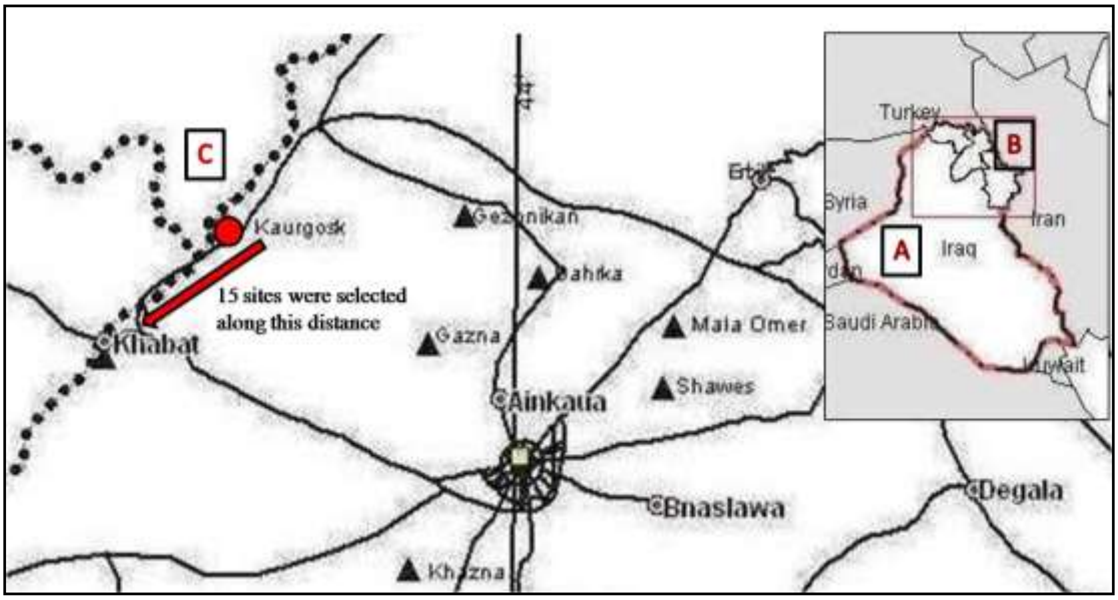
Industrialisation, urbanization and increased population have rapidly increased environmental pollution (51). These industries are more hazardous to the environment upon its existence within the limits of the cities, or its existence within urban area, and or near the agricultural terrines such as Kawrgosk Oil Refinery, west of Erbil city. Naturally, soil is the richest reservoir of microorganisms and a key component of ecosystems because environmental sustainability depends largely on a sustainable soil ecosystem (2). Fungi are one of the dominant groups present in soil, which represent the main reservoir of fungi (41). Soil fungi can be grouped into three general functional groups based on how they get their energy, decomposers, mutualizes and pathogens (8 and 44). Fungal hyphae physically bind soil particles together, creating stable aggregates that help increase water infiltration and soil water holding capacity (20). Whenever soil is polluted, the ecosystem is altered and agricultural activities are affected (19). The demand for crude oil as a source of energy and primary raw material for industries has increased. This has led to an increase in production, transportation and refinery, which have therefore resulted in grossing pollution of the environment (3). Tons of hydrocarbons release into the environment through oil spill, tank leakages or wastewater disposal (48).

Oil spills occur at all stages of production, transportation, and handling of petroleum products. Thus, it could results from pipeline ruptures, accidents, and dumping of waste engine oil (29). These pollutants are toxic and hazardous to life. Their release into the environment has led to many environmental problems that are of global concern (48). Nevertheless, some of the soil microorganisms that participated in soil processes as transformation of nutrients are active hydrocarbon degraders (35). Crude oil occurs naturally as a complex mixture of hydrocarbon and non-hydrocarbon compounds, which contains a measurable toxicity towards living organisms (3). Generally, soil conditions of agricultural land, microorganisms as well as plants are damaged or altered by any contact with crude oil. Beyond 3% oil concentration in an environment, crude oil becomes increasingly toxic to soil biota and crop growth (33). One major environmental concern of soil contaminated with crude oil or petroleum product is an increase in organic carbon of the soil with a concomitant decrease in soil nitrate and phosphorous, thus imposing a condition that impaired oil degradation in the soil (31). Different species and different life stages of organisms have been

demonstrated to have different susceptibilities to pollution. The extent of pollution depends on the quantity of oil and the damage of the environment. In heavily polluted areas, there will be an immediate detrimental effect on the life of flora and fauna (28). Due to higher increase in oil industry activities in Kurdistan of Iraq, since there are a little information of the environmental status of the areas around the refinery locations, and the concerns of a possible environmental pollution that will cause health and life threats to living organisms particularly fungi. Therefore, fungal population were studied to investigate their relations to pollution of soil in various distances from Kawrgosek Oil Refinery in Erbil.

Materials and methods

Erbil Refinery is located in Khabat district, at Kawrgosek, 40 km west of Erbil city, and it occupies a land of 2.5 Km² to the left of upper Zab River. The refinery is composed at this stage of three production lines for crude Oil refining, the production, storage, distribution and supply of petroleum products as per applied standards, which represent the first plant for crude Oil refining in Kurdistan region. Construction of this refinery started in 2005. The refinery produces the following oil products: naphtha, kerosene, gasoil (diesel), fuel oil, gasoline (automobile's benzene) and liquid gas (after operating the second production line). These products are stored and distributed in storage tanks and then transported through loading stations by tankers, or may be pumped through a pipe to Erbil Depot according to the request (22). The studied sites



in the present study were different distances at Kawrgosk Oil Refinery (Figure 1).

Figure 1 Map showing A. Iraq, B. Kurdistan Region and C. Erbil City with the studied area.

Sixteen sites according to distance from the refinery centre were taken for this study which were: site 1: 0-m (Oil Refining centre), site 2: 50-m, site 3: 100-m, site 4: 150-m, site 5: 200-m, site 6: 250-m, site 7: 300-m, site 8: 350-m, site 9: 400-m, site 10: 450-m, site 11: 500-m, site 12: 1000-m, site 13: 3000-m, site 14: 5000-m and site 15: 10000-meters away from the refinery centre and the last site (site 16) was the College of Science Greenhouse which was used as control.

During December 2014, soil samples (0-10 cm depth) from these sites were collected into polyethylene bags. From each site, three samples were taken. The soil samples were then taken to the laboratory for analysis. Soils were air-dried, crushed and sieved, for various physico-chemical parameters, through 2-mm stainless sieve to remove debris (36). For fungal study, soil samples with three replicates were taken from each study site with disinfected spatula and the samples were placed in sterilized packets then stored in a cool box until they reached the laboratory. In the laboratory, the samples were milled and sieved twice to remove large stones and debris to obtain soil samples with small particles. The samples then were processed in an isolated process of fungi using the standard plate method.

Soil moisture content was determined by gravimetric method in which the samples were dried to constant weight as described by (21). Particle size distribution and soil texture was determined by hydrometer method according to (42). The pH and EC of the soils were determined using a calibrated pH-meter (JENWAY 3505) and an electrical conductivity meter (JENWAY 4510) in 1:1 (soil: water suspension) in line with the method proposed by (42). Walkly-Black procedure (1934) was followed for determination of soil total organic carbon (36). The micro-Kjeldahl procedure was used as mentioned by (42) for estimation of total nitrogen. Ascorbic acid combined with potassium antimonyl tartrate of Murphy and Riley (1962) which described by (36) was used for determination of total phosphorus. Total potassium was determined by flame photometric method according to (36). Total sulfur was determined by turbid metric method as described by (36). Toluene extraction method was used for determination of oil residues (30).

For counting, isolation and identification of soil total fungi, potato dextrose agar was prepared by adding 39 g of powdered medium into distilled water and bringing the volume to 1 litre. The medium composition is as follow: [potato, 200 g; dextrose, 20 g; and agar, 15 g] as described in (34). Then mixed thoroughly and gently heating and bringing to boiling in flasks. The medium was then autoclaved. Then 0.2 mg Chloramphinicol was added (9). Serial dilution was prepared by adding one gram of each soil sample to 9 ml of sterile distilled water in sterilized test tubes, shaken well, a serial dilution of 10^{-3} were made in the same method. One ml of 10^{-3} dilution was poured in each Petri dish containing prepared medium (PDA + Chloramphinicol) by sterile pipette, each sample made by three replication plates and incubated at 25 °C for 10 days (6). The fungal isolates were transferred to sterilized plates for purification and identification. The grown fungi were mounted on a slide, stained with lactophenol-cotton blue to detect fungal structures (6), covered with a cover slip, examined under microscope and identified on the basis of their colony morphology and spore characteristics (40). The texts (Books) used for identification of soil fungi, depending on their taxonomic keys are as follows: (24; 38; 16; 18; 49; 46; 26 and 39).

Statistical analysis for the obtained data was performed using descriptive statistics and ANOVA accompanied with Duncan's test for comparing of the means at the level of significant of 0.05 by SPSS version 11.5 and Microsoft excel 2010. Person's correlation was done to test the relationship among fungal population, oil residues and the soil physicochemical characteristics from the different sites at ($p<0.05$) level was considered statically significant.

Results and discussion

The results of soil physical properties are presented in table 1. The highest moisture content of 5.73±0.017 was observed at site 13 which characterized by clay loam texture class and the observation is closed to those of (47) at different contaminated soils from Ota, Nigeria. Regarding to moisture changes, (43) states that moisture regimes are apt to change both in degree and frequency across wide geographical regions in the face of global climate changes. In general, it has been proposed that fungi will exhibit less of a response to changes in moisture because of their chitinous cell walls make fungi more resistant and resilient to changes in moisture content of soil. The lowest content of moisture (0.50±0.035) by the present study was at site 11 which has a sandy loam soil class. The results in table 1 showed significant differences ($p<0.05$) between the sites 2, 3, 4, 9, 12, 13, 14 and 15 as compared with the control soil; however the soil texture class for these sites were respectively: silt loam, loam, sandy loam, sandy loam, silty clay loam, clay loam, clay, clay and loam for garden soil (control).

Table 1 Physical properties of the studied soils

Site No.	*Distance (m)*	*Moisture %*	*Particle size distribution*			
			Sand %	*Silt %*	*Clay %*	*Texture class*
1	***0 (Oil Refining Centre)***	0.84±0.023^{hi}	26.89	32.78	40.34	Clay
2	***50***	4.34±0.017^{c}	34.66	54.88	10.45	Silt Loam
3	***100***	3.27±0.035^{d}	48.31	43.94	7.75	Loam
4	***150***	3.39±0.040^{d}	63.77	36.23	0.00	Sandy Loam
5	***200***	1.63±0.017^{fg}	54.26	43.20	2.54	Sandy Loam
6	***250***	2.30±0.017^{e}	69.29	30.71	0.00	Sandy Loam
7	***300***	1.68±0.017^{f}	46.60	45.77	7.63	Loam
8	***350***	0.96±0.032^{hi}	52.04	40.39	7.57	Sandy Loam
9	***400***	5.50±0.058^{ab}	68.25	31.75	0.00	Sandy Loam
10	***450***	1.16±0.023^{gh}	57.00	40.47	2.53	Sandy Loam
11	***500***	0.50±0.035^{i}	59.80	35.18	5.03	Sandy Loam
12	***1000 (1-Km)***	5.04±0.012^{b}	18.38	42.12	39.49	Silty Clay Loam
13	***3000 (3-Km)***	5.73±0.017^{a}	17.79	39.78	42.43	Clay Loam
14	***5000 (5-Km)***	4.25±0.662^{d}	24.28	23.50	52.22	Clay
15	***10000 (10-Km)***	3.75±0.012^{d}	22.08	36.36	41.56	Clay
16	***Control (garden soil)***	2.09±0.017^{ef}	48.93	33.19	17.87	Loam

From the present finding in table 2 is obvious that there were no significant differences between pH of the studied soils from different sites when compared with control. Soil pH is one of the main parameter for determining the extent of pollution, it was ranged between 7.39±0.049 to 8.47±0.017 in the site 15 (10-Km away from the refinery centre) and site 2 (50-m away from the refinery centre) respectively, indicating neutral to slightly alkaline soils, and this finding come in agreement with those obtained by (51) in Jodhpur-Rajasthan and observation of (7) from petroleum hydrocarbon contaminated site in Manglia. In general, fungi have been found to be more acid-tolerant leading to increased fungal dominance in acidic soils. However, this does not appear to be a conclusive pattern since alterations in pH can result in

increased, decreased, or unchanged levels of fungal dominance (43). Works conducted across a land-use gradient in the Southeastern U.S. found the relative abundance of fungal taxa were more strongly related to soil P and C:N ratios than any other edaphic characteristic (25).

Table 2 Chemical properties (mean ± S.E.) of the studied soils.

Site No.	***pH***	***EC µS.cm^{-1}***	***Total organic C g.Kg^{-1}***	***Total – N g.Kg^{-1}***	***Total – P mg.Kg^{-1}***	***Total – K g.Kg^{-1}***	***Total – S g.Kg^{-1}***
1	8.24±0.023^{bcd}	21±4.619^{g}	7.804±0.001^{i}	1.545±0.006^{cd}	17±0.006	17±0.000^{k}	15.4±0.058^{d}
2	8.47±0.017^{a}	55±2.887^{f}	21.811±0.577^{d}	1.528±0.000^{cd}	18±0.001	19.7±0.058^{i}	1.853±0.015^{k}
3	8.37±0.040^{ab}	53±1.732^{f}	7.804±0.001^{i}	2.298±0.000^{a}	19±0.001	22±0.000^{ef}	6.896±0.002^{i}
4	8.11±0.012^{de}	57±1.732^{ef}	11.806±0.058^{h}	1.455±0.000^{d}	14±0.001	19.9±0.058^{i}	0.0±0.000^{l}
5	7.97±0.012^{ef}	64±1.155^{de}	23.812±0.173^{bc}	1.468±0.000^{d}	16±0.000	20.7±0.173^{h}	0.0±0.000^{l}
6	7.84±0.023^{fgh}	76±0.577^{c}	24.812±0.012^{b}	2.313±0.000^{a}	16±0.002	16.4±0.115^{l}	0.0±0.000^{l}
7	8.16±0.058^{cd}	167±4.041^{b}	9.805±0.001^{i}	0.756±0.000^{e}	18±0.002	21.5±0.231^{g}	7.424±0.115^{h}
8	7.79±0.058^{gh}	231±1.155^{a}	16.808±0.173^{f}	1.675±0.000^{c}	19±0.003	22.5±0.115^{d}	15±0.000^{e}
9	7.89±.046^{fg}	59±2.887^{ef}	44.822±0.058^{a}	1.549±0.000^{cd}	18±0.002	23.6±0.115^{c}	14.6±0.058^{f}
10	7.78±0.017^{gh}	70±1.155^{cd}	44.822±0.577^{a}	1.529±0.000^{cd}	20±0.001	25.4±0.173^{b}	19.8±0.058^{c}
11	7.7±0.115^{h}	14±1.155^{i}	22.811±1.155^{cd}	2.293±0.058^{a}	23±0.002	19.1±0.058^{j}	15.2±0.115^{de}
12	7.78±0.115^{gh}	25±1.155^{g}	14.807±0.577^{g}	1.459±0.173^{d}	18±0.006	21.7±0.058^{fg}	20.4±0.173^{b}
13	7.76±0.058^{gh}	27±1.732^{g}	18.809±0.002^{e}	1.529±0.058^{cd}	20±0.006	16.4±0.058^{l}	7.701±0.115^{g}
14	7.53±0.017^{i}	75±2.887^{c}	17.809±0.001^{ef}	2.295±0.001^{a}	16±0.002	16.7±0.115^{kl}	2.068±0.017^{k}
15	7.39±0.049i	27±2.887^{g}	12.806±1.155^{h}	1.457±0.000^{d}	21±0.001	22.3±0.173^{de}	26.7±0.115^{a}
16	8.31±0.023^{abc}	75±1.155^{c}	9.648±1.732^{i}	1.845±0.012^{b}	18±0.001	29.5±0.058^{a}	4.396±0.115^{j}

Soil electrical conductivity is a measure of soluble salt content in the soil and is used as an overall indicator of the level of macro- and micronutrients in the soil. By this study, the highest EC of 231±1.155 µS cm^{-1} was obtained in site 8 (350-m away from the refinery centre) and the lowest EC of 14±1.155 µS.cm^{-1} was in site 11 (500-m away from the refinery centre); and there were significant differences between the sites 6, 7 and 8 in comparing with control soil as shown in table 2. The highest EC of 231±1.155 µS.cm^{-1} was close to the observation of (51) who found 250 µS cm^{-1} as highest EC in Mogra polluted site. However, low EC values were observed in sites 1, 11, 12, 13 and 15 and this may be an indicator of contamination which affected soil structure and modified its physicochemical properties (17). The reduction in salt concentration which are suitable terminal electron acceptors may affecting the indigenous microbial growth and metabolism (45). This could be the reason for the lower fungal growth in contaminated soil samples.

The highest total organic carbon was 44.822±0.577 and 44.822±0.058 g.Kg^{-1} in both the sites 10 (450-m away from the refinery centre) and 9 (400-m away from the refinery centre), whereas the lowest organic carbon content was 7.804±0.001 and 7.804±0.001 in both the sites 3 (100-m away from the refinery centre) and 1 (the refinery centre). Significant differences between all the studied sites were observed except for the sites 1, 3 and 7 (table 2). Total organic carbon range of this study comes in agreement with the findings of (7; 23 and 11) and higher than the findings of (17) in six polluted sites in Jodhpur-Rajasthan who found a range of 1.0-3.8 g Kg^{-1} and this may refer to the nature of the studied soil.

Total nitrogen was ranged between 0.756±0.000 and 2.313±0.000 g Kg^{-1} in both the sites 7 (300-m away from the refinery centre) and 6 (250-m away from the refinery centre) respectively. Statistical analysis revealed no significant differences between the studied sites when compared with control as given in table 2. Moreover, total phosphorus content of the studied soils showed no significant differences between all of the sites and the range was between 14±0.001 and 23±0.002 mg Kg^{-1} from the sites 4 (150-m away from the refinery centre) and 11 (500-m away from the refinery centre) respectively. The obtained total nitrogen (0.756-2.313 g Kg^{-1}) and total phosphorus (14-23 mg Kg^{-1}) in this study are lower than the obtained results by (37) in the chemical factory near the city of Kėdainiai in Central Lithuaniaand, and this may refer to their studied sites because the factory's major product by the present is nitrogen phosphorous fertilizer. However present findings of total nitrogen contents were similar to the observations of (11) from the mechanic workshops. Total potassium of the studied soils were between 16.4±0.115 and 29.5±0.058 g Kg^{-1} in both the sites 6 (250-m away from the refinery centre) and 16 (control) and there were no significant differences between the studied sites in comparing with garden soil. The highest total sulfur content 26.7±0.115 g Kg^{-1} was recorded at site 15 (10-Km away from the refinery centre) and zero values were recorded in sites 4, 5 and 6 as the lowest level. Significant differences were obtained between the studied sites except for the sites 2, 4, 5, 6 and 14.

The residual oil content of the soil samples were determined and presented on the figure 2. The highest oil residue was in site 14 (5-Km away from the refinery centre) which was 0.0022 mg.Kg^{-1}; whereas, the lowest oil residue 0.0007 mg Kg^{-1} was in site 5 (200-m away from the refinery centre) and there were no significant statistical differences between the studied sites in comparing with control.

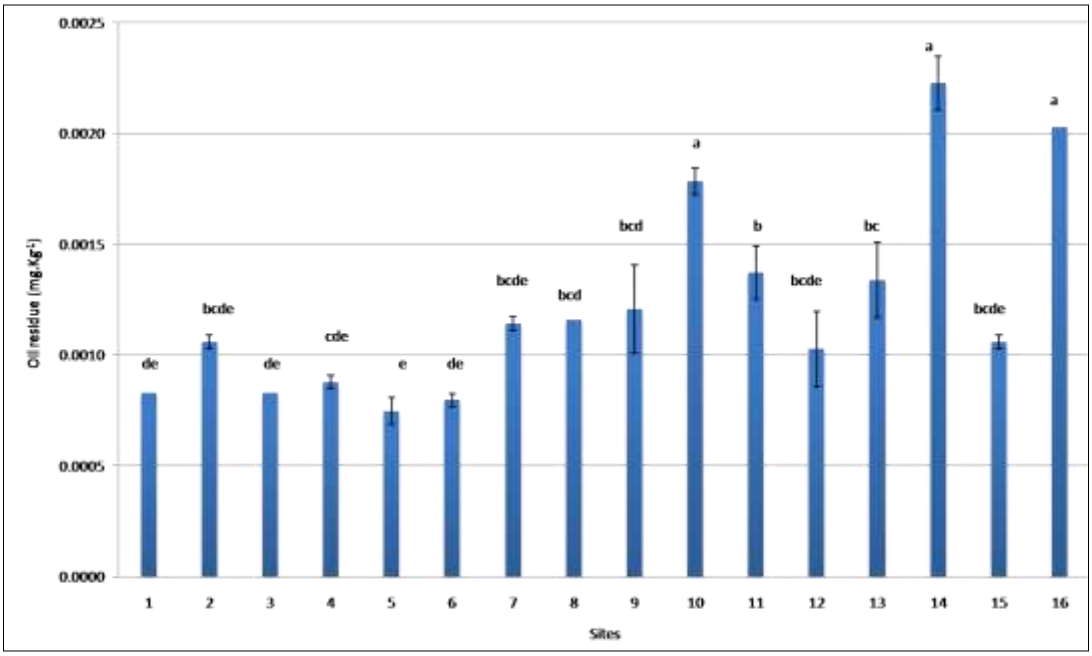


Figure 2 Residual oil content (mg Kg^{-1}) in the studied sites. Different letters mean significant differences among the studied sites.

The highest oil residue 0.0022 mg Kg^{-1} observed at site 14 was lower than that observed by (47) which was 10.10 mg Kg^{-1} in contaminated soils of Nigeria, and this may refer to the type of oil source in these area in comparing with the present site, whereas, they obtained zero values in non-contaminated soils, however the lowest oil residue 0.0007 mg Kg^{-1} at site 5, 200-m away from the refinery centre, by the present study possibly indicate no contamination with oil in this site according to the statement of (47). On the other hand site 14, characterized by only yeast population of 16×10^{3} cfu g^{-1} which may have no the sufficient capacity to degrade the oil content in this area, while site 5 which has the lowest oil residue, characterized by *Aspergillus niger* (5×10^{3} cfu g^{-1}) and *Penicillium* spp. (4×10^{3} cfu g^{-1}) which have a specialized enzyme system for degrading of petroleum compounds; in this regard (15) reported that indigenous microorganisms are more capable of degrading indigenous crude oil due to the fact that native microorganisms are best adapted to intrinsic environmental conditions.

Fungal communities in soils are an important component, because they participate in regulating microbial activity in polluted soils (37). The results of fungal study were tabulated in table 3 which shows the identity and the total colony forming units (cfu) of fungi on PDA medium.

Table 3 Fungal flora isolated from sixteen sites located at different distance from Kawrgosk Oil Refinery. Total cfu.g^{-1} dry soil expressed as (mean ± S.E.) and multiplied by 10^{3}.

Site No. / *Fungi*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*Total no. of species isolated from all sites*
Alternaria raphani	0	0	0	0	0	0	0	0	3	0	0	0	2	0	0	0	**5**
Aspergillus niger	0	0	0	0	5	0	2	1	0	0	0	0	0	0	0	4	**12**
A. ochraceous	0	0	2	0	0	31	1	2	0	7	0	0	0	0	0	0	**43**
A. terreus	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**
Circinella muscae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	**3**
Cladosporium oxysporum	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	**4**
Cunninghamella elegans	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	**6**
Fusarium sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	**6**
Mycelia sterilia	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	**3**
Penicillium spp.	0	6	3	0	4	1	2	1	6	0	0	0	0	0	0	0	**23**
Rhizoctonia solani	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	**5**
Rhodotorula sp.	0	0	0	0	0	0	4	0	0	0	0	0	10	0	10	0	**24**
Taeniolella exitis	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	**3**
Yeasts	2	16	0	8	8	7	0	19	0	5	6	4	0	16	5	0	**96**
Total cfu.g^{-1} dry soil of isolated fungal species from each site	**2 ± 0.577^{k}**	**22 ± 1.154^{d}**	**6 ± 0.000^{i}**	**8 ± 0.000^{h}**	**17 ± 1.154^{ef}**	**41 ± 0.000^{a}**	**13 ± 0.577^{g}**	**26 ± 0.000^{b}**	**9 ± 0.577^{h}**	**13 ± 1.154^{g}**	**6 ± 0.000^{i}**	**4 ± 0.577^{j}**	**18 ± 0.577^{e}**	**16 ± 0.000^{f}**	**24 ± 0.000^{c}**	**9 ± 0.000^{h}**	**234±1.426**

A total of twelve different fungal genera and fourteen species were isolated and this is similar with the observations of (10) who isolated thirteen fungal genera from oil-contaminated soils with different oil contamination from Khuzestan, Iran and

the thirteen isolated fungal genera included *Aspergillus*, *Penicillium*, *Fusarium*, *Acremonium*, *Candida*, *Rhodotorula*, *Mucor*, *Aureobasidium*, *Cunninghamella*, *Rhizopus*, *Alternaria*, *Beauveria* and *Paecilomyces*. Investigation of the isolation of fungi from oil-contaminated environments showed that abundance and fungal population in different stations were significantly different and this in consistence with the present findings.

By the present study, the total number of isolated fungi from the sixteen selected sites was 243×10^3 (2.43×10^5) cfu.g^{-1} dry soil, but (47) isolated slightly higher count 4.3 to 8.3×10^5 cfu g^{-1} and this may either refer to the moisture content in the studied soils as stated by (12), or it may refer to the organic matter and nitrogen content in the soils (37). Maximum population of fungi 41×10^3 cfu g^{-1} dry soil was observed in site 6 (250-m away from the refinery centre), while the minimum population of fungi 2×10^3 cfu g^{-1} dry soil was isolated in the refinery centre as in figure 3 and the difference in count of the sites may be due to the difference in sites receiving domestic effluents and agricultural runoff (6) who reported that variation in counts in sites will be due to the industrial and domestic discharges to the sites; moreover, the lower fungal population may refer to the least amount of total organic carbon in the refinery center as in table 2 which affect the population and diversity of fungi.

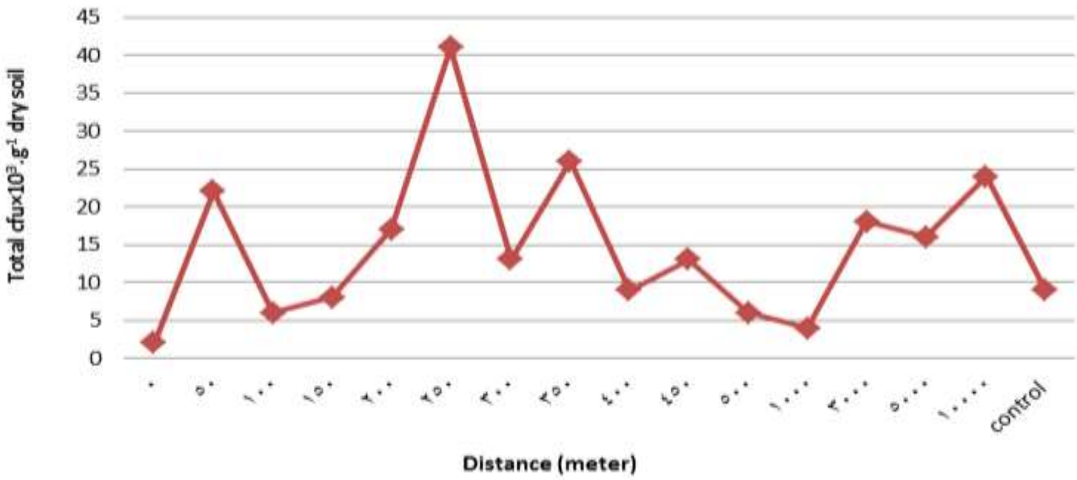


Figure 3 Total cfu of fungi per a gram of dry soil along different distances from Kawrgosk Oil Refinery.

All of the studied fungi were identified on the basis of their cultural and morphological characteristics. The most frequently isolated fungi were yeasts 96×10^3 cfu.g^{-1} dry soil which have a percentage of occurrence of 41.03% as in figure 3, followed by *Aspergillus ochraceous* 43×10^3 cfu.g^{-1} dry soil (18.38 %), *Rhodotorula* sp. 24×10^3 cfu g^{-1} dry soil (10.26 %), *Penicillium* spp. 23×10^3 cfu.g^{-1} dry soil (9.83 %) and *A. niger* 12×10^3 cfu g^{-1} dry soil (5.13%), in this circumstance our results come in agreement with those found by (27) who found that the major genera of fungi in polluted soils of Calabar-Nigeria were *Aspergillus*, *Penicillium* and *Mucor*; as well as agree with findings of (32) in Ondo oil-polluted soils and water who isolated mostly *Penicillium italicum*, *Aspergillus niger*, *P. oxalicum*, *Streptothorix atra*, *Articulosporium inflata*, *Geniculosporium serpens*, *A. flavus*, *Halosprangium pavum*, *A. fumigates and A. rapens*. However, (7) identified *Aspergillus*, *Penicillium*,

Fusarium, *Rhizopus*, *Mucor*, *Cladosporium*, *Morssonina*, *Chaetomium*, *Curvularia*, *Helminthosporium*, *Alternaria* and *Trichoderma* species in petro-polluted soil in Manglia. For the fungal isolates observed by (13), *Penicillium* spp. had the highest occurrence of 33.3% while *Mucor*, *Rhizopus*, *Aspergillus* and *Cephalosporium* spp. had the least occurrence of 16.7% in Nigeria. However, (5) isolated lower counts of *Aspergillus ochraceous*, *Penicillium* spp. and *A. niger* in the grounds of several automobile stations at different places within the city of Chennai, India polluted by PAHs, and the dominant fungal species by their study were *Mucor racemosus* (6.3 cfu.g^{-1}), *Rhizopus stlonifer* (7.2 cfu.g^{-1}), *Aspergillus niger* (3.4 cfu.g^{-1}) and *Penicillium chrysogenum* (5.3 cfu.g^{-1}); since they grow the fungal isolates on medium containing 1 % (w/v) of the following carbon sources such as diesel, kerosene, petrol, grease, motor oil and contaminated soil from garages. Whereas, (47) isolated *Penicillium chrysogenum*, *Aspergillus* sp. and *Candida* sp. dominantly in contaminated soils from Nigeria. Furthermore, (17) mostly isolated *Penicillium* sp. and *Aspergillus* sp. in soils contaminated with fresh and old crude oil spills at different location at the Badia Region-Jordan; whereas (4) mainly isolated *Aspergillus*, *Penicillium* and *Rhizopus* species. These organisms have been previously implicated with petroleum product degradation.

The least frequently isolated fungal species by this study was *A. terreus* $1×10^3$ cfu g^{-1} dry soil (0.43 %) as shown in figure 4. Moreover, results of statistical multiple comparisons between the studied sites when compared with control showed significant differences, except for the sites the refinery centre, 100-m away from the refinery centre, 500-m away from the refinery centre and 1-Km away from the refinery centre and no differences between these sites come in agreement with the observation of (4) who found no significant differences between five studied sites each with 1-Km intervals in Kukawa local government area of Borno State, Nigeria according to Turkey's HSD at $P < 0.05$ during December 2012; however significant differences between all the other sites come in agreement with (4) who revealed that the counts between five sample sites in Nigeria were significantly different ($P≤0.05$). The microscopic nature of some of the isolated fungal genera by this study is shown in figure 5.

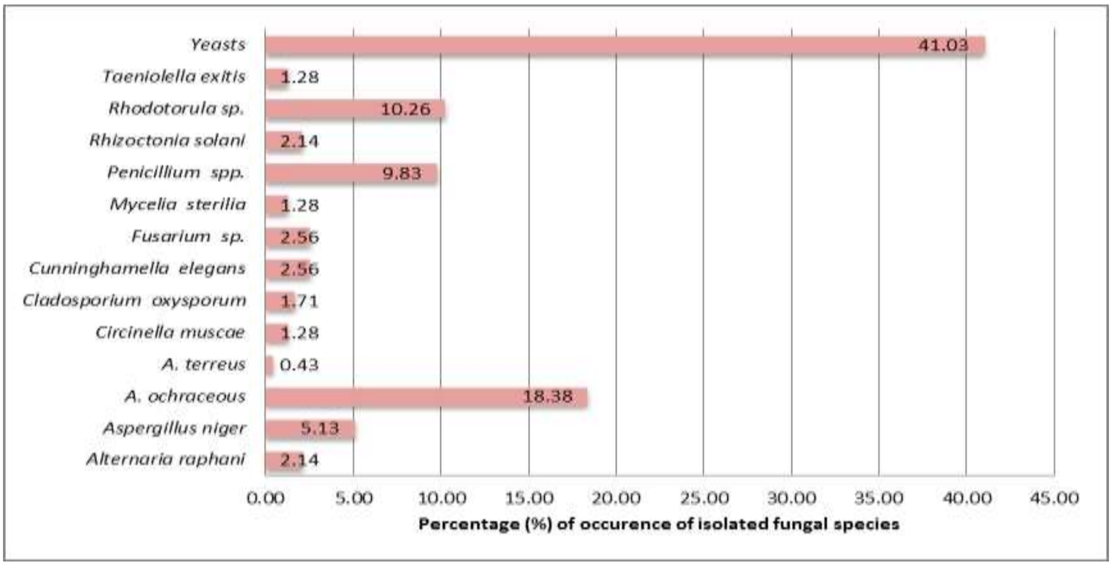


Figure 4 Percentage (%) of occurrence of fungal isolates from the studied sites.

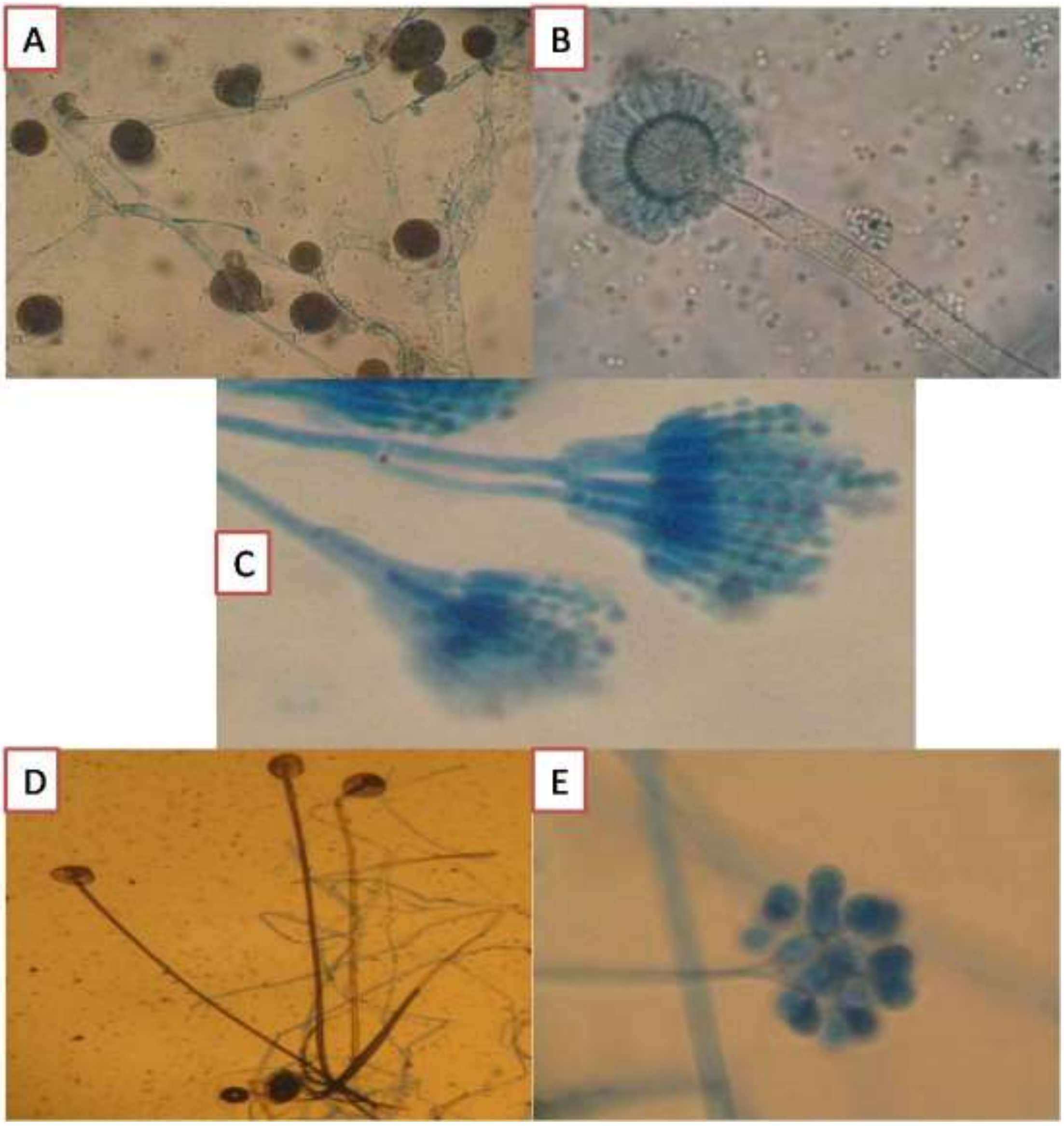


Figure 5 The isolated fungal genera on PDA medium in the studied soils. A/ *Circinella muscae*; B/ *Aspergillus* sp.; C/ *Penicillium* sp.; D/ *Rhizopus* sp. and E/ *Cunninghamella elegans*.

After computation of Pearson's correlation between the physical and chemical properties with the oil residues and fungal population in all of the studied sites as shown in table 4, a significant negative correlation was obtained between sand and clay content of the studied soils, as well as a significant positive correlation was obtained between total phosphorus and total sulfur contents of the studied soils at 0.05 level of significance. Moreover, the correlation between total cfu of fungi with oil residue was negative by r value of -0.092 (figure 6) and this may refer to the ratios of carbon and nitrogen in the studied sites as stated by (25). The correlation between total cfu of fungi with silt and clay contents, pH, total P, K, and S were also negative by r values of -0.005, -0.135, -0.290, -0.090, -0.255 and -0.227 respectively at 0.05 level of significance, however, (37) also obtained a negative correlation between fungal population and pH by r values of -0.71; as well as they obtained negative correlations between number of fungal genera with total P and total K by r values of -0.71 and -0.70 respectively in the studied soils of chemical factory in Kėdainiai city.

The possible stress factor behind this negative correlation that the microorganisms can be subjected to could be substrate availability as a result of the higher proportion of phosphorus, potassium and sulfur in the unavailable form that is not substrate for microorganisms (50). While, the correlation between total cfu of fungi with moisture, sand, EC, total organic C and total N were positive by r values of 0.005, 0.143, 0.355, 0.161 and 0.152 respectively at 0.05 level of significance, and (37) also obtained positive correlations between fungal population with moisture content, organic matter and total nitrogen by r values of 0.46, 0.74 and 0.77 respectively in the studied soils of chemical factory in Kėdainiai city. When considering the response of fungi to changes in moisture, (14) found that fungal biomass and fungal dominance were both positively related to soil moisture in cultivated settings. Moreover, (1) obtained a high positive correlation (r = 0.84, P ≤ 0.0001) between soil organic carbon and total nitrogen and microbial properties at the 5-10 cm soil depth in southern Nigeria.

Table 4 Person's correlation among physical and chemical parameters, total cfu of fungi and residual oil content in the studied soils.

	Moisture	*Sand*	*Silt*	*Clay*	*pH*	*EC*	*Total organic C*	*Total – N*	*Total – P*	*Total – K*	*Total – S*	*Total cfu of fungi*	*Residual*
Moisture	1												
Sand	-0.405	1											
Silt	0.016	-0.095	1										
Clay	0.382	-0.921**	-0.300	1									
pH	-0.127	0.192	0.489	-0.367	1								
EC	-0.345	0.283	0.150	-0.330	0.064	1							
Total organic C	0.100	0.447	-0.077	-0.399	-0.290	-0.055	1						
Total – N	-0.067	0.183	-0.482	0.013	-0.147	-0.242	0.028	1					
Total – P	-0.135	-0.162	0.177	0.086	-0.324	-0.138	0.155	0.096	1				
Total – K	-0.148	0.261	0.156	-0.312	0.198	0.229	0.152	-0.216	0.203	1			
Total – S	-0.048	-0.295	-0.021	0.291	-0.479	-0.127	0.165	-0.211	0.662**	0.275	1		
Total cfu of fungi	0.005	0.143	-0.005	-0.135	-0.290	0.355	0.161	0.152	-0.090	-0.255	-0.227	1	
Residual oil	0.029	-0.131	-0.408	0.285	-0.286	0.080	0.211	0.235	0.210	0.326	0.048	-0.092	1

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

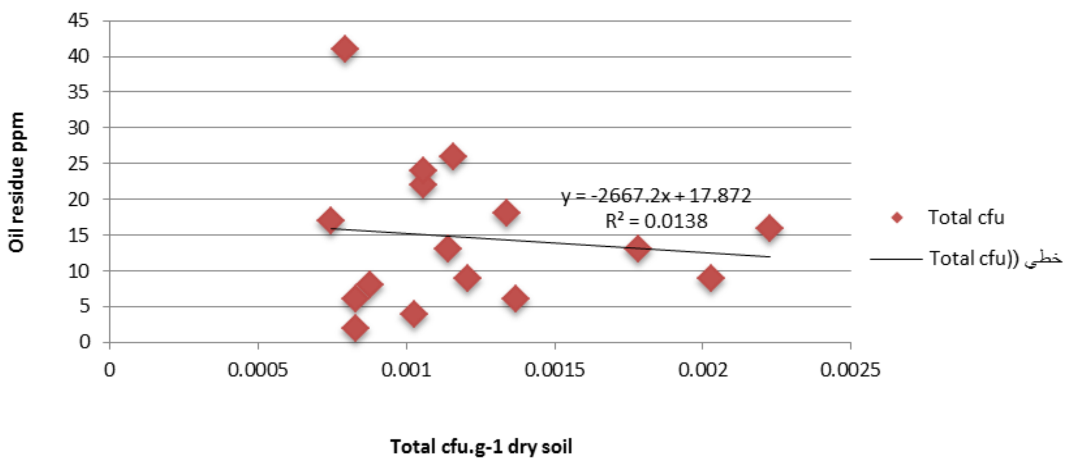


Figure 5 Correlation between total cfu.g^{-1} of isolated fungal species and oil residue from different studied sites.

The study concluded that, the detected oil residues were lower than that observed in the previous studies conducted in oil polluted soils in different parts of the world and this may possibly indicate no contamination for nowadays in the studied area because the refinery is still new-established and newly works and not faced the level of soil contamination. Soil fungi are important component because they participate in the regulation of soil microbial activity in polluted areas. Although oil pollution in the studied area around the oil refinery is low, it influences the community of soil fungi to some degree and the fungal population was negatively correlated with oil residue. The results of this study will be helpful to decision-makers, planners, scientists, and the local communities to protect the environment in areas surrounding various oil refineries established in Kurdistan Region of Iraq for the future.

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