Ilkogretim Online - Elementary Education Online, 2021; Vol 20 (Issue 5): pp. 7763-7769
http://ilkogretim-online.org
doi: 10.17051/ilkonline.2021.05.881

Bacterial and Fungal Species of Oil Contaminated Soil of Banda Dauood Shah, KP Pakistan

Waseem Shoukat, Department of Chemical Sciences, Bahauddin Zakariya University, Multan 60800, Pakistan.
Shujaat Hussain, Department of Chemical Sciences, Bahauddin Zakariya University, Multan 60800, Pakistan.
Dr. Asma Noureen, Department of Zoology, Institute of Molecular Biology & Biotechnology (IMBB) The University Of Lahore Road, Campus, Lahore.
Gulab Khan, Directorate of Cereal Crops Agriculture Research Institute ARI Quetta.
Javed Ahmad Ujjan, Associate Professor Department of Zoology, Shah Abdul Latif University Khairpur Sindh Pakistan.
Farhana, Department of Zoology, Ghazi University, Dera Ghazi Khan.
Anam Zohra, Department of Zoology, University of Veterinary and Animal Sciences, Lahore.
Hidayat Ullah, Department of Microbiology,Hazara University Mansehra, Pakistan.
Hina Saeed, PhD Scholar Department of Biochemistry and Biotechnology, IUB, Bahawalpur.

Abstract- Current study focus to evaluate the oil contaminant soil of the Banda Dhoud Shah in terms of biological diversity (bacteria and fungi), Soil sample were collected from three different depths that is 0-1.5, 1.5-2.5 and 2.5-3.5m from different places of the Banda dauood shah (M.CPF, M. OF, G. P, SB.DS, A.WDS and N. OGDCL). samples were processed through microorganism isolation and identification. Result revealed that upper surface (0-1.5m) was inhibit by diverse and large number of strains (bacterial and fungal) while the deeper layer (2.5-3.5m) was inhibited by least number of organisms. further, sampling site no 3 (Gorgary Plant (G. P)) contain highest number of bacterial count (n=9 and average value 3), while in case of fungal strains sampling site 6 (N. OGDCL) contained greater quantity of the organism (n=5 and ave=1.66). After gram staining and microscopy it was found that most of the isolated bacteria were gram negative 51.3%. Besides this after microscopy it was found that most of the gram positive were cocci (n=12) while in gram negative bacillus was found at highest frequency (n=21). After biochemical tests it was found that Pseudomonas spp, Bacillus cereus, Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Acinetobacter sp and Streptococcus pneumonia. Among these isolates dominated spp was Pseudomonas spp (n=15; %=30), followed by the Bacillus cereus (n=12; %=24). In term of fungi upper layer contains 8 fungal strains with average value of the 1.33. besides this second layer of the depth contains 6 CFU/g (average 1) and the last layer/depth contain only 4 cfu/g (average 0.66). furthermore, sampling site 6 (N. OGDCL) contained greater quantity of the organism (n=5 and ave=1.66. On the basis of colony morphology Aspergillums niger was found at least frequency (n=4; %=21.05). all other strains (Penicillium citrinum, Alternaria tenuissima and Aspergillus tubingensi), were in equal number and frequency that is n=5 and %= 26.3.

Keywords: Oil contaminated soil, Microbiology of soil, Fungi, bacteria

I. INTRODUCTION

Soil is the mini-ecosystem harbor immense number of life forms such as several invertebrate like worms and insects. Many micro-organisms such as bacteria, virus, fungi and protozoan. all of these life forms are inter connected with each other and to the quality of soil, responsible to create a continuous change in the environment (Eilers et al., 2012; Swer et al., 2011). These microbes not only present in the soil but also in another part of this world (biosphere) like, deepest region of the ocean, boiling springs, at very high altitude and deep inside the rocks (Hoorman and Islam, 2010; Collins, 2010; Dastager et al., 2010). In every habitat they play significant role in ecosystem like air borne microbes paly role in precipitation and changing weather. Same bacteria present in the hydrocarbon rich environment paly role in hydrocarbon degradation (Hardy, 1990; Magdoff, 2010). 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 environment (Holliger et al., 2001; Watson and Kelsey, 2006; Mandal, 2003). 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 (Alvarez and Vogel., 2005). Current study aimed to evaluate bacterial and fungal species population in these environment.

II. METHODOLOGY

Soil Collection: Oil contaminated soil samples were collected from the different location of Banda Dauood Shah from three different depths that is 0-1.5m, 1.5-2.5m and 2.5-3.5m in polyethylene bags. Before collecting soil sample first surface was clean from any large size impurity like piece of plants, different wastes like polyethylene bags etc. From each site 500×3g. collected sample were recorded according to the

date, time and amount. And transfer to the Environmental Microbiology lab, Department of Microbiology, KUST and will be stored at 40C for further use.

Isolation 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. 100g of each depth soil was added into the sterile mineral medium which were supplemented with the analyte. In order to provide sufficient amount of the minerals to both of microbes (fungi and bacteria), M9 Mineral media (Difco, Sparks, MD, United States) was used for bacteria while for fungi Czapek media was used. After then the flasks containing bacteria cultures were incubated at 30 degrees on the rotary shaker with 180rpm. While that cultures that contain fungi were incubate at 24 degrees on the rotary shaker with 120rpm. Both of the cultures were incubated for 7 days. After incubation period 5mL amount of each culture was transfer into another flask containing MM and analyte. Three repeated sub-cultures were prepared in the same manner. For final isolation of the microbes those cultures that were morphologically different were transfer to another plates containing Malt Extract Agar (MEA), which were used for fungi while Tryptone Soy Agar (TSA) were used for the bacterial strains. Further for identification several biochemical test and gram stating were performed.

III. RESULTS

Bacteria colonies count: After isolation of the bacteria on the basis of the dept. it was revealed that top layer 0-1.5m contain more bacteria count (mean value of 3.66) and less number of bacteria were recorded for the layer of 2.5-3.5m (mean value 0.83), besides this, sampling site no 3 (Gorgary Plant (G.P)) contain highest number of bacterial count (n=9 and average value 3). On the other hands lowest count was recorded for site no 1 (M.CPF) that is1.66. Table 1 shows detail of the result.

Table 1: Total CFU/g of the bacteria in depths and sampling sites

Site no	**Sampling site**	**Depth 0-1.5m**	**1.5-2.5m**	**2.5-3.5m**	**TOTAL**	**Average**
	Colony Forming Unit (CFU/g)					
1	**M.CPF**	4	1	0	5	1.66
2	**M.OF**	4	3	1	8	2.66
3	**G.P**	5	4	0	9	3
4	**SB.DS**	2	5	1	8	2.66
5	**A.WDS**	4	2	2	8	2.66
6	**N.OGDCL**	3	5	1	9	3
	Total	22	20	5	47	
	Average	3.66	3.33	0.83		

Gram staining and microscopy: Further after gram staining and microscopy it was found that most of the isolated bacteria were gram negative 51.3% while gram positive bacteria were 48.97%. Besides this after microscopy it was found that most of the gram positive were cocci (n=12) while in gram negative bacillus was found at highest frequency (n=21). Table 2 shows the detail of the test while figure 1 shows gram staining and figure 2 shows shapes of the bacteria under the microscope.

Table 2: Gram staining and Microscopy

Gram Staining	**Frequency (%)**	**Shapes of Bacteria**	**Frequency %**
Gram positive	48.97%	+cocci	**12**
Gram negative	51.3%	-cocci	**4**
		+ Bacillus	**12**
		-Bacillus	**21**

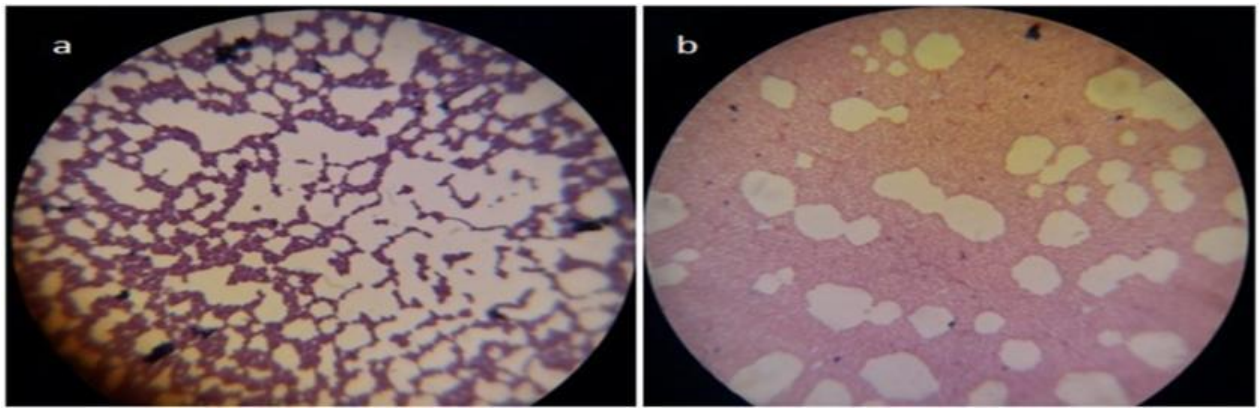

Figure 1: Gram Staining Method a) Gram Positive Bacteria (b) Gram Negative Bacteria

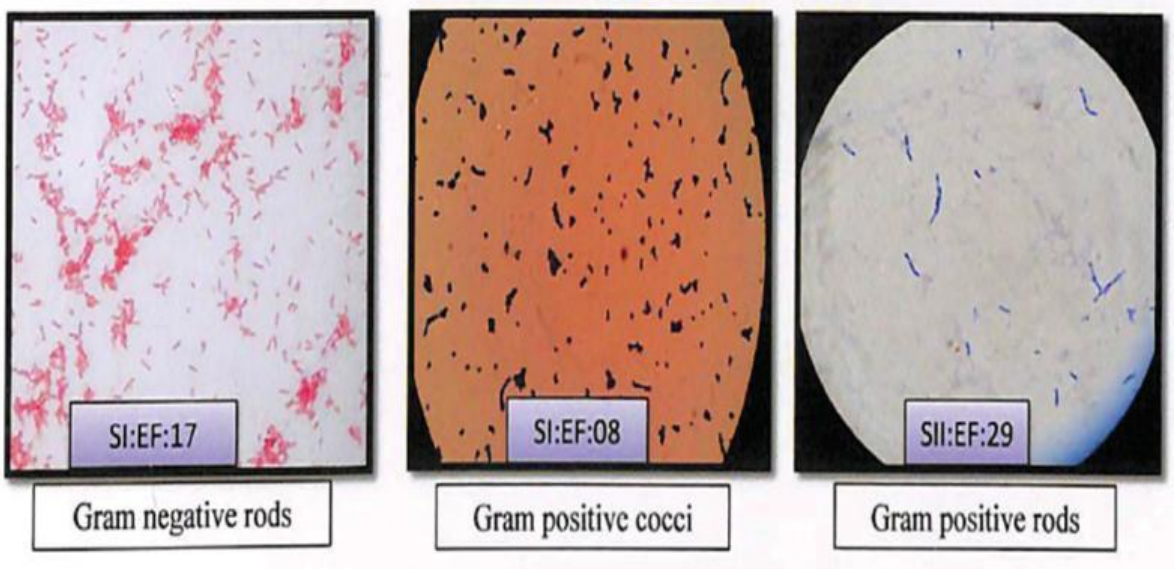

Figure 2: Shape of the cultured bacterial strain under the 100X microscope

Biochemical tests: After gram staining and microscopy isolated strains were further subjected to biochemical tests such as MacConkey, Simon Citrate, Mannitol Salt Agar, Oxidase, Catalase and Starch Test for identification. Obtained result is summarized on the table 4.4 and figure 3 it was revealed that overall dominated species was Pseudomonas spp (n=15; %=30), followed by the Bacillus cereus (n=12; %=24). Figure 4 shows applied tests.

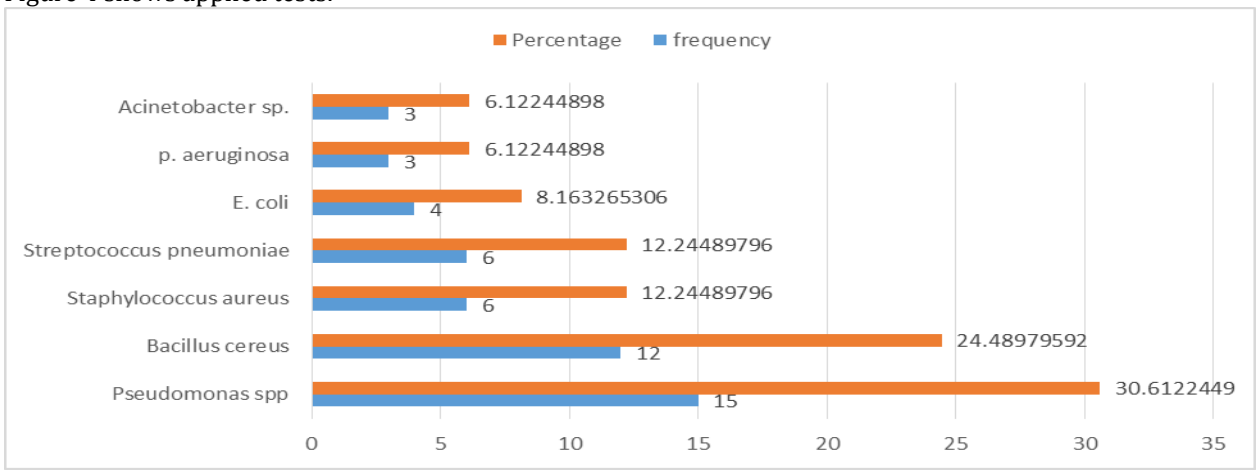

Figure 3: Occurrence of the bacterial strain (frequency and percentage)

The identification of the bacterial strain in different strata (layer/depth) of the soil is given in the table 3. Result revealed that top soil was inhibit by diverse number of the bacteria that is Pseudomonas spp, Bacillus

cereus, Staphylococcus aureus, E. coli and Pseudomonas aeruginosa as compared to the lower layer. The only absent bacteria were Acinetobacter sp. The last layer at depth 2.5-3.5m very limited number of strains were isolated such as E. coli, Acinetobacter sp and Pseudomonas aeruginosa.

Table 3: Distribution of the bacterial strain in strata (layers/depth)

Bacterial strain	0-1.5m	1.5-2.5m	2.5-3.5
Pseudomonas spp	+	+	-
Bacillus cereus	+	+	-
Staphylococcus aureus	+		-
Streptococcus pneumoniae	+	+	-
E. coli	+	+	+
Pseudomonas aeruginosa	+	+	+
Acinetobacter sp.	-	-	+

FUNGI

CFU/g count: After identification of the bacteria strains soil sample were subjected to isolate the fungi from the soil and it was revealed that upper layer contains 8 fungal strains with average value of the 1.33. besides this second layer of the depth contains 6 CFU/g (average 1) and the last layer/depth contain only 4 cfu/g (average 0.66). Furthermore, sampling site 6 (N. OGDCL) contained greater quantity of the organism (n=5 and ave=1.66). lowest fungal strain was counted in the sampling Site1 (M.CPF) that is n=1 and ave=0.33. result is depicted in the table 4.

Table 4: Site wise distribution of the fungal strains

Site no	**Sampling site**	**Depth 0-1.5m**	**1.5-2.5m**	**2.5-3.5m**	**TOTAL**	**Average**
	Colony Forming Unit (CFU/g)					
1	**M.CPF**	1	0	0	1	0.333333
2	**M.OF**	1	0	2	3	1
3	**G.P**	1	0	1	2	0.666667
4	**SB.DS**	0	3	0	3	1
5	**A.WDS**	2	1	1	4	1.333333
6	**N.OGDCL**	3	2	0	5	1.666667
	Total	8	6	4	18	
	Average	1.333333	1	0.666667		

Identification through morphological characteristics: Identified fungal strains is listed in the table 4. and it was found that Aspergillums niger was found at least frequency (n=4; %=21.05). all other strains (Penicillium citrinum, Alternaria tenuissima and Aspergillus tubingensi), were in equal number and frequency that is n=5 and %= 26.3. figure 4 shows the percentage and frequency of each strain in the samples.

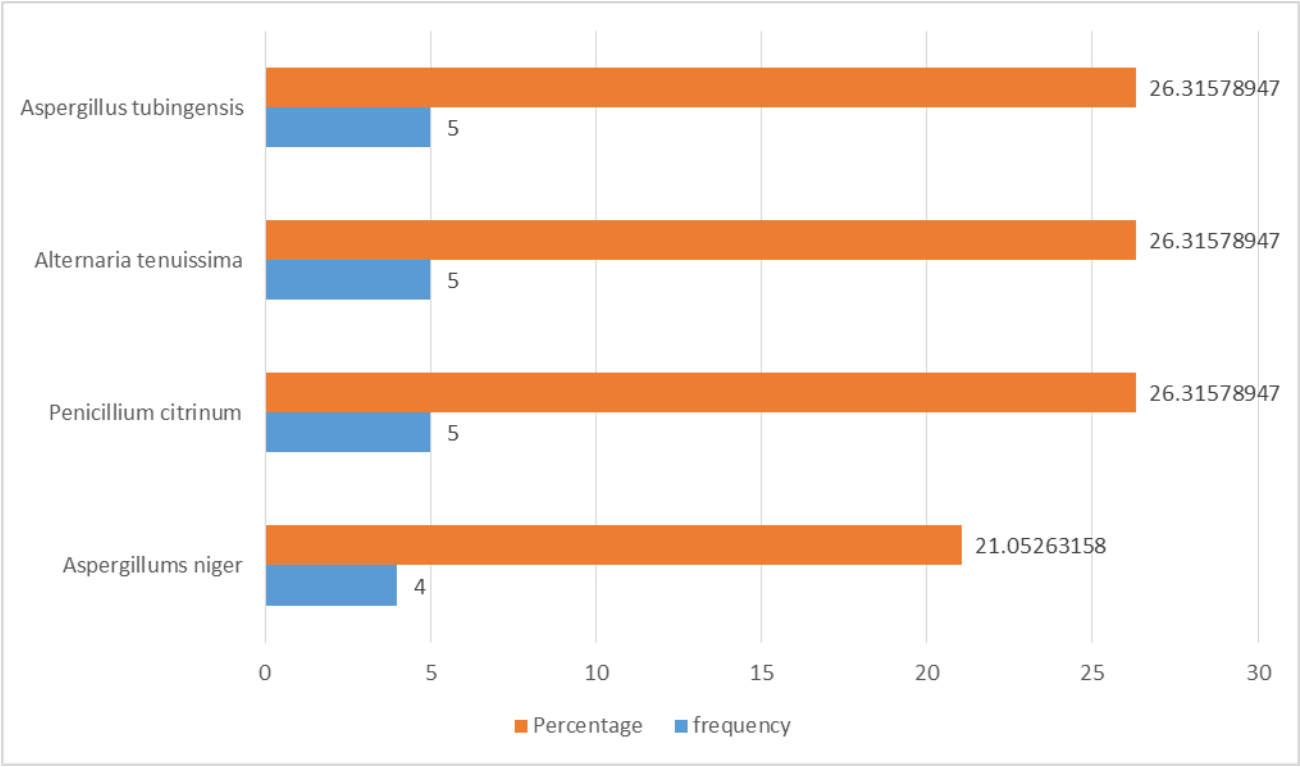

Figure 4: Percentage and frequency of the fungal strains identified

Distribution of the fungal species in strata (depth/layer): When distribution of the species were examined in the different strata of the soil than it was revealed that top (0-1.5m) and mid layer (1.5-2.5m) were inhibited with the same kind of species that is Aspergillums niger, Penicillium citrinum Alternaria tenuissima and Aspergillus tubingensis while last(2.5-3.5m) was inhibited by the Aspergillus tubingensis. Table 5 shows the detail of each strata.

Table 5: Fungal distribution in each strata (layer/depth)

Fungal strain	0-1.5m	1.5-2.5m	2.5-3.5
Aspergillums niger	+	+	-
Penicillium citrinum	+	+	-
Alternaria tenuissima	+	+	-
Aspergillus tubingensis	+	+	+

IV. DISCUSSION

Current study focused to evaluate different soil and soil depths (layers) sample from the Banda Dauood Shah district Karak contaminated with the oil from different sources, for the fungal and bacterial strains. After isolation and identification through different procedure it was revealed that for bacteria top layer 0-1.5m contain more bacteria count (mean value of 3.66) and less number of bacteria were recorded for the layer of 2.5-3.5m (mean value 0.83), besides this, sampling site no 3 (Gorgary Plant (G.P)) contain highest number of bacterial count (n=9 and average value 3). On the other hands lowest count was recorded for site no 1 (M.CPF) that is1.66. and after gram staining, microscopy and biochemical test it was found that majority of the isolated bacteria were gram negative 51.3% while gram positive bacteria were 48.97%. Besides this after microscopy it was found that most of the gram positive were cocci (n=12) while in gram negative bacillus was found at highest frequency (n=21). And on the basis of the chemical analysis it was found that Pseudomonas spp, Bacillus cereus, Staphylococcus aureus, E. coli, Pseudomonas aeruginosa and Acinetobacter sp it was revealed that overall dominated species was Pseudomonas spp (n=15; %=30), followed by the Bacillus cereus (n=12; %=24). Furthermore, it was found that top soil was inhibit by diverse number of the bacteria that is Pseudomonas spp, Bacillus cereus, Staphylococcus aureus, E. coli and Pseudomonas aeruginosa as compared to the lower layer. The only absent bacteria were Acinetobacter sp.

The last layer at depth 2.5-3.5m very limited number of strains were isolated such as E. coli, Acinetobacter sp and Pseudomonas aeruginosa. In term of fungal strain revealed that upper layer contains 8 fungal strains with average value of the 1.33. besides this second layer of the depth contains 6 CFU/g (average 1) and the last layer/depth contain only 4 cfu/g (average 0.66). furthermore, sampling site 6 (N. OGDCL) contained greater quantity of the organism (n=5 and ave=1.66). lowest fungal strain was counted in the sampling Site1 (M.CPF) that is n=1 and ave=0.33. and after examination of the morphometric keys it was found that Aspergillums niger, Penicillium citrinum, Alternaria tenuissima and Aspergillus tubingensi were isolated and Aspergillums niger was found at least frequency (n=4; %=21.05). all other strains (Penicillium citrinum, Alternaria tenuissima and Aspergillus tubingensi), were in equal number and frequency that is n=5 and %= 26.3. when distribution of the species were examined in the different strata of the soil than it was revealed that top (0-1.5m) and mid layer (1.5-2.5m) were inhibited with the same kind of species that is Aspergillums niger, Penicillium citrinum Alternaria tenuissima and Aspergillus tubingensis while last(2.5-3.5m) was inhibited by the Aspergillus tubingensis. same kind of the organism were also isolated from same environment by many other researchers such as Okerentugba and Ezeronye, (2003) isolate Streptococcus, Pseudomonas, Streptococcus Bacillus and Staphylococcus species. Jones et al. (2011) reported Burkholderia, Arthrobacter, Pseudomonas, Mycobacterium, Rhodococcus and Sphingomonas from the oil contaminated soil from various samples at Nigeria. Another study from the same country was reported by the Adebusoye et al, (2007) and report nine strains of the hydrocarbon degrading bacteria such as P. aeruginosa, Pseudomonas fluorescens, Bacillus sp., Alcaligenes sp., Bacillus subtilis, Flavobacterium sp., Acinetobacter lwoffi, Corynebacterium sp and Micrococcus roseus. similarly, Bartha and Bossert, (2007) reported 22 genera of bacteria and 31 genera of fungi from the soil sample contaminated with crude oil. Furthermore, in current study we found larger number and diverse microorganism at the top soil (0-1.5m), it is due to the presence of the large quantity of the humus at the surface and availability of the oxygen. While the lower sub layers were deprived of diversity and quantity of the organism is due to reverse condition of these layer as compared to upper layer. Very small number of microbes are isolated from the sublayer and those are mostly aerobes. Furthermore, identification and isolation of these organism from the soil, indicate strategic evolution which help them to adjust and adopt to the oil contaminated environment and are also capable of using these substances as source of energy. Among isolated bacteria presence of the Bacillus can be endorsed to their capacity of forming spores which enable them to survive in harsh environment such as oil pollutant environment (Ghazali et al., 2004; Stine and Weil, 2002; Cocks and Torsvik, 2002).

V. CONCLUSION

In current studies we found that upper layer contains rich and diverse variety of fungi and bacteria species and among bacteria most of the bacteria were gram negative. Dominated species of bacteria was Pseudomonas spp followed by the Bacillus cereus. Among fungi Aspergillums niger was found at least frequency. All other strains (Penicillium citrinum, Alternaria tenuissima and Aspergillus tubingensi), were in equal number. Future research should be carried out to identify these on molecular level and test these isolates as potential biodegrades.

REFERENCES

1. Eilers KG, Debenport S, Anderson S, Fierer N (2012) Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. Soil Biology and Biochemistry50: 58-65.
2. Hoorman JJ, Islam R (2010) Understanding soil microbes and nutrient recycling. Agricultural and natural resource 16(10).
3. Hardy J (1990) The rain making bacteria. ASCP, California, USA.
4. C. Holliger, S. Gaspard, G. Glod et al., "Contaminated environments in the subsurface and bioremediation: organic contaminants," FEMS Microbiology Reviews, vol. 20, no. 3-4, pp. 517–523, 2001.
5. P. J. J. Alvarez and T.M. Vogel, "Substrate interactions of benzene, toluene, and para-xylene during microbial degradation by pure cultures and mixed culture aquifer slurries," Applied and Environmental Microbiology, vol. 57, no. 10, pp. 2981– 2985, 2005.
6. Okerentugba, PO; Ezeronye, OU (2003). Petroleum degrading potentials of single and mixed
7. microbial cultures isolated from rivers and refinery effluent in Nigeria. African Journal of Biotechnology, 2: 288-292.
8. D. M. Jones, A. G. Douglas, R. J. Parkes, J. Taylor, W. Giger, and C. Schaffner, "The recognition of biodegraded petroleum-derived aromatic hydrocarbons in recent marine sediments," Marine

Pollution Bulletin, vol. 14, no. 3, pp. 103–108, 2011
9. S. A. Adebusoye, M. O. Ilori, O. O. Amund, O. D. Teniola, and S. O. Olatope, "Microbial degradation of petroleum hydrocarbons in a polluted tropical stream," World Journal of Microbiology and Biotechnology, vol. 23, no. 8, pp. 1149–1159, 2007.
10. R. Bartha and I. Bossert, "The treatment and disposal of petroleum wastes," in Petroleum Microbiology, R. M. Atlas, Ed., pp. 553–578, Macmillan, New York, NY, USA, 2007.
11. Ghazali, FM; Rahman, RNZA; Salleh, AB; Basri, M (2004). Degradation of hydrocarbons in soil by microbial consortium. Int. Biodeterioration and Biodegradation, 54: 061-067.
12. Stine MA, Weil RR (2002) The relationship between soil quality and crop productivity across three tillage systems in south central Honduras. American Journal of Alternative Agriculture17(1): 2-8.
13. Cocks LRM, Torsvik TH (2002) Earth Geography from 500 to 400 million years ago: Faunal and palaeomagnetic review. J Geol Soc Lond 159: 631-644.
14. Watson GW, Kelsey P (2006) The impact of soil compaction on soil aeration and fine root density of Quercus palustris. Urban Forestry & Urban Greening4(2): 69-74.
15. Mandal SN (2013) Brief Introduction to Soil. Department of Agriculture, Lalitpur, Nepal.
16. Collins H (2010) Impacts of Fumigation and Crop Rotation on soil Microbial Populations. USDA-ARS Irrigated Research Center, USA.
17. Magdoff F, ES VH (2010) Building Soils for Better Crops: Sutainable Soil Management. (3rd edn), Sustainable Agriculture Research and Education, USA, pp. 294.
18. Swer H, Dkhar MS, Kayang H (2011) Fungal population and diversity in orginically amended agricultural soils of meghalaya, India. Journal of Organic Systems6(2): 3-12.
19. Dastager SG, Deepa CK, Pandey A (2010) Isolation and characterization of novel plant growth promoting Micrococcus sp NII-0909 and its interaction with cowpea. Plant Physiol Biochem48(12): 987-992.