

Original Research Article

Seasonal Fluctuations in Microbial Diversity in Hydrocarbon Contaminated Areas of Thar Desert

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A B S T R A C T

The rhizosphere effect is pronounced in deserts both, qualitatively and quantitatively than in other soils providing intense microbial diversity. Microbial diversity are more prominent in the oil contaminated areas rather than unstressed sites which underwent seasonal fluctuations with consecutive months. Current study aimed at studying the seasonal fluctuations in the microbial diversity of oil contaminated sites in the great Indian Thar Desert. Sampling was done in three consecutive months from the oil spilled as well as non-oil spilled sites and cultured using Mineral Salt medium. Plate count method was employed to study succession and abundance of the microbial flora as Hydrocarbon utilizing Bacteria (HUB) & Hydrocarbon Utilizing Fungi (HUF). In oil contaminated soils, both samples showed highest concentration of *Bacillus* sp. and *Pseudomonas* sp. in each of the three months (HUB). Bacterial diversity was found almost similar for unpolluted soil in all the months. Similarly for Hydrocarbon utilizing fungi (HUF), both samples showed highest concentration of *Fusarium* sp. and *Aspergillus* sp. in each of the three months. Fungal diversity was found almost similar for unpolluted soil in all the months. Nutrient agar and Potato dextrose agar were used to further maintain cultures for studies of bioremediation technology

Keywords

Bioremediation,
Hydrocarbon,
Mineral Salt
Medium,
Rhizosphere,
Seasonal
fluctuations

Introduction

The Thar Desert is a large, arid region lying in the northwest of the Indian subcontinent. It owes multiple oil drilling sites and petroleum plants. Soil contamination by crude oil is an important environmental problem in context to the toxic, mutagenic and carcinogenic properties of the petroleum. (Liu *et al.*, 2009) Rhizosphere is an important site of microbial activity in desert soils, since it provides ample carbon

substrate in an otherwise organic matter poor arid soil. The rhizosphere effect is therefore more pronounced in deserts both, qualitatively and quantitatively than in other soils (Bhatnagar and Bhatnagar *et al.*, 2005). Bacteria are considered to be the predominant agents of hydrocarbon degradation in the environment and hydrocarbon degrading bacteria are ubiquitous (Pucci *et al.*, 2009a,b).

Biodegradation of crude oil requires a mixture of different bacterial groups or consortia which can degrade a wider range of hydrocarbons (Bordenave *et al.*, 2007; Cagnon *et al.*, 2011).

Contaminated environments are inhabited by a range of microorganisms that are capable of tolerating and degrading pollutants impacting the environment. This in turn leads to the dominance of pollutant-tolerant bacteria. Hence, bacterial communities in contaminated sites are typically less diverse than those in non-stressed systems. (Harayama *et al.*, 2004)

Microbes are vital to the stream food web and changes in their structure or composition can vary on a temporal scale, (Hullar *et al.*, 2006) which can have unintended consequences for stream biotic health (Findlay *et al.*, 2002). Constant researches have been focused on the characteristics of bacterial community structures in oil-contaminated, as well as changes in these community structures associated with oil contamination (Labb'e *et al.*, 2007; Popp *et al.*, 2006). Initial degradation of the hydrocarbon is an oxidation process, catalyzed by the oxygenases and peroxidases (Ron and Rosenberg, 2002) Microorganisms utilize the hydrocarbons as source of carbon and energy causing their break down to simpler non-toxic compounds such as CO₂ and H₂O.

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 (Van Hamme *et al.*, 2003). Hydrocarbon toxicity towards microbiota, age of the oil spill and concentration of the pollutant control the microbial at the oil contaminated site. 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 (Brito *et al.*, 2006) and fungi (Chaillana *et al.*, 2004). Accounts of different weather conditions impart a great effect over the abundance and the succession of the microbial diversity in desert soils. Seasonal changes invoke variations in the biodiversity and microbial population indicating the potential of soil for supporting microbial growth in extreme salinity, drought and low/high temperatures. This in turn significantly affects the biodegradation potential of these microorganisms varying their enzyme systems.

Current study aimed at studying the effect of the seasonal fluctuations on the microbial diversity in oil contaminated areas of the Thar Desert. A comparative analysis has been performed in accordance of oil spilled and non-oil spilled areas on monthly basis. The potent microorganisms were further examined for bioremediation framework studies.

Materials and Methods

Collection of soil samples

Oil contaminated areas and non-contaminated areas in the Thar Desert were undertaken to determine the seasonal fluctuations 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). One sample of each of the non-oil spilled area was collected for the comparative studies.

Soils were collected at the end of three consecutive months viz. December, January

and February by digging the surface up to 1 cm. Sample were collected 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's weather fluctuations.

Enrichment and isolation

Samples from oil spilled areas (S1 & S2)

Mineral Salt Medium for bacteria

Enrichment of soil microbial community of the oil contaminated areas was carried using Mineral Salt (MS) medium, composed of Na_2HPO_4 , 5.9 g/L; KH_2PO_4 , 1.5 g/L; NH_4Cl , 0.3 g/L; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g/L; KNO_3 , 2.0 g/L and 5 ml of Trace element solution was added, comprising: EDTA, 50.0 g/L; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 22.0 g/L; CaCl_2 , 5.54 g/L; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 5.06 g/L; $\text{FeSO}_4 \cdot 4\text{H}_2\text{O}$, 4.99 g/L; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 1.10 g/L; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 1.57 g/L; $\text{COCl}_2 \cdot 6\text{H}_2\text{O}$, 1.61 g/L (Raza *et al.*, 2010). From each of the soil sample 1gm was added to 99ml of MSM supplemented with 1ml of crude oil as carbon source. Cultures were incubated in rotating flask incubator at 37°C for 96±2 hours at 100 rpm. Further the isolates were streaked on nutrient agar plates and observed for microbial diversity.

Same procedure was repeated for soil samples in the end of each consecutive month and a comparative analysis was done over diversity and succession of microflora.

Mineral salt medium for fungi (oil agar plates): For fungi MSM comprised of NaCl , 10g/L; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.42g/L; KCl , 0.29g/L; KH_2PO_4 , 0.83g/L; NaH_2PO_4 , 1.25g/L; NaNO_3 , 0.42g/L; agar, 20g/L;

maintaining pH to be 7.2. Filter sterilized 1% crude oil was added to the sterile MSM and inoculated with serial dilutions of soil samples (Obire and Anyanwu, 2009).

Soil heterotrophic fungi were estimated by soil dilution plate count method and further maintained on potato dextrose agar plates. Same procedure was repeated for soil samples on monthly basis and a comparative record was determined.

Samples from non-oil spilled area: For the consecutive months the samples from non-polluted sites were studied for microbial diversity. Soils were serially diluted and inoculated on nutrient agar plates by pour plating. Plate counts were carried out and estimation was done in context to the microbial flora of that area in different weather conditions.

Identification of the microbial flora: Microbial diversity obtained was identified on the basis of colony morphology, staining techniques and biochemical analysis (Bergey's Manual of Systematic Bacteriology).

Result and Discussion

Diversity, abundance and succession studies of hydrocarbon utilizing microorganisms

Our study aimed at studying the seasonal fluctuation on microbial population in oil contaminated areas of Thar Desert. The Diversity and Abundance of hydrocarbon utilizing bacteria (HUB) and Fungi (HUF) in soils were calculated.

Although, hydrocarbon degraders may be expected to be readily isolated from an oil associated environment, the same degrees are isolated from a total related environment

such as soil. A Comparative analysis of the diversity and abundance of the microorganisms was performed by sampling in three consecutive months and a total of 24 isolates were obtained.

Figures 1–9 indicate the variations in diversity of Hydrocarbon utilizing bacteria (HUB) in both samples of each of the three months. In oil contaminated soils, both samples showed highest concentration of *Bacillus sp.* and *Pseudomonas sp.* in each of the three months. Bacterial diversity was

found almost similar for unpolluted soil in all the months.

Figures 10–18 indicate the variations in diversity of Hydrocarbon utilizing fungi (HUF) in both samples of each of the three months. In oil contaminated soils, both samples showed highest concentration of *Fusarium sp.* and *Aspergillus sp.* in each of the three months.

Fungal diversity was found almost similar for unpolluted soil in all the months.

**Hydrocarbon Utilizing Bacteria HUB
December**
Fig.1

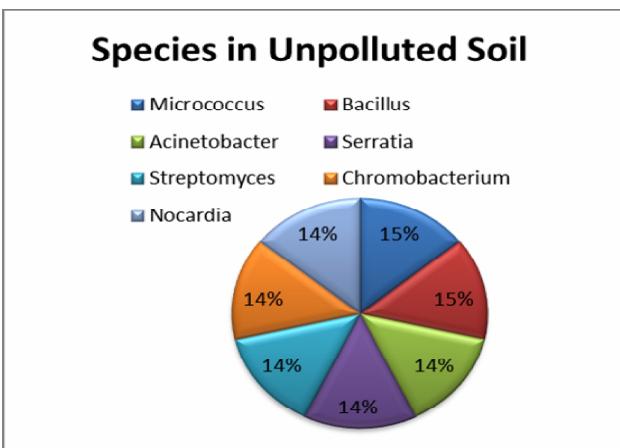


Fig.2

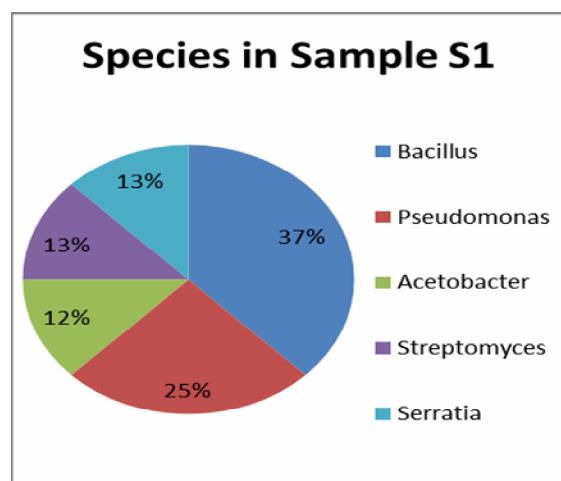
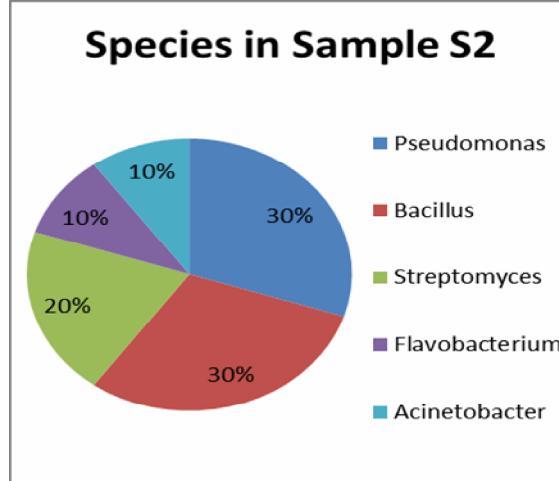


Fig.3



January
Fig.4

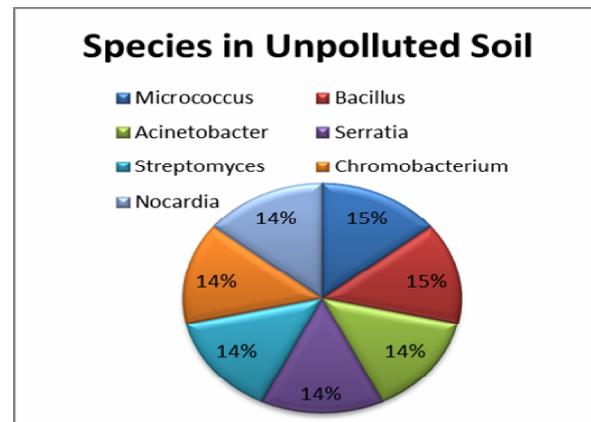


Fig.5

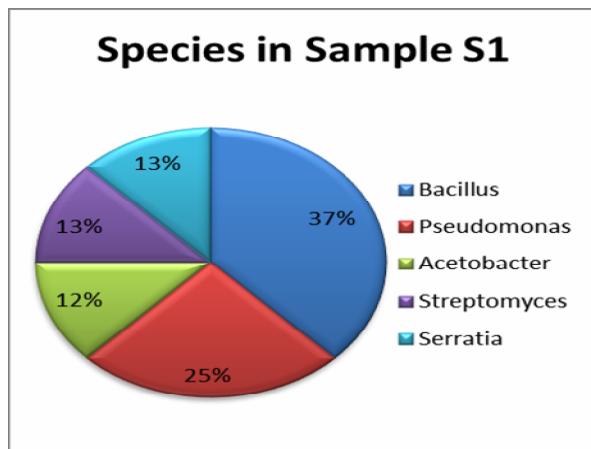
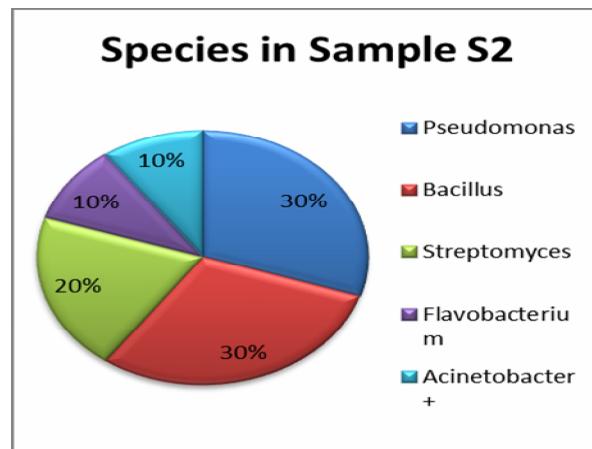


Fig.6



February
Fig 7

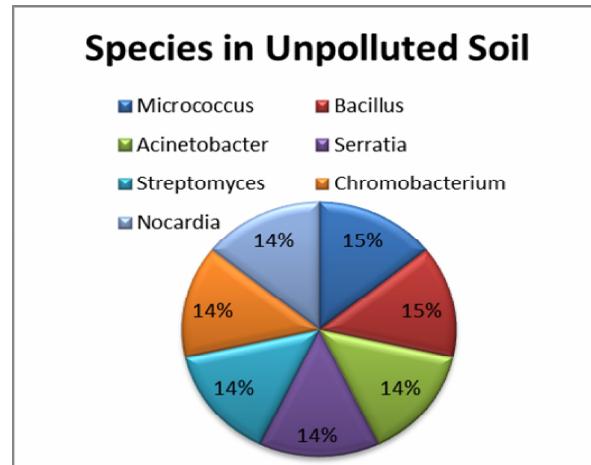


Fig.8

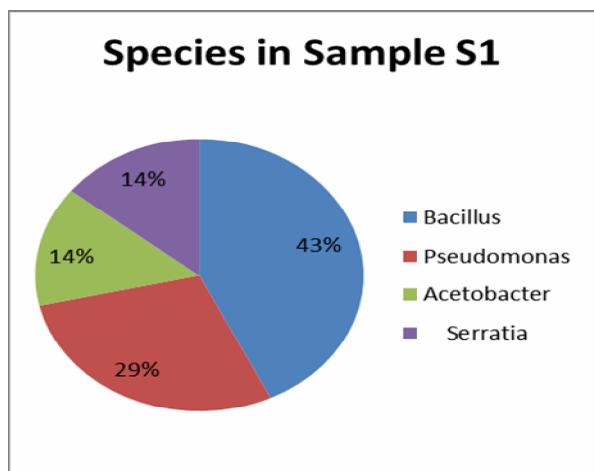
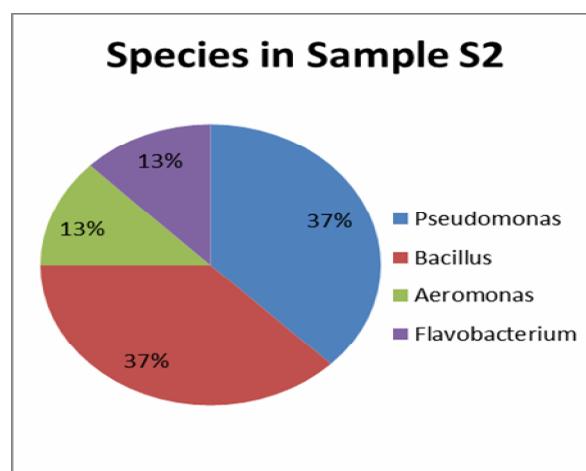


Fig.9



Hydrocarbon Utilizing Fungi HUF

December

Fig 10

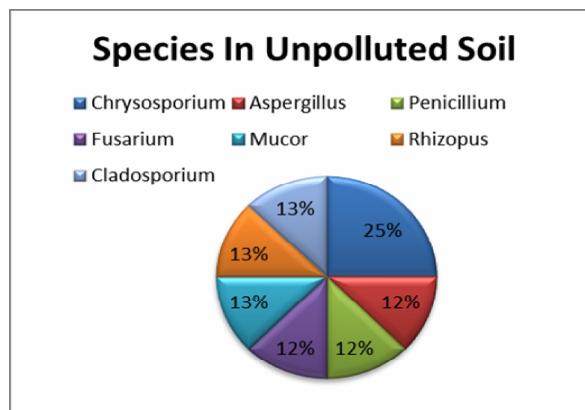


Fig 11

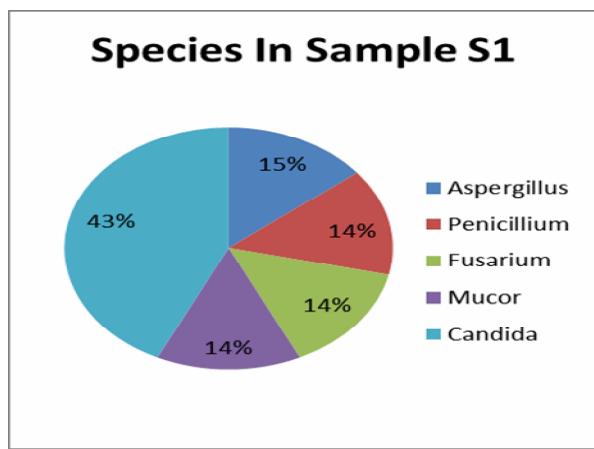
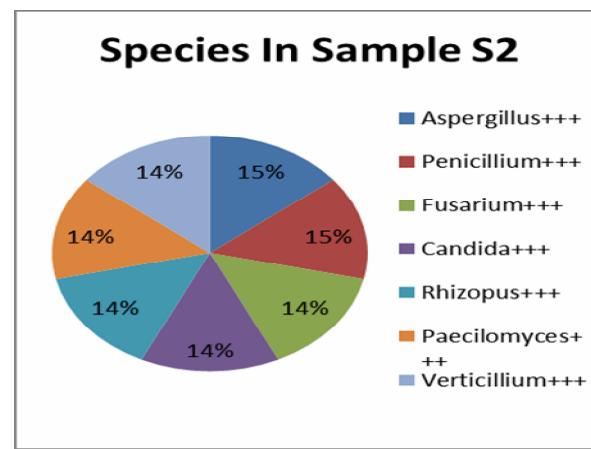


Fig 12



January
Fig.13

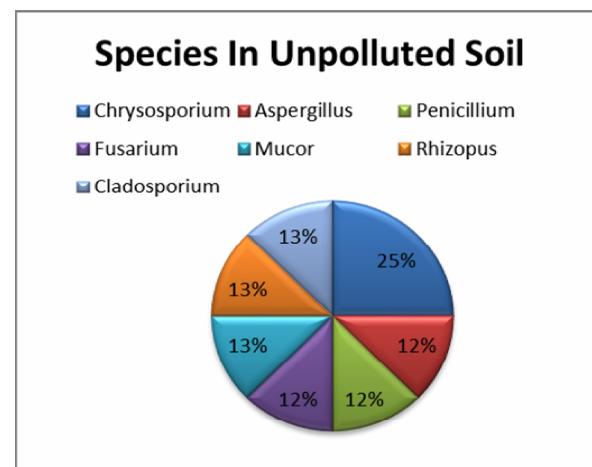


Fig.14

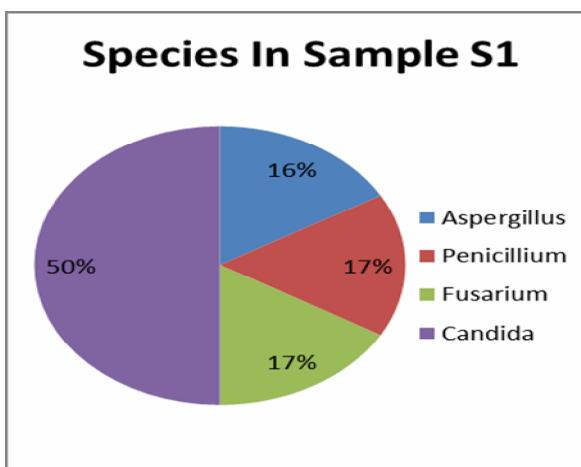
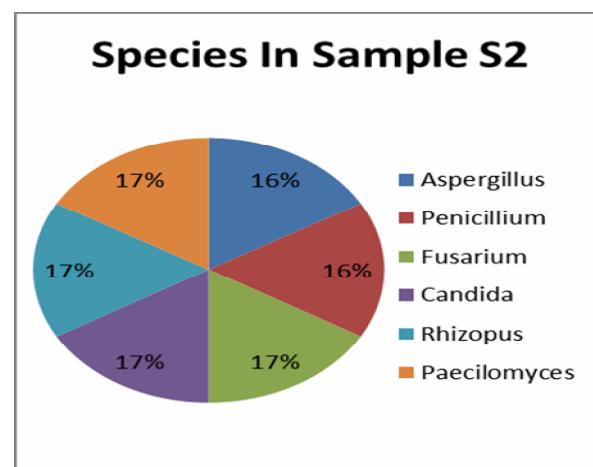


Fig.15



February
Fig.16

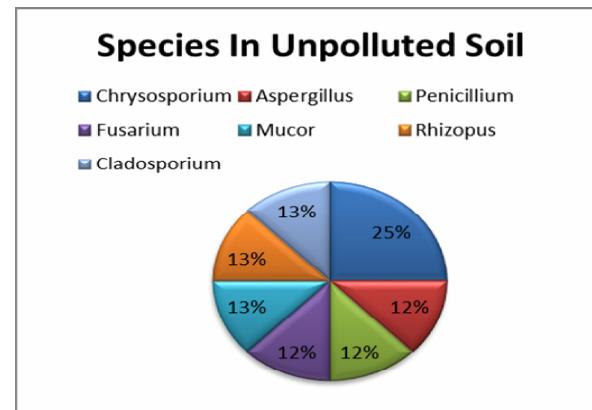


Fig.17

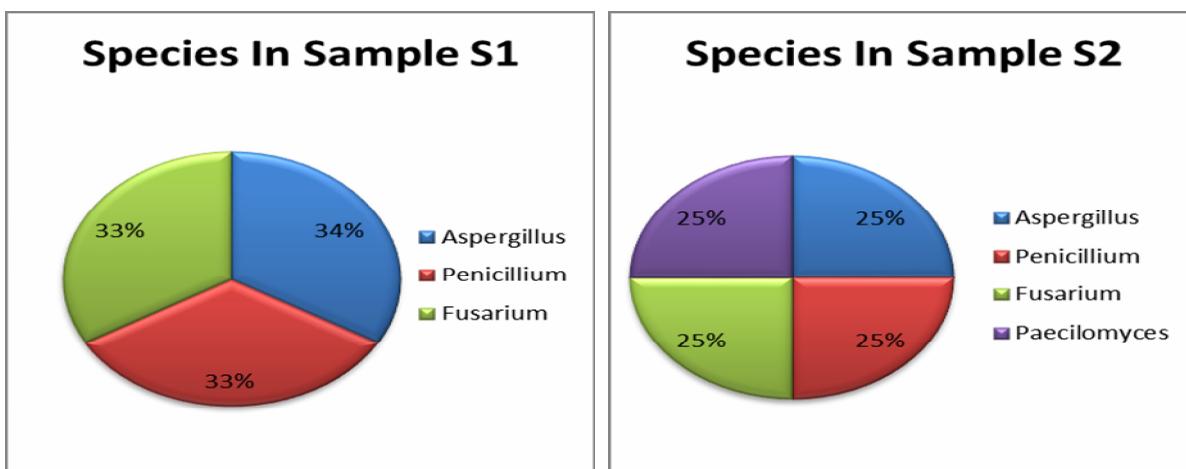


Fig.18

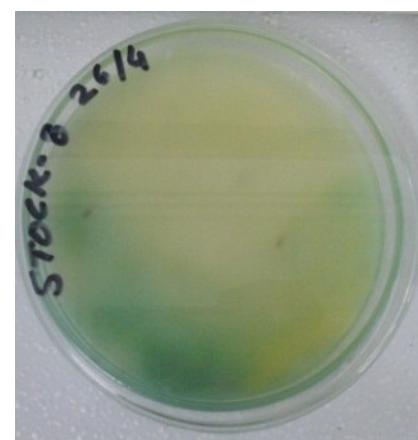
S1: Diesel Shed; S2: Barmer; HUB: Hydrocarbon Utilizing Bacteria; HUF: Hydrocarbon Utilizing Fungi

Hydrocarbon Utilizing Bacteria HUB

Fig.19



Fig.20

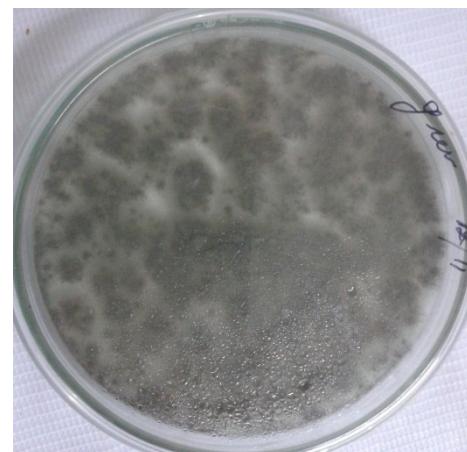


Hydrocarbon Utilizing Fungi HUF

Fig.21



Fig.22



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