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Keyword: Fungal population; Crude oil-utilizers; Heterotrophic soil fungi

Introduction

Oil products are continually used as a main source of energy in industry and daily life [1]. Release of hydrocarbons into the environment, whether accidentally or due to anthropogenic activities, is a major cause medium was composed of NaCl of 10.0 g; $MgSO_4 7H_2O$ of 0.42 g; KCl of 0.29 g; KH_2PO_4 of 0.83 g; Na_2HPO_4 of 1.25 g; $NaNO_3$ of 0.42 g; agar of 15 g; distilled water of 1 L and nal pH of 7.2. Streptomycin antibiotic was added to the media to prevent bacterial growth [20,21]. en the fungi that grew on these media were considered as oil-degrading ones and subcultured on PDA and identi ed according to macroscopic and microscopic characteristics [22-24].

Statistical analysis

Data were analyzed by statistical SPSS-14 so ware. To study the signi cant di erence, variance analysis and the di erence were calculated in a signi cant level (p   0.05).

Result and Discussion

Analysis of soil and population of soil fungi

Table 1 shows the results of soil analysis as well as population of total heterotrophic and oil-utilizing fungi. A er enumeration of the indigenous fungi, the average counts of total heterotrophic fungi on PDA media and the average counts of crude oil-utilizing fungi in oil agar media were expressed as $\times 10^2$ CFU/g soil. In the soil samples, the counts of total heterotrophic fungi ranged from 0.41 ± 0.16 to 3333.33 ± 288.00 while the counts of crude oil-utilizing fungi ranged from 0.26 ± 0.10 to 2860.00 ± 163.20. Figures 1 and 2 show the population of heterotrophic and crude oil-utilizing fungi. Due to the signi cant di erence in fungal population of soils 1 and 2, the corresponding population graphs are presented separately. e highest populations of heterotrophic fungi (3333.33 ± 288.00) and crude oil-utilizing fungi (2860.00 ± 163.20) were related to soil 2. e least total fungal populations of 0.41 ± 0.16 and 0.50 ± 0.10 belonged to soils 5 and 8 respectively, and the least amount of crude oil-utilizing fungi of 0.26 ± 0.10 was related to soil 6. According to the results, population of crude oil-utilizing fungi was more than heterotrophic fungi in soils 3 and 5 (Figure 2). Figure 3 shows the ratio (%) of oil-degrader fungi to heterotrophic fungi. e highest amount of these fungi belonged to soil

Figure 2: Average counts ($\times 10^2$ CFU/g soil) of heterotrophic and oil-utilizing fungi in soils 3, 4, 5, 6, 7 and 8.

soil 2 which reduced the population of oil-degraders. Westlake et al. [25] also reported that specific microbes show increase in population because of using petroleum hydrocarbons as a nutrient [25].

Soils 4 and 5 had organic carbon of 18.35% and 12.22% respectively, and neutral pH. They also had the same phosphorus amounts as soils 1 and 2; however, their fungal population was very low which could be attributed to the short time exposure to oil spill. Soil 5, due to shorter time of exposure to the oil spill, also had a higher electrical conductivity which was a limiting factor for fungal growth (Table 1). An excess of dissolved salts (anions and cations) in the soil is detected by electrical conductivity [26]. Electrical conductivity is one of the characteristics that promote anti-microbial activity. Excessive heavy metal concentration in the soils was reported to cause decrease in microbial population [27]. Most metal ions have the ability to create oxygen radicals, thus forming molecular oxygen which is highly toxic to bacteria and fungi [28].

$$2H_2O + \frac{1}{2}O_2 \xrightarrow{Metallon} 2H_2O_2 \to H_2O + (O)$$

The electrical conductivity of soils varies depending on the amount of moisture held by soil particles. Increasing soil solution salinity (EC) will decrease available water. This will have a negative effect on crop yield and microorganisms such as fungi, also may affect their survival [29,30].

Sudden discharge of large quantities of oil to these areas has affected most of the microorganisms and has reduced the microbial population. Because of a short time exposure to oil spill, the remaining microorganisms did not have enough time to adapt to the environment. Low population of soils 4 and 5 can be explained by the fact that primary reduction in fungal population occurs whenever the crude oil is added to the environment, probably because of crude oil toxicity; then, some organisms are killed or controlled by toxic components of crude oil while other oil degrading heterotrophic organisms are increased in number [31]. In soils 6, 7 and 8, a lower fungi population was observed because of low amount of organic carbon. It seems that the percent of oil hydrocarbon consumers can be an index for existence of hydrocarbons in the environment. In heavily polluted environments, immediate harmful effects will be observed in biological forms [21]. The effect of petroleum pollution depends on chemical composition of oil product and the species of microorganisms [25]. These results are in agreement with findings of Obire and Anyanwu that treated soil with different concentrations of crude oil and counted fungal populations within eighteen weeks. Fungal population was decreased in control soil (without oil), a primary reduction and then considerable increase were observed in high concentrations of oil [21]. e toxicity of crude oil or petroleum products varies widely, depending on their composition and concentration. Moreover, the scale of pollution depends on the amount of oil and the damage done to the environment [31]. An interesting observation in this study was a signi cant increase in oil-utilizing population in soil 3 in comparition to heterotrophic population which was recognized to be Aspergillus species (Figure 2). is species have been never observed previously on PDA medium at the time of counting and isolation.

In studies conducted by Chaudhry et al. [32] population of heterotrophic fungi in all samples was more than diesel utilizing fungi [32]. In this study the same results were observed in soils 1, 2, 4, 6, 7 and 8, and the opposite results were observed in soils 3 and 5.

Although, in this study, the heterotrophic fungal population was expected to be greater than oil-utilizing fungal population due to use of enrichment medium (PDA), opposite results were observed in two areas. is could be attributed to adaptation of oil-utilizing fungi to the amount of hydrocarbon in the environment and there by their increase in two regions. However, it should be noted that nutrients found in the oil can also, stimulate the growth of fungi.

Shaw found that organisms break down hydrocarbons and use the energy to synthesize cellular components. A er being completely broken down, the reaction releases carbon (IV) oxide, water and energy used to create cellular biomass [33]. e results of studying heterotrophic fungi and petroleum-utilizing fungi in some soil samples suggest that the petroleum-utilizing fungi were adapted to the quantity of hydrocarbons in the environment, and thereby increased the number of petroleum-utilizing fungi in polluted areas.

In soil 8, despite the lack of crude oil pollution, the ratio of degrading fungi to heterotrophic fungi was 72.0% showing isolation of oil-degrading microorganisms from irrelevant environments. Microbes which can use hydrocarbons are found naturally in the environment. However, they increase a er oil spill because an additional carbon resource (hydrocarbons) is being prepared [34].

Soil 2, despite of having the greatest population and the largest amount of organic carbon, showed a low fungal diversity composed of a species of Penicillium (dominant soil population) and two species of Fusariumand Aspergillus terreus (both heterotrophic and crude oil-utilizing fungi). e highest fungal diversity belonged to soil 8 which had the least organic carbon.

Obire and Anyanwu reported that reduction in diversity of species (fungal genus) with an increase in concentration of added crude oil was

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12. Llanos C, Kjßller A (1976) Changes in the þora of soil fungi following oil waste application. Oikos 27: 377-382.

13. Chaillan F, Le Flèche A, Bury E, Phantavong YH, Grimont P, et al. (2004) Identiýcation and biodegradation potential of tropical aerobic hydrocarbon-degrad4C0014(3t5oroorg)]Tsms.ions M5orobiol 619: 587-595 -1.62 -2.01 Td (13.)T4 ET BT /Span <</ActualText (þÿ)>>BDC 7 0 0 7 42.5197 722.8152 Tm 1.39 -3.217-2.0