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Evaluating Fungal and Bacterial strains as hydrocarbon degrader from the soil of workshops

WaseemShoukat, Department of Chemical Sciences, BahauddinZakariya University, Multan 60800,Pakistan.
ShujaatHussain, Department of Chemical Sciences, BahauddinZakariya University, Multan 60800, Pakistan.
Dr. AsmaNoureen,Department of Zoology, Institute of Molecular Biology & Biotechnology (IMBB) The University of Lahore Road, Campus, Lahore.
Dr. Khalid Hussain Rind, Department of Molecular Biology & Genetics Shaheed Benazir Bhutto University ShaheedBenazirabad Pakistan.
Saba Manzoor, Department of Zoology, Wildlife and Fisheries University of Agriculture Faisalabad.
SafiaGul,Department of Botany SBKWU Quetta
FareehaMaham,Department of Botany SBKWU Quetta
AdeelMubarik,Department of Chemistry, Government College University Faisalabad 38000, Pakistan.
MonibaZahidMahmood,Department of Environmental Science, COMSATS University Islamabad Abbottabad Campus.
Shamsher Ali,Department of Soil and Environmental Sciences (AMKC Mardan) The University of Agriculture Peshawar, Pakistan.
Waseem Akhtar Qureshi,Department of Forestry, Range and Wildlife Management, Bagdad-ul-Jadeed Campus, The Islamia University of Bahawalpur, Pakistan.
Hina Saeed,Department of Biochemistry & Biotechnology IUB Bahawalpur.

Abstract-The most dangerous pollution in the environment are the unwanted hydrocarbon in form of oil and petroleum which is the result of leak from the coastal oil refiners and to overcomes these situations use of microbes are the only ecofriendly method and they are the main contributor to maintain a safer environment. Current study aimed to evaluate the fungi and bacteria as a bio-degrader of hydrocarbon, for his bacteria and fungi were first isolate and identify from the oil contaminated soil collected from the different workshops of Sargodha city. Result reveled that upper layer (0-2m) contain diverse and large number of bacteria and fungi specie (bacteria 50%; fungi 50%), while lowest layer (3-4m) contain less diverse and low number of bacterial and fungi species (bacteria 6.66%; fungi 13.6%). After identification it was found that total 4 strains of fungi were isolate in which Aspergillustubingensis and Alternariatenuissima were dominantIn case of bacteria 7 strains were identified. Among which E. coli were dominant. Followed by the Pseudomonas spp. after biodegradation test it was found that fungal strain Aspergillustubingensis was the excellent bio-degrader (1.69×109) followed by the Aspergillums niger (1.40×108). On the other hands among the bacterial strains Staphylococcus aureus utilizes high amount of the hydrocarbon as energy source (1.76×107) followed by the Pseudomonas aeruginosa (1.67×109). in conclusion Introducing these microbes to the oil contaminated environment can solve the problems of hydrocarbon pollution. But future research should be conducted to better understand the molecular mechanism of hydrocarbon degradation in order to better utilize these microorganisms for the welfare of human beings.

Keywords: fungal strains, bacterial strains, soil samples, hydrocarbon degradation, workshops, soil depths

I. INTRODUCTION

From the last decade several ecosystem has been changed due to anthropogenic activities. Many of that change is effecting human life in negative ways, so a need has been emerged to protect this ecosystem in order to sustain better life [1, 2]. Pollution in the environment are the major problem emerged due to human activity and has to be controlled. The most dangerous pollution in the environment are the unwanted hydrocarbon in form of oil and petroleum which is the result of leak from the coastal oil refiners. Which leads several researchers to investigate its distribution and effects in the environment and impact on human. This contamination effect almost every ecosystem (marine, fresh and terrestrial). Annually 5millions tons of crude oil comes from the human activity into the environment. The main source of this pollution are the spills from the tankers, vessels (pipelines) and barges.

These spills got attention of many researchers including chemists, engineers, biotechnologists and environmentalists [3]. The interest has been increased to promote the ecofriendly mechanisms in the procedure of decontamination of the oil polluted areas. This type of methods is less costly which are not introducing the extra chemical agents in the environment. While comparing the chemical and physical procedures the bioremediation is offering much suitable alternate to the oil spills response [4].

II. MATERIALS AND METHODS

***Collection*:**

Soil sample that was contaminated with the oil was collected from the 4 different location of Sargodha workshops from the three different depths that is 0-2 m, 2-3m and 3-4m. before collecting sample the upper layer of soil was cleaned up from the other impurities like plants and other wastes (animals waste, polyethylene bags etc.). form each site samples were collected in triplicates of amount 500g (500×3g). each collected sample were recorded according to the time, amount and date. Collected sample in the bag of polyethylene were transfer to the laboratory of Sargodha for analysis and was stored at 4^0C until further procedure.

***Isolation and identification of the fungi and bacteria*:**

For the isolation of the microbes' different cultures were setup using the contaminated soil as inoculum. And petrol as the sole source of the carbon. After obtaining the pure culture isolates were identified on the basis of colony morphology (color, shape, edge etc.), gram staining and biochemical tests such as Mannitol Salt Agar, Starch Test, Simon Citrate, Oxidase, Catalase and MacConkey, (General Microbiology Laboratory Manual by Rachel Watson)

***Hydrocarbon Degradation Test*:**

After isolation and identification the identified bacteria and fungi were subjected to the hydrocarbon degradation test. For this Bushnell Hass broth of 100ml was prepared in four conical flasks which was supplemented with 1ml of spent engine oil. After then the media was inoculated with the loop full of fungi and bacteria. the flasks were placed in the shaker. Using spectrophotometer culture absorbance was measured. Pour plate and serial dilution was done on each culture in the conical flask every two days, in order to enumerate the colonies number which, bloom on the broth (Bushnell Hass broth-engine oil mixture.

III. RESULTS.

Microorganism colonies count: After isolation of the bacterial and fungal isolates from the different depth it was revealed that upper layer (0-2m) contain diverse and large number of bacteria and fungi specie (bacteria 50%; fungi 50%), while lowest layer (3-4m) contain less diverse and low number of bacterial and fungi species (bacteria 6.66%; fungi 13.6%). After identification it was found that total 4 strains of fungi were isolate in which *Aspergillustubingensis and Alternariatenuissima* were dominant, as shown in the figure 2. In case of bacteria 7 strains were identified. Among which E.coli were dominant. Followed by the *Pseudomonas* spp. as shown in the figure 2. Table 1 shows the detail of each layer and site of the sample. Table 2 shows the detail of the diversity of each layer of the sample. Table 3 shows the detail of colonies

Table 1: Distribution of the microorganisms in each depth and Site of the sample

Site no	**Sampling site ID**	**Depth 0-2m**		**Depth 2-3m**		**Depth 3-4m**		**TOTAL**		**%**	
	Colony Forming Unit (CFU/g)										
		Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria
1	**SS1**	8	4	1	1	0	0	9	5	40.90	16.66
2	**SS2**	2	4	5	3	2	1	9	8	40.90	26.66
3	**SS3**	1	5	1	4	1	0	3	9	13.63	30.00
4	**SS4**	0	2	1	5	0	1	1	8	4.54	26.66
Total		11	15	8	13	3	2	22	30		
	%	50.00	50.00	36.36	43.33	13.63	6.66				

Table 2: Diversity of the microorganism in each layer and depth

	Bacterial strain	Fungal strain	0-1.5m		1.5-2.5m		2.5-3.5	
			Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
1	*Pseudomonas* spp	*Aspergillums niger*	+	+	+	+	-	-
2	*Bacillus cereus*	*Penicilliumcitrinum*	+	+	+	+	-	-
3	*Staphylococcus aureus*	*Alternariatenuissima*	+	+		+	-	-
4	*Streptococcus pneumoniae*	*Aspergillustubingensis*	+	+	+	+	-	+
5	E. coli		+		+		+	
6	*Pseudomonas aeruginosa*		+		+		+	
7	*Acinetobacter*sp.		-		-		+	

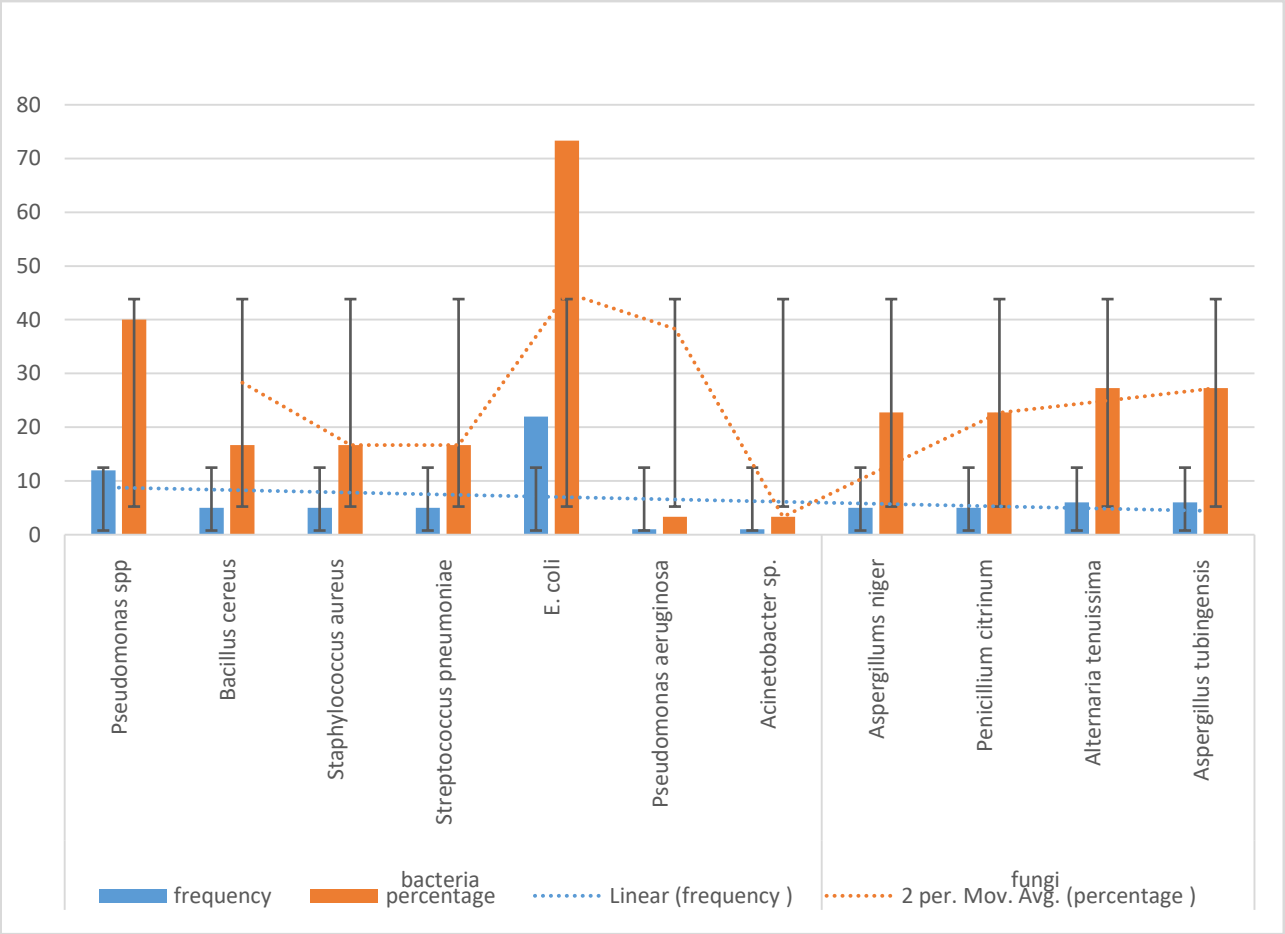

Figure 1: percentage and frequency of each microbe isolate during experimental procedure.

Table 3: Colonies morphology and Biochemical testes result.

Fungal strain	Hyphae	Surface Color	Reverse color
Aspergillums niger	conidia.	Black	sulfur-yellow
Penicilliumcitrinum	phialospores	bluish-green	pale yellow
Alternariatenuissima	macroconidia	Black	greenish-black
Aspergillustubingensis	conidia	white to pink	light- yellow
Bacterial strain			
	Gram	Biochemical tests	

	staining						
		Urease Test	**Simon Citrate**	**Mannitol Salt**	**Oxidase**	**Catalase**	**Indole test**
Pseudomonas spp	- Bacilli	-	+	+	-	-	-
Bacillus cereus	+ Bacilli	+	-	+	+	+	-
Staphylococcus aureus	+ Cocci	+	+	-	-	-	-
Streptococcus pneumoniae	+ Cocci	+	+	+	+	+	+
E. coli	- Bacilli	-	-	-	-	-	-
Pseudomonas aeruginosa	- Bacilli		-		+		
*Acinetobacter*sp.	- Bacilli	-	-	-	+	+	+

*Hydrocarbon degradation test :*When the isolates were subjected to the biodegradation tests it was found that on the basis of day 6th among fungal strain *Aspergillustubingensis*was the excellent bio-degrader (1.69×10^9) followed by the*Aspergillums niger (*1.40×10^8). On the other hands among the bacterial strains *Staphylococcus aureus*utilizes high amount of the hydrocarbon as energy source (1.76×10^7) followed by the *Pseudomonas aeruginosa*(1.67×10^9). Detail of the experiment is given in the table 4.

Table 4: hydrocarbon degradation per day.

Fungal strain	Day 2	Day 4	Day 6
Aspergillums niger	1.22×10^5	1.37×10^7	1.40×10^8
Penicilliumcitrinum	1.16×10^4	1.28×10^5	1.35×10^8
Alternariatenuissima	1.57×10^2	1.29×10^3	1.37×10^3
Aspergillustubingensis	1.66×10^5	1.68×10^8	1.69×10^9
Bacterial strain			
Pseudomonas spp	1.14×10^8	1.23×10^8	1.67×10^9
Bacillus cereus	1.29×10^3	1.29×10^5	1.44×10^7
Staphylococcus aureus	1.68×10^3	1.67×10^4	1.76×107
Streptococcus pneumoniae	1.67×10^5	1.57×10^8	1.34×10^7
E. coli	1.36×10^4	1.47×10^7	1.59×10^7
Pseudomonas aeruginosa	1.34×10^5	1.48×10^6	1.67×10^8
*Acinetobacter*sp.	1.44×10^2	1.44×10^4	1.33×10^7

IV. DISCUSSION

Discharge of hydrocarbons into the environment either naturally or due to anthropogenic activity is considered as the main source of pollution of both aquatic and terrestrial environments. These pollutants can accumulate in the organism (both plant and animal) tissues and can affect other organism through food chain or even cause death or mutation in the organism in which they are accumulated (1,2) Serval methods and technology have been used in the remediation of the hydrocarbon in these ecosystems such as, evaporation, burying, mechanical, washing and dispersion. But unfortunately these techniques/technology are very expensive or unavailable in most of the cases and besides this it also leads to incomplete decomposition or breakdown of the hydrocarbon (contaminant) of the environment (5). So alternative method known as bioremediation are used which can be defined as 'use of microorganisms to detoxify or remove pollutants'. Due to their capacity or capabilities of their metabolic functions. This technique is one of the most evolving procedure to remove or detoxify the pollutant in the environment even includes petroleum industries products. Furthermore, this technique is also better in term of cost and non-invasive (6, 7). Current study aimed to isolate fungal and bacterial strains from the oil contaminate environment and evaluate them as potential bio-degrader. Result revealed that After isolation of the bacterial and fungal isolates from the different depth it was revealed that upper layer (0-2m) contain diverse and large number of bacteria and fungi specie (bacteria 50%; fungi 50%), while lowest layer (3-4m) contain less diverse and low number of bacterial and fungi species (bacteria 6.66%; fungi 13.6%). After identification it was found that total 4 strains of fungi were isolate in which *Aspergillustubingensis and Alternariatenuissima* were dominant. In case of bacteria 7 strains were identified. Among which *E. coli* were dominant. Followed by the *Pseudomonas* spp. When the isolates were subjected to the biodegradation tests it was found that on the basis of day 6th among fungal strain

*Aspergillustubingensis*was the excellent bio-degrader followed by the*Aspergillums niger.* On the other hands among the bacterial strains *Staphylococcus aureus*utilizes high amount of the hydrocarbon as energy source followed by the *Pseudomonas aeruginosa.* These microbes both bacteria and fungi are present in the soil and degrade the hydrocarbons present in the soil. Though both of the species have strong capabilities to degrade the hydrocarbon but fungal strains got less attention (8). Fungal strain can grow in the soil in the hyphal form which help them to reach and penetrate deeply into the contaminated soil (9). besides several beneficial properties associated with the fungal strains there are also some limitation in the application of the fungi, which should be kept in mind, such as fungal strains degradation can be a very slow process, due to the reason that they need longer period to adapted to environment. Besides this sometimes degradation of the hydrocarbons leads to the incomplete degradation and remain in the soil as intermediate (10, 11, 12). Study of the fungi in interaction with the bacteria strains has not been studied broadly. But this aspect might be very useful for the improvement of the microbe's performances as bioremediation. Several researchers report bacteria as the potential hydrocarbon degrader such as Geetha et al (13); Nalinee et al (14); Arpita*et al* (15). On the other hands among these researcher bacterial strains like Bacillus cereus, *Alcanivora*x, *Marinobacter*, *Pseudomonas*, and *Acinetobacter*were the common isolates. Besides this other researchers' like Maqbool*et al*., (12); Michael *et al*., (16).

V. CONCLUSION:

Hydrocarbons are the wide ranged compounds contaminate the soil and effect the soil quality. These hydrocarbons are released to the environment by industrial process and several accidental oil spill. In order to overcome these pollutions bioremediation has been followed. Our study aimed to evaluate the isolates from these contaminate environment as hydrocarbon degraders. Result revealed that, upper layer of the soil contains diverse and large number of microbes due to high amount of oxygen. Among fungal isolates *Aspergillustubingensis and Alternariatenuissima* were dominant and among bacteria*E. coli* were dominant. Followed by the *Pseudomonas* sppamong fungal strain *Aspergillustubingensis*was the excellent bio-degrader. On the other hands among the bacterial strains *Staphylococcus aureus*utilizes high amount of the hydrocarbon as energy source. Introducing these microbes to the oil contaminated environment can solve the problems of hydrocarbon pollution. But future research should be conducted to better understand the molecular mechanism of hydrocarbon degradation in order to better utilize these microorganisms for the welfare of human beings.

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CONFLICTS OF INTEREST.
The authors declare no conflict of interest.

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