

Microbial Diversity in Oil Contaminated Areas of Indian Thar Desert

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Abstract: The ability to isolate high numbers of certain oil degrading microorganisms from oil polluted environment in desert ecosystem is commonly taken as evidence that these microorganisms are the active degraders of the environment. Soil counts cannot be used for analysis of biodegradability of the spilled hydrocarbons, the diversity and the number of microorganisms at a given site may help to characterize that site with respect to the toxicity of these hydrocarbons to the microbiota, age of the spill and concentration of the pollutant. From 5 different sampling sites total 10 soil samples were collected. Samples were subjected to cultural experiments for the diversity and abundance of Hydrocarbon Utilizing Bacteria HUB and Hydrocarbon Utilizing Fungi HUF by sampling in three consecutive months. Although, hydrocarbon degraders may be expected to be readily isolated from an oil associated environment, the same degree of isolated from a total related environment such as soil. Therefore sampling experiments were performed both for oil spilled and non-oil spilled areas. A total of 24 isolates were isolated and it was reported that the introduction of oily wastes into soil caused appreciable increases in the number of both the groups.

Keywords: Desert Ecosystem, Hydrocarbon, Microbiota

Introduction

The tropical desert of Asia extends to India through Rajasthan and Gujarat where it is called the Thar also known as “The Great Indian Thar Desert”. The Thar lies in west of Aravali Range in Rajasthan and covers an area of about 196,150 km². It is the world's 18th largest subtropical desert.⁽¹⁾

Temperature, moisture and the availability of organic carbon control the microbial activity in desert soils. It is due to extreme weather conditions viz. extreme variations in temperature and moisture, organically poor soils, with limited amounts of bioavailable inorganic nutrients, the plants are highly adaptable to dry as well as salinity. So the under explored microbial flora also has high adaptability to cope up with frequent droughts and stresses such as starvation, high osmolarity, high temperature, and desiccation and variation according to the desert ecosystem.⁽²⁾

Populations of aerobic bacteria in deserts across the world are reported to be < 10 in Atacama desert and 1.6×10^7 g⁻¹ in Nevada desert. Thar sand dunes are reported to have relatively smaller

microbial population ($1.5 \times 10^2 - 5 \times 10^4 \text{ g}^{-1}$ soil). Since the beginning of commercial oil production accumulation of biodegraded oils has been a problem for petroleum production. Microorganisms destroy hydrocarbons and other components to produce altered denser heavy oils.⁽³⁾⁽⁴⁾ Crude oil consists of four major groups: aliphatic hydrocarbons, aromatic hydrocarbons, resins and asphaltenes. Biodegradation of resins and asphaltenes is complicated but aliphatic and aromatic are easily degraded by bacteria and fungi.⁽⁵⁾⁽⁶⁾ Initial degradation of the hydrocarbon is an oxidation process which is catalyzed by the oxygenases and peroxidases.⁽⁷⁾ Indigenous microorganisms can utilize the total petroleum hydrocarbons of crude oil as source of carbon and energy and break them down to simpler non-toxic compounds such as CO_2 and H_2O .

Biodegradability of the spilled hydrocarbons is determined by the diversity and the number of microorganisms at a given site which in turn is controlled by the toxicity of these hydrocarbons to the microbiota, age of the spill and concentration of the pollutant. Microbial isolates from the soils that have been previously exposed to hydrocarbon pollution exhibit a higher potential of biodegradation than others with no such exposure in past. Therefore, in oil polluted soil, the biodiversity and microbial population may indicate the potential of soil for supporting microbial growth. Fresh oil spillage with high toxicity often kills or inhibits large part of the soil microbial population; whereas soils with lower levels or old pollution show greater numbers and diversity of microorganisms viz bacteria⁽⁸⁾ and fungi⁽⁹⁾

Different strategies are currently being framed to combat the growing threat of hydrocarbon contamination. Current study aimed at evaluating microbial diversity of the Great Indian Thar Desert.

The remediation of polluted soils in desert region requires the study of the microorganisms' diversity in the environment and the determination of the ability of different microbes and their consortia to degrade pollutants in the presence of high salt concentration.⁽⁴⁾ As it was observed that the introduction of a single oil-oxidizing strain into the oil-spilled environment does not assure a complete clean-up, our study aimed at isolating the microbial diversity of the oil contaminated areas of the Thar Desert. These isolates formed the basis for the further environment cleaning programme.

Materials & Methods

Collection of Soil Samples

Soil samples used for this study were collected from two different locations of Thar. A comparative soil collection was performed choosing soils from polluted as well as non-polluted sites. Surface was dug upto 1cm depth and soils were kept in pre sterilized bags, kept at 4°C prior to experimentation.

The samples were labeled accordingly (SP1-SP5 and SU1-SU5). Standard microbiological procedures were employed in collection and handling of the soil samples and analysis was done within 24 hours of collection.

Isolation and Screening

In the laboratory, 10g of each of the soil samples was weighed and transferred into 250ml flask containing 90ml of sterile distilled water. Flasks were kept on rotary shaker at 200rpm for 30min. Each solution was allowed to stand for about 1 hr after which the suspension was decanted into another 250ml flask.

Serial dilution of each suspension was made by the standard serial dilution technique and 1ml of aliquot was inoculated into Nutrient Agar plates by pour plating technique.

The plates were incubated at 37° C for 48hr. Total Plate count (APC) was carried out using a colony counter (Scientific -Cock Limited) model M.E. 16.

Plates for culturing fungi were made of Potato Dextrose Agar (PDA) and were incubated at 30° C. for 3-4 days. Identification of bacterial genera was done according to Bergey's Manual of Determinative Bacteriology.

Diversity, Abundance and Succession of Hydrocarbon Utilizing Microorganisms.

Oil contaminated areas and non-contaminated area in the nearest vicinity were under taken to determine the changes in microbial flora in soil samples due to oil contamination. For this purpose soil samples were collected from various oil contaminated areas viz. Diesel shed, Jodhpur and Petroleum producing areas of Barmer (S1 & S2 respectively).

Soils were collected at the end of three consecutive months in sterile polythene bags and labeled legibly. Abundance, diversity and the succession of hydrocarbon utilizers in the samples was analyzed in laboratory by standard culture techniques for each month.

Results

Total microbial population of soil samples from Thar Desert

The microbial population in a sample was expressed as colony forming units (CFU) that determined an estimate of viable cells of microorganisms. Plate counting aimed at confirmation that every colony is separate and formed by single viable cell. 5 samples each of oil spilled and non-oil spilled areas were studied and following observations were undertaken.

Oil Spilled Area

SP1

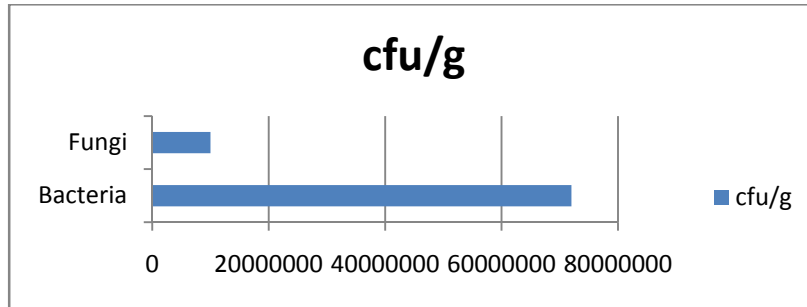


Fig. 1

SP 2

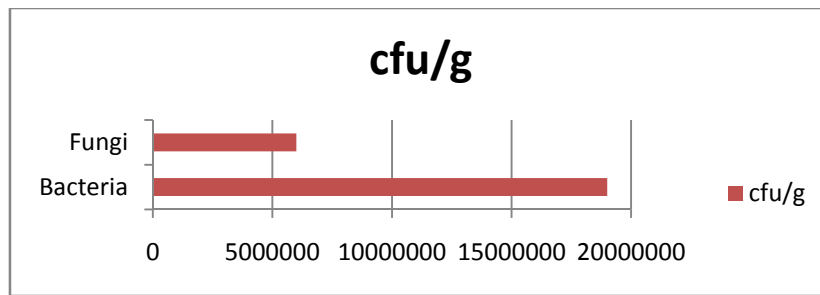


Fig. 2

SP 3

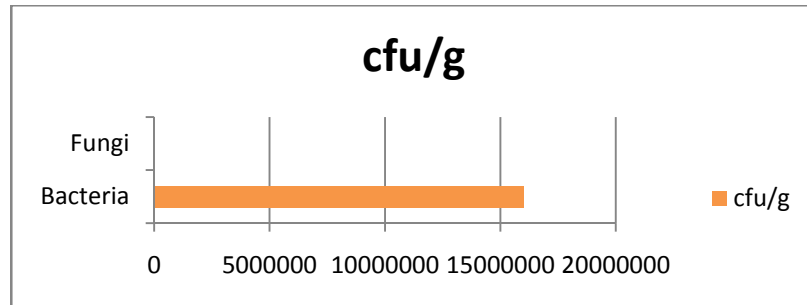


Fig. 3

SP 4

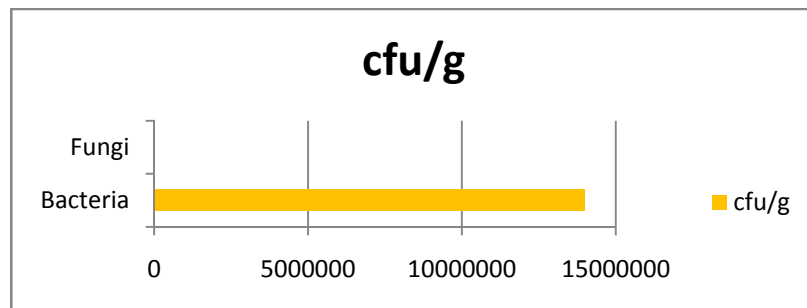


Fig.4

SP 5

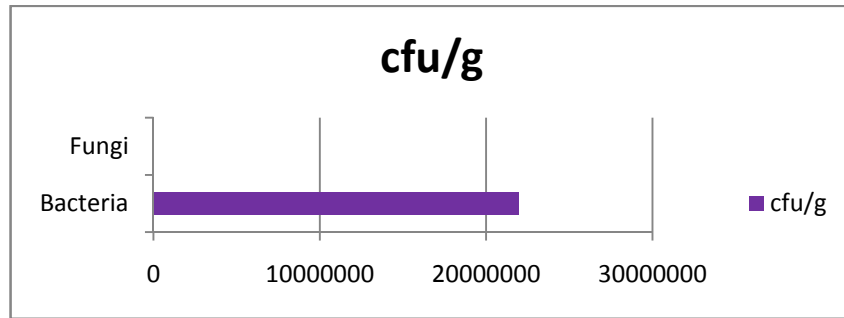


Fig. 5

Plate counts (cfu/g) calculated for each of the five samples from oil spilled areas are shown in fig. 1-5

The highest plate counts of bacteria were observed in sample SP1 as 7.2×10^7 and for fungi 6×10^6 were observed in sample SP2. For samples SP3, SP4 & SP5 no fungal colonies were obtained. Samples SP1 and SP2 shown microbial diversity for both bacteria and fungi.

Non-Oil Spilled Area

SU 1

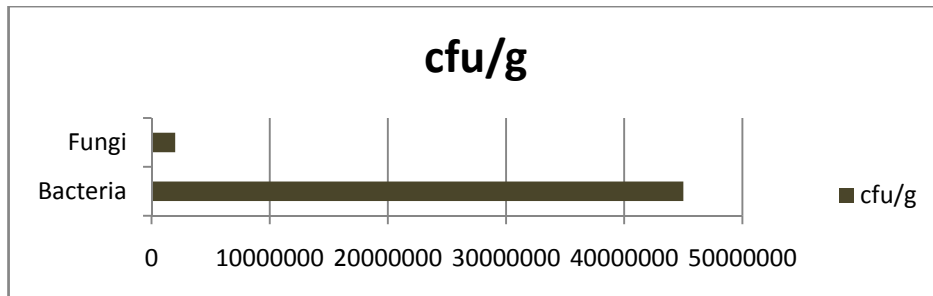


Fig 6

SU2

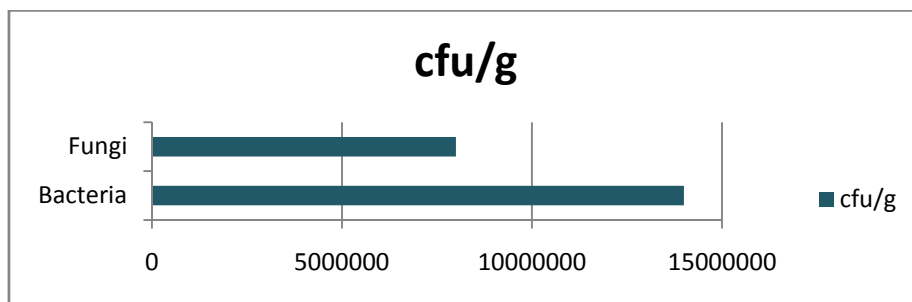


Fig. 7

SU3

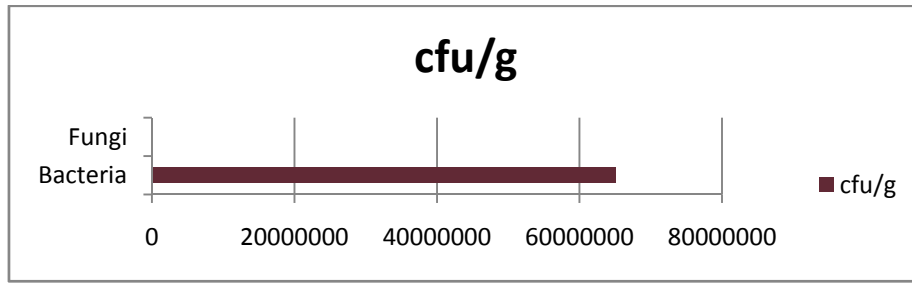


Fig 8

SU 4

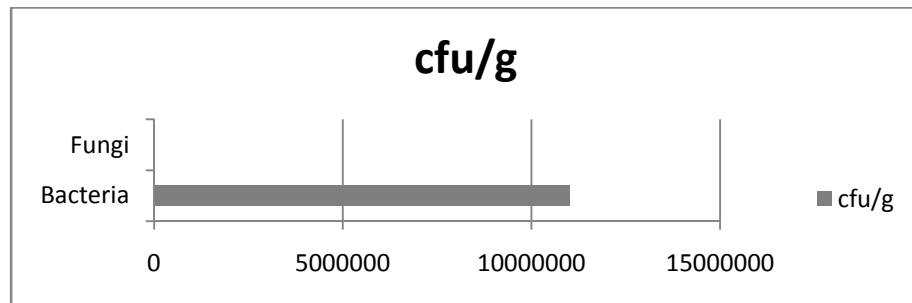


Fig.9

SU5

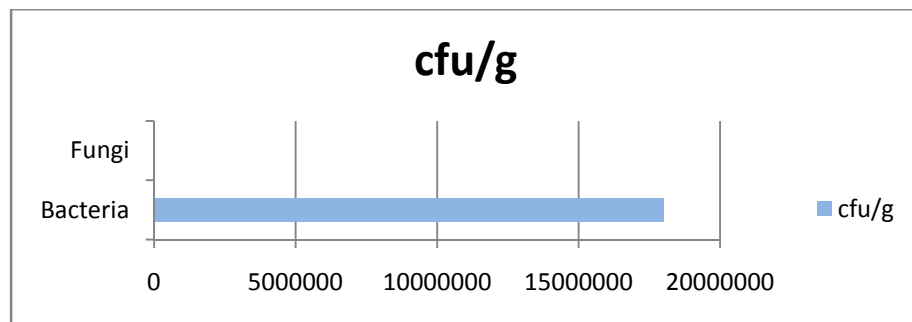


Fig. 10

Similar plate count studies (cfu/g) were done with each of the five samples from non-oil spilled areas.(fig-6-10) The highest plate counts of bacteria were obtained for sample SU3 6.5×10^7 and for fungi highest plate count was obtained for sample SU2 as 8×10^6 . For samples SP3, SP4 & SP5 no fungal colonies were obtained. Samples SP1 and SP2 shown microbial diversity for both bacteria and fungi.

Average Microbial Population of Soil Samples from Thar Desert

Oil Spilled Area

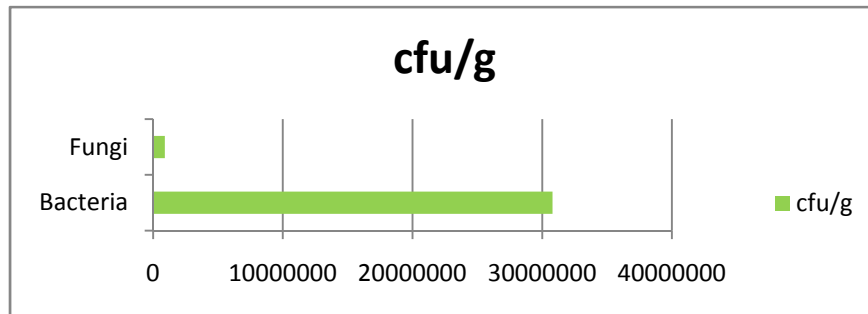


Fig. 11

Non-Oil Spilled Area

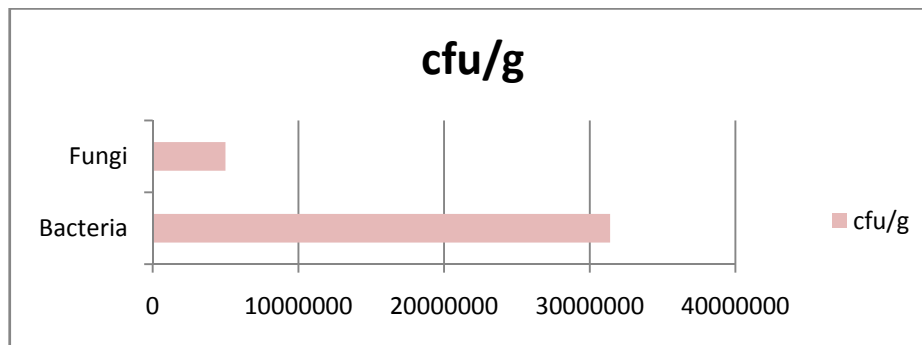


Fig 12

Comparative study of the microbial diversities of oil spilled and non-oil spilled areas showed a great variations. In case of Oil Spilled Area, bacterial counts were found to be 30.8×10^7 and fungal counts as 9×10^6 whereas for Non-Oil spilled areas it was found to be 31.4×10^6 and 5×10^6 for bacteria and fungi respectively. It is clear from the Figs-11-12 that presence of oil spills had a considerable increase in the presence of both the groups.

Most frequently isolated microbial strains from oil spilled area of Thar

On the basis of Gram's staining, and biochemical characteristics bacterial isolates were identified using Bergey's Manual. Identification of fungi was done by mounting and examining mycelia under microscope. A comparative study was done for isolates obtained from both oil

spilled and non-oil spilled areas.

Oil Spilled Area

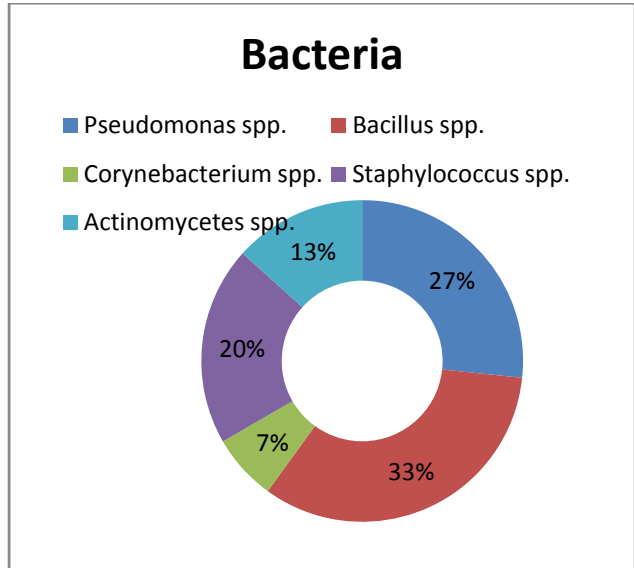


Fig 13

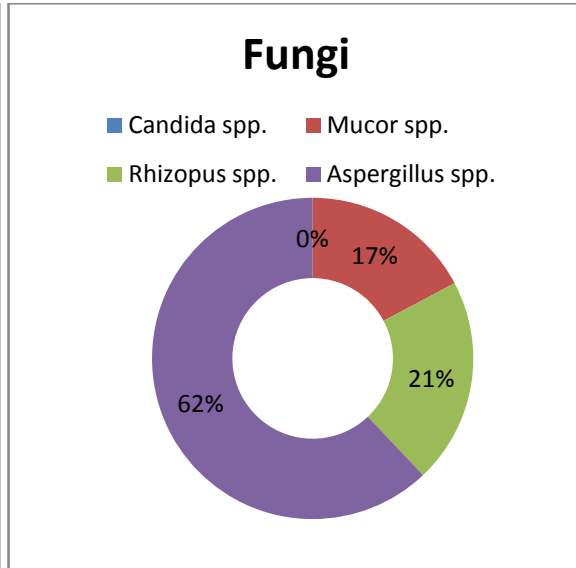


Fig 14

Non-Oil Spilled Area

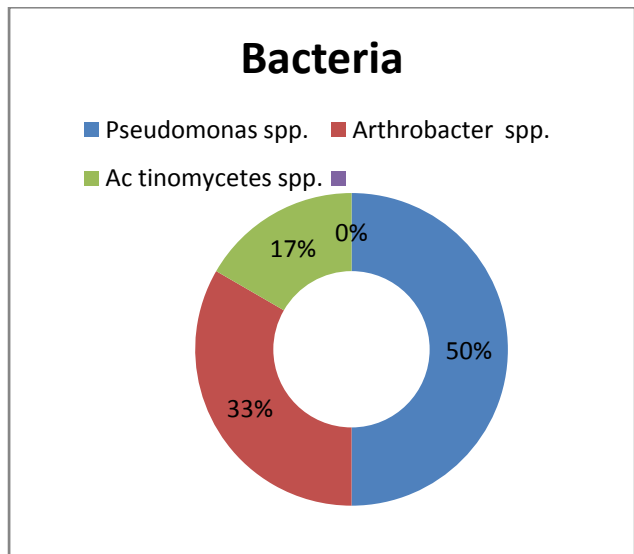


Fig 15

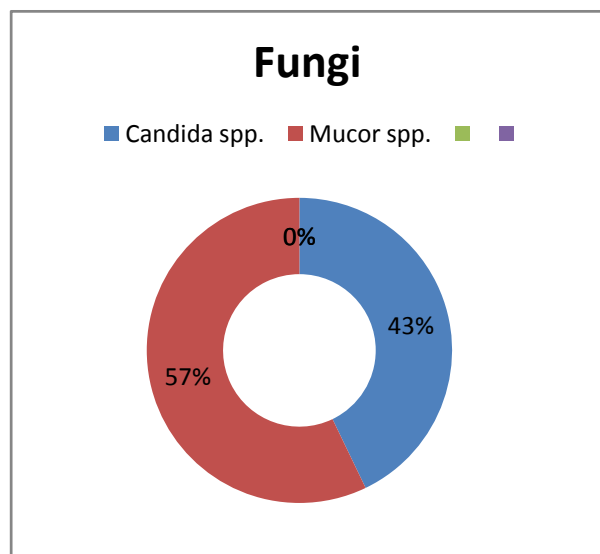


Fig 16

As shown in Fig 13 & 15, there is a great difference amongst the microbial diversity obtained from oil spilled area than that of non-spilled area. In oil spilled area around 5 different bacterial groups were noticed whereas non-oil spilled showed 3 bacterial groups.

Similarly three different fungal groups were obtained in oil spilled area than that of two for non-oil spilled area.

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