

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; $MgSO_4$ of 0.42 g; KCl of 0.29 g; K_2HPO_4 of 0.83 g; $NH_4H_2PO_4$ of 1.25 g; NaN_3 of 0.42 g; agar of 15 g; distilled water of 1 L and final pH of 7.2. Streptomycin antibiotic was added to the media to prevent bacterial growth [20,21]. The fungi that grew on these media were considered as oil-degrading ones and subcultured on PDA and identified according to macroscopic and microscopic characteristics [22-24].

Statistical analysis

Data were analyzed by statistical SPSS-14 software. To study the significant difference, variance analysis and the difference were calculated in a significant 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. After 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 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 significant difference in fungal population of soils 1 and 2, the corresponding population graphs are presented separately. The highest populations of heterotrophic fungi (3333.33 ± 288.00) and crude oil-utilizing

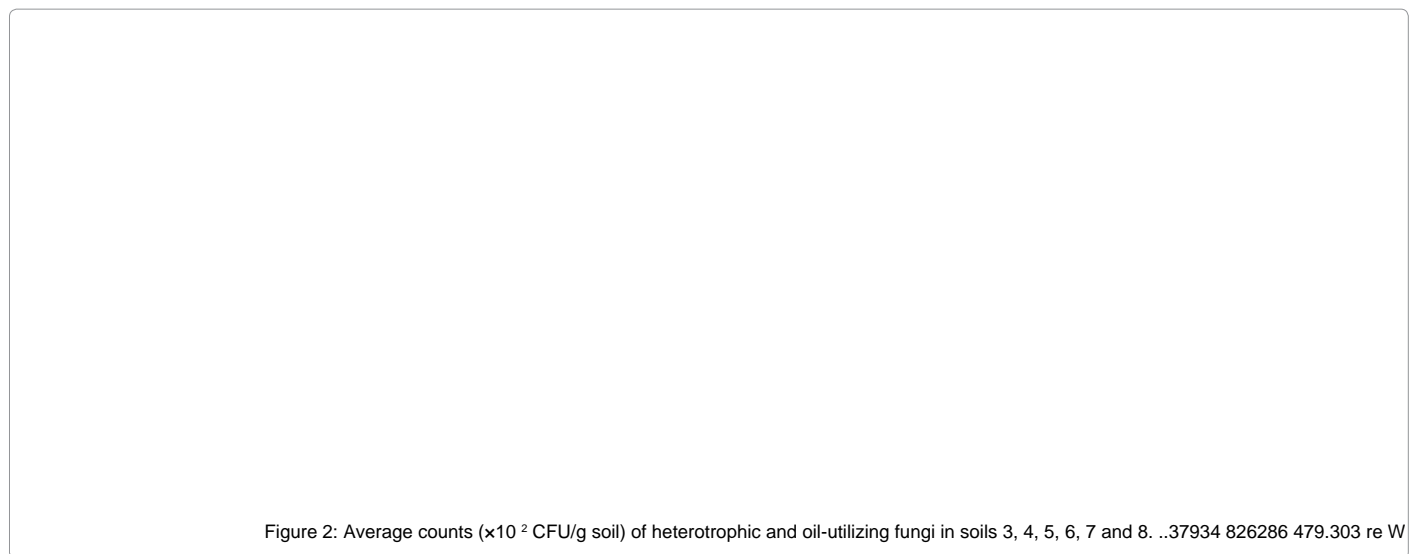
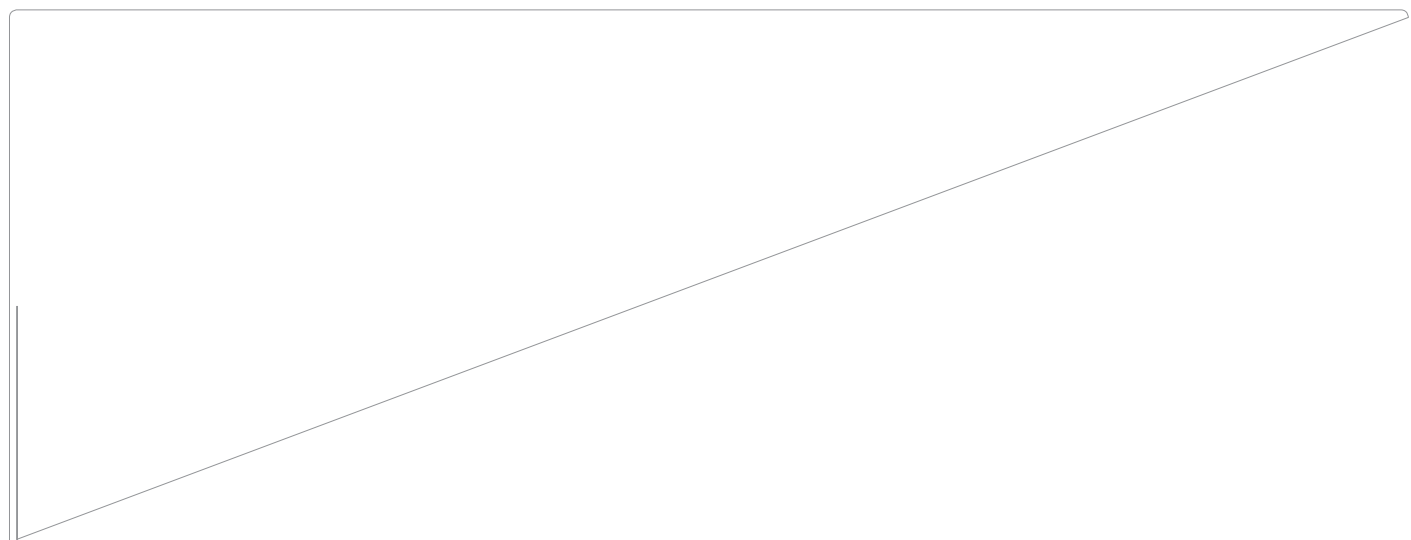
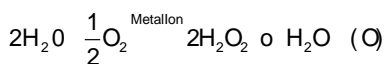


Figure 2: Average counts ($\times 10^2$ CFU/g soil) of heterotrophic and oil-utilizing fungi in soils 3, 4, 5, 6, 7 and 8. ...37934 826286 479.303 re W n q /GS0



soil 2 which reduced the population of oil-degraders. Westlake et al. Increasing soil solution salinity (EC) will decrease available water. [25] also reported that specific microbes show increase in populations will have a negative effect on crop yield and microorganisms such because of using petroleum hydrocarbons as a nutrient [25]. as fungi, also may affect their survival [29,30].

Soils 4 and 5 had organic carbon of 18.35% and 12.22% respectively, Sudden discharge of large quantities of oil to these areas has 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].



The electrical conductivity of soils varies depending on the amount of moisture held by soil particles. In heavily polluted environments, 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]. The 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 significant increase in oil-utilizing population in soil 3 in comparison to heterotrophic population which was recognized to be *Aspergillus* species (Figure 2). These 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. This could be attributed to adaptation of oil-utilizing fungi to the amount of hydrocarbon in the environment and thereby 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. After being completely broken down, the reaction releases carbon (IV) oxide, water and energy used to create cellular biomass [33]. The 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 after 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 *Fusarium* and *Aspergillus terreus* (both heterotrophic and crude oil-utilizing fungi). The 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

the heterotrophic and

have been (heterotrophic and two species) 0.2 (heterotrophic and micro) 0.2 (after pollution 35, the ratio of) 0.2 0.2

12. Šjæ) [•ÁÔÉŠice||^!ÁCE!CFJĪ ĪD!Ô@æ} *^•Áá)Ác@^! [!æÁ[-!•[á!~ } *á!-[! [, á) *Á[á! , æ•c^! application. Oikos 27: 377-382.
13. Chaillan F, Le Flèche A, Bury E, Phantavong YH, Grimont P, et al. (2004) (á^} c! , &æá [}! á) á! [á^*!æáæá [}! [c^} cíæ! [-! c! [] á&á! æ^! [á&á! @~ á! [&æ! à [] É 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