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Fungal diversity analysis in wastewater and agricultural soils irrigated with wastewater of Nullah Lai

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ABSTRACT

Peri-urban cultivated areas of many cities in Pakistan are being irrigated from municipal and industrial wastewater since long. Similarly wastewater of Nullah Lai is also being used for irrigation in Rawalpindi. Wastewater and soil samples were collected from six locations of Rawalpindi districts for investigating fungal diversity. Eight fungal species were found in these soils. The most common fungal strains were Aspergillus sp., Acremonium sp., and Chaetomium sp. To isolate the fungi in different soil samples, sterilization technique, serial dilution and spread plate technique were used. Presence of fungi was detected in the different soil samples on the bases of morphological characteristics, percentage frequency, growth rate and colony forming units. The results obtained showed that most widely distributed fungi in soil samples were Aspergillus sp. Aspergillus niger and Aspergillus flavus grew well in contaminated soil containing heavy metals. This ability of Aspergillus makes it attractive potential candidate for further investigation regarding its ability to remove metals from contaminated soil. The usage of untreated wastewater in agricultural land put harmful effects on soil physical, chemical properties and biodiversity. To recognize health risk due to wastewater usage for irrigation, treat the wastewater to the recommended level.

Introduction

Many countries worldwide are entering a period of severe water shortage increasing competition for water among urban centers, industry and irrigated agriculture together with rapidly growing population will put current agriculture and irrigation practices under severe pressure because irrigation is by far the largest uses of water (UNDP, 1998). The use of wastewater in irrigation of agricultural land is practiced throughout the world at different scales, and involves different degree of soil contamination (McGrath and Lane, 1989). The use of waste water instead of expensive fertilizers in agriculture is considered convenient solution in developing countries (Martin-Ortega, 2011). Soil health is correlated with the soil biota as they provided essential nutrients to the soil and plants by different processes (Kibblewhite et al., 2008).Various potentially toxic elements including heavy metals are present in wastewater and these toxic elements at elevated concentration are known to affect soil microbial populations and their associated activities. When wastewater is applied to soil, it reduces the permeability of soil. It affects the micro flora of soil (McGrath et al., 1988; Chaudhari et al., 1993).

Waste water and soil both are the rich habitats containing variety of microorganism, fungi is one of them (Mueller & Bills, 2004). Fungi provide many valuable services to the mankind and in the soil ecosystem and also are the cause of different types of diseases (Manoch, 1998; Sheppard et al., 2004). In addition, they produce chemicals with different odours and tastes in water (Kelly et al., 2003). The continuous irrigation of soils with wastewater results dramatic change in the soil nutritional status may favor certain fungal groups while hampering the growth of others. Specific groups of saprotrophic fungi like the white-rot fungi may exploit the availability of carbon sources present in wastewater and are favored by mineral-N scarcity. Some saprotrophic fungi belonging to important plant pathogens may be suppressed by soil conditions established by wastewater addition (Rousidou et al., 2010). The development of saprophytic fungi was significantly higher in the wastewater irrigated soils, whereas photosynthetic rates and the amount of the total root-soluble carbohydrate were decreased significantly after application of wastewater (Mechri et al., 2008). With the use of wastewater for irrigation, mycorrhizal fungi have reduced activity and some time its number also reduces.

The objective of the study is isolation and identification of fungi from the waste water and soil irrigated with waste water of Nullah Lai at different locations of Rawalpindi.

Materials and methods

Study area and collection of soil and wastewater samples

Nullah Lai is natural stream flowing through the city of Rawalpindi. 65% of the waste water of Rawalpindi is disposed off into open drainage that ultimately drain off into Nullah Lai which is the main natural drainage channel passing through the city. The Lai Nullah Basin has a catchment area of 234.8 km^2, extending basically to the twin cities of Islamabad and Rawalpindi. Nullah Lai starts from the Inter Junction Principal (IJP) Road in Islamabad at the administrative boundary between the twin cities of Rawalpindi and Islamabad. Apart from flow from Islamabad area, 11 main drains of Rawalpindi city also contributes to Nullah Lai. Wastewater of Nullah Lai is used for irrigation of agricultural areas in different near by sites of its flow in Rawalpindi (Islam-ul-haq et al., 2007).

Wastewater samples were taken from some depth of Nullah and were taken in plastic bottles. The samples were collected

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from the five points i.e. Mareer Hassan (MH), Sawan Stop (SS), Adiala Jail (AJ), I-9 Islamabad (I-9) and Ali Trust Islamabad (AT). Soil samples were taken from the depth of 0-10 cm and then were placed in plastic bags individually. The samples were collected from the six locations i.e. Nursery near Harley Street (NHS),Dhok Lakhn (DL), Misrieal Raod (MR), Dhok Hassu (DH I), Dhok Hassu (DH II) and Dhok Hassu (DH III).For isolation of fungi sterilization technique, serial dilution and spread plate technique were used.

Sterilization technique

Petri plates, media bottles, distilled water, syringes were sterilized in autoclave. For sterilization purpose all apparatus was autoclaved for 30 minutes at 121˚C. After autoclaving all sterilized material was dried in an oven at 90˚C.

Media preparation

Potato Dextrose Agar (PDA) media was used for fungal cultures growth (Razak et al., 1999). Two hundred grams of potato were peeled, sliced, boiled and then sieved through a clean Muslim cloth to get a broth in which agar and glucose were added. The media was then autoclaved for 30 minutes at 121˚C. To suppress the bacterial growth 0.5 ml/L streptomycin was added in the medium (Martin, 1950).

Dilution preparation

The purpose of serial dilution was to determine the occurrence and frequency of fungi. One ml of wastewater was taken from each sample. Serial dilution was set up by carefully taking the 10 ml of distilled water in McCartney bottles. Then these bottles were autoclaved for 30 minutes at 121˚C. From the sample of wastewater 1 ml was dissolved in 10 ml of sterile distilled water in McCartney bottle to give (1:10) and shaked well. The McCartney Bottle 2 was inoculated with 1 ml from bottle 1 to give 1:100 dilutions. McCartney Bottle 2 was also shaked well. McCartney Bottle 3 was inoculated with 1 ml from bottle 2 to give 1:1000 dilutions. McCartney Bottle 4 was inoculated with 1 ml from bottle 3 to give 1:10000 dilutions. Same dilution method was applied in the case of soil samples making dilutions upto 1:1000. To complete the serial dilution a micropipette was used with sterilized tips. Estimation of fungal population was done by standard spread plate dilution method described by Seeley and Van Denmark (1981) in triplicates.

Isolation of fungi

Spread plate technique was used for enumeration of fungi from given samples. From each McCartney bottle 0.5 ml of sample was taken separately with the help of micropipette along with sterilized blue tips. Then these diluted samples were inoculated on sterile PDA plates with the help of micropipette and L shape rod was used to spread the diluted sample on the PDA plate. Repeat the same step with all other wastewater and soil samples. Then these plates were incubated at 30°C for 7 days and then the colonies were counted (Adesemoye et al., 2006).

Identification of fungi

The cultures were identified at genus level on the basis of macroscopic (colonial morphology, color, texture, shape and appearance of morphology) and microscopic characteristics (septation in mycelium, presence of specific reproductive structures, shape and structure of conidia) (Zafar et al., 2006).

Results

The isolated fungi were represented by morphological characteristics, frequency percentage and on the bases of occurrence, growth and colony forming units.

Morphological characteristics of fungi

From the collected wastewater samples three major species of Aspergillus were isolated while eight species were isolated from the soil samples. Tables 1 shows the morphological characteristics and this indicates that Aspergillus niger is typically black and different from the other species of the Aspergillus. Aspergillus flavus is yellow green while Aspergillus fumigatus is green. While other five species Curvularia, Chaetomium, Acremonium, Ttrichoderma and Fusarium were different in color and morphological characteristics.

Occurrence of fungi in different sites

From the collected soil samples, occurrence of species in different samples is shown in Table 2. Table shows the presence (+) and absence (–) of fungi.

Total eight fungi were isolated from the six soil samples of different locations of agricultural areas of Rawalpindi irrigated by wastewater of Nullah Lai. Table indicates the presence of fungal strains that includes Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus and Acremonium and were more abundant in soil samples while Curvularia, Chaetomium, Trichoderma and Fusarium were lower in number in different soil samples.

Table shows that higher fungal diversity was found in Dhok Hassu II that had five different fungi. Misrieal Road and Dhok Hassu III had lower number of fungi in terms of diversity and contained only two kinds of fungi. The present study shows that Harley Street, Dhok Lakhn and Dhok Hassu I had three different kinds of fungi.

Table revealed that Aspergillus was found in all six soil samples while Curvularia was found in two soil samples. Chaetomium was found in three soil samples and Acremonium was found in four soil samples. Results shows that Trichoderma and Fusarium were found in only one soil sample and were absent in other five samples.

Table 3 shows the presence of fungal strains that includes Aspergillus niger, Aspergillus fumigatus and Aspergillus flavus and are the most common fungal species at different sites of collected wastewater samples.

Site 1: Aspergillus niger is isolated in all the samples, while Aspergillus flavus is isolated in sample 1 and 4 and Aspergillus fumigatus is isolated in sample 2 and 3.

Site 2: Aspergillus niger is isolated in all four samples. Aspergillus flavus is isolated in sample 2 and 3. Aspergillus fumigatus is isolated in sample 1 only.

Site 3: Aspergillus niger is isolated in all samples while Aspergillus flavus and Aspergillus fumigatus are not isolated at all.

Site 4: Aspergillus niger is isolated in all the four samples. Aspergillus flavus is isolated in the three samples. Aspergillus fumigatus is isolated in the sample 3 and 4.

Site 5: Aspergillus niger is isolated in all the four samples and Aspergillus flavus is isolated in the sample 2 and 3. Aspergillus fumigatus is identified in sample 3 and 4.

The present data shows that the occurrence of Aspergillus niger is high among the other two fungi.

Frequency percentage

Table 4 showing the number of cases of isolation and percentage frequency of fungal isolates from the collected soil samples. From all of six soil samples 413 colonies of fungi were isolated. The frequency of Aspergillus niger was high in all the locations and it was highest in Misriael Road that was 63%. Frequency of Aspergillus flavus was high in Dhok Hassu III that was 36% and

frequency of Aspergillus fumigatus was high in Dhok Hassu I and Dhok Hassu III. Precentage of Chaetomium was highest in Dhok Lakhn that was 24 %. Trichoderma was found only in Dhok Hassu II and percentage was 15. Percentage of Acremonium was highest in Dhok Hassu II that was 25 %. Curvularia was found in Misrieal Road and percentage was 13%. Fusarium was only found in Dhok Hassu III and percentage was 8 %.

The frequency percentage of Aspergillus niger is high in Adiala Jail site that is 100% while the frequency of Aspergillus flavus is high in site 2 that is Sawan Stop and the frequency is 18.46%. The frequency of Aspergillus fumigatus is high 47.22% in I-9 site (Table 5).

Growth of fungi

Table 6 shows the growth of fungi during 7 days from the wastewater samples of Nullah Lai (Islamabad and Rawalpindi). The growth of fungi was more after 24 hours and growth increased during the incubation of seven days. The growth was zero after 24 hours of incubation. The fungal growth was more after 48 hours of incubation. Table shows that the growth increased with the passage of the days. So the growth was higher after 178 hours of incubation.

Table 7 showing the growth of fungi during seven days in different samples of soil irrigated with wastewater of Nullah Lai. Growth of fungal species was increasing with the passage of days. Growth was zero after 24 hours of incubation. The fungal growth was more after 48 hours of incubation. Growth rate was highest after seven days of incubation.

Colony Forming Unit (CFU) of isolated fungi

Fig. 1 indicates the colony forming unit of isolated fungi from the collected soil of different sites irrigated with wastewater of Nullah Lai .The CFU of Dhok Lakhn was highest among all other sites. Dhok Lakhn was followed by Dhok Hassu I and Dhok Hassu II that had almost similar CFU and Nursery near Harley Street had lowest CFU.

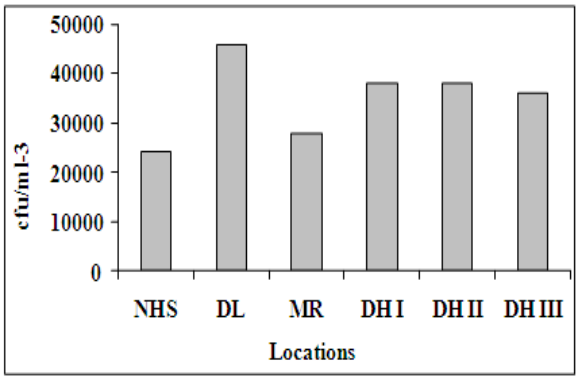


Fig. 1 Colony Forming Unit (CFU) of isolated fungi.

Fig. 2 showing the colony forming unit of isolated fungi from collected wastewater samples. The analysis of CFU was taken at third dilution. The CFU of I-9 sample was higher in all collected samples of wastewater followed by the samples of Adiala Jail, Ali trust, Sawan Stop and Mareer Hassan. The wastewater samples of the Mareer Hassan showed lower CFU.

Discussion

The wastewater of Nullah Lai (Islamabad and Rawalpindi) is badly affected by domestic waste, municipal waste, industrial effluents, raw sewage and garbage (Buckley and Schmidt, 2001; Fliessbach et al., 1994). The wastewater of Nullah Lai is often use as source of water for irrigation in nearby areas. This may affect the whole biological community including species diversity and accumulation of toxic elements in food chain (Bailkey and Nasr, 2000).

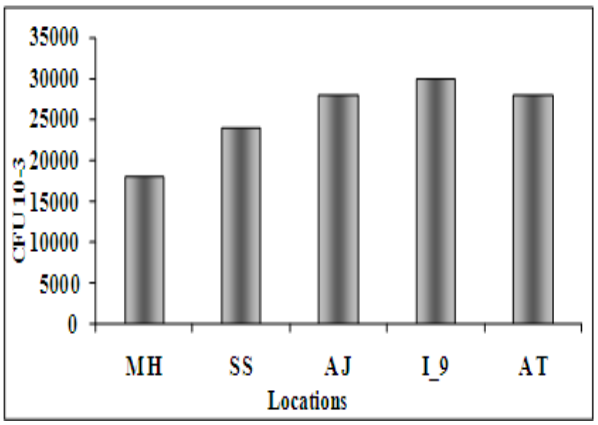


Fig. 2 Colony Forming Unit (CFU) of Isolated Fungal Species.

In present study, soil samples were collected from the agricultural areas that were receiving wastewater of Nullah Lai for several years. Different 8 species were isolated from soil samples. Some fungi appeared in all land uses while some appeared in few samples.

Different species isolated from soil were different in colors. Aspergillus species were mostly green to yellow while other species were from grey to white. Difference in color is due to the adaptation of fungi to different environmental stress.

Aspergillus niger, Aspergillus flavus, Chaetomium and Acremonium were dominant fungi in all soil samples. Metal resistance fungi (Aspergillus, Fusarium and Trichoderma) can be isolated from the agricultural soil irrigated with wastewater (Nazina et al., 2002).

Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus were dominant species in all wastewater samples. Wastewater contains many effluents and heavy metals. Aspergillus niger has ability to tolerate the heavy metals present in wastewater so its number is more in wastewater (Michael et al., 2000).

Some of the species isolated from wastewater are reported to be either well known agents of mycosis such as Aspergillus flavus and Aspergillus fumigatus was the second dominant specie in wastewater. This specie has been cited as one of the fungi which are present in wastewater of various areas of the world (Moallaei et al., 2006).

In the present study Total Viable Counts (TVC) indicates that wastewater of Nullah Lai and soil irrigated with with wastewater of Nullah Lai has the more fungal load after 48 hrs of incubation and the value was higher than the recommended value (Baxter-Potter and Gilliland, 1988). The mean total fungi were high for samples and in comparison and by international standard, any water contaminated to this level is neither good for domestic use nor is it supposed to be discharged directly into the environment without treatment (Makinde., et al 2006).

The soil samples collected from polluted sites were more affected by wastewater irrigation which affected the population densities of fungi. The difference between the sampled sites in terms of richness of fungal isolates is closely related to the heavy metal pollution present in wastewater. Generally, the pollution of heavy metals to soil and water may lead to a decrease in microbial diversity.

This is due to the extinction of species sensitive to the stress imposed and enhanced growth of other resistance species. As the Figure 3.1 shows that colony forming unit of site 2 (Dhok Lakhn) was higher than other 5 sites.

Table 1 Morphological characteristics of isolated fungi from the wastewater and the soil irrigated with wastewater of Nullah Lai

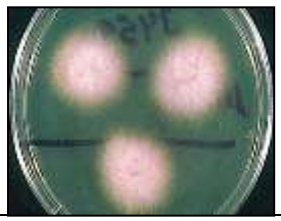
Species	Morphological Characteristics
Aspergillus niger 	Typically black powdery colony, conidia large, globose, irregularly roughened, uninucleate, mostly 4-5 mm diameter.
Aspergillus flavus 	Yellow green to brown colony, conidiophores hyaline, conodia globose, finely echinulate, and rough walled.
Aspergillus fumigatus 	Green colony, strictly columnar conidial heads, pigmented conidiophores, conidia globose to subglobose, echinulate
Chaetomium sp. 	Colony color is from white, grey to red and brown, hyphae are septate, hyaline to pale brown.
Acremonium sp. 	White, grey, pink, rose or orange in color, hyphae are fine and hyaline, globose to cylindrical.
Trichoderma sp. 	Mostly white color colony, conidiophore are yellow in color highly branched loosly or compactly tufted.
Fusarium sp. 	White, red and pink colour colony, hyaline septate hyphae.

Table 2. Occurrence of species in different soil samples of Rawalpindi.

Sites	Samples	Isolates of fungi					
		Aspergillus	*Curvularia*	*Chaetomium*	*Acremonium*	*Trichoderma*	*Fusarium*
NHS	Sample 1	+	–	–	+	–	–
	Sample 2	+	–	–	+	–	–
	Sample 3	+	–	+	–	–	–
DL	Sample 1	+	–	–	–	–	–
	Sample 2	+	–	+	–	–	–
	Sample 3	+	–	–	+	–	–
MR	Sample 1	+	+	–	–	–	–
	Sample 2	+	–	–	–	–	–
	Sample 3	+	–	–	–	–	–
DH I	Sample 1	+	–	–	–	–	–
	Sample 2	+	–	–	–	–	–
	Sample 3	+	+	+	–	–	–
DH II	Sample 1	+	–	+	–	–	–
	Sample 2	+	–	–	+	–	–
	Sample 3	–	–	–	+	+	+
DH III	Sample 1	+	–	–	+	–	–
	Sample 2	+	–	–	+	–	–
	Sample 3	+	–	–	+	–	–

Table 3 Occurrence of species in different water samples of Rawalpindi and Islamabad.

Sites	Samples	Isolates of Fungi		
		Aspergillus niger	*Aspergillus flavus*	*Aspergillus fumigatus*
Mareer Hassan	Sample 1	+	+	-
	Sample 2	+	-	+
	Sample 3	+	-	+
	Sample 4	+	+	-
Sawan Stop	Sample 1	+	-	+
	Sample 2	+	+	-
	Sample 3	+	+	-
	Sample 4	+	-	-
Adiala Jail	Sample 1	+	-	-
	Sample 2	+	-	-
	Sample 3	+	-	-
	Sample 4	+	-	-
I-9 (Islamabad)	Sample 1	+	+	-
	Sample 2	+	+	-
	Sample 3	+	+	+
	Sample 4	+	-	+
Ali Trust(Islamabad)	Sample 1	+	-	-
	Sample 2	+	+	-
	Sample 3	+	+	+
	Sample 4	+	-	+

Table 4. Frequency percentage of isolates fungi from the agricultural soils of Rawalpindi

	NHS		DL		MR		DH I		DH II		DH III		Total	
Species	NCI	%F	NCI	%F	NCI	%F	NCI	%F	NCI	%F	NCI	%F	NCI	%F
Aspergilus niger	47	61	42	49	33	63	24	34	20	33	28	40	194	46.9
Aspergilus flavus	8	10	10	11	10	19	36	5	11	18	25	36	100	24.2
Aspergilus fumigatus	2	2	4	4	2	3	4	5	0	0	4	5	16	3.4
Chaetomium sp.	2	2	21	24	0	0	2	2	0	0	0	0	25	6.4
Trichoderma sp.	0	0	0	0	0	0	0	0	9	15	0	0	9	2.2
Acremonium sp.	18	23	8	9	0	0	0	0	15	25	10	14	51	12.56
Cuvularia sp.	0	0	0	0	7	13	4	5	0	0	2	3	13	3.14
Fusarium sp.	0	0	0	0	0	0	0	0	0	0	5	8	5	1.2
Total	77	100	85	100	52	100	70	100	60	100	69	100	413	100

NCI No of cases of isolation (out of 6)
%F Percentage frequency of occurrence (calculated per 6 samples).

Table 5. The number of cases of isolation and frequency percentage of fungi from the collected wastewater samples.

	Marer Hassan		Sawn Stop		Adiala Jail		I-9 Islamabad		Ali Trust Islamabad	
Species	NCI	%F	NCI	%F	NCI	%F	NCI	%F	NCI	%F
Aspergillus niger	15	78.94	50	78.92	60	100	27	37.5	40	72.72
Aspergillus flavus	02	10.52	12	18.46	0	0	11	15.27	5	9.09
Aspergillus fumigatus	02	10.52	03	46.15	0	0	34	47.22	10	18.18

- NCI = Number of cases of isolation.
- %F = Percentage frequency of occurrence.

Table 6. Growth of fungi during incubation of seven days

	Fungal Incubation (Hours)						
Sites	24	48	72	96	130	154	178
Mareer Hassan	0	5	7	10	15	17	19
Sawan Stop	0	10	20	35	42	57	65
Adiala Jail	0	13	19	29	45	54	60
I-9	0	17	32	41	54	66	72
Ali Trust	0	9	15	29	37	50	55

Table 7. Growth of fungi in seven days from the soil samples of Rawalpindi.

Sites	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
NHS	0	15	40	50	63	70	77
DL	0	19	27	40	60	75	85
MR	0	7	20	26	38	48	52
DH I	0	6	26	33	49	65	70
DH II	0	9	21	38	46	53	60
DH III	0	11	24	30	51	60	69

The present data indicated that wastewater samples collected from the Nullah Lai (Islamabad and Rawalpindi) seems to have different population diversity of fungi. This was shown by the difference among the CFU of isolated fungi in different locations of Nullah Lai. According to Cooke (1997), water pollution affects the fungal diversity; it tends to reduce the diversity of fungal species while increasing the number of those that are more less sensitive (Tan and Lim, 1984).

Conclusion

The present study has built initial knowledge on fungal diversity in polluted water and soil environments. At the end, it can be concluded that the usage of untreated wastewater in agricultural land put harmful effects on soil physical, chemical properties and biodiversity. For the safety of environment particular care should be adopted. To treat the wastewater to the level recommended. From the irrigation point of view, apply crop restrictions which can be the most effective measures to protect he consumers and to promote safe crop production areas supported by monitoring and control.

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