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Temporal assessment of microbial growth in drainage system of Nullah Lai

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ABSTRACT

This study based on physical and microbial analyses of wastewater samples collected from Nullah Lai, Islamabad and Rawalpindi. Total viable count was done on sterilized media by spread plate technique. In physical analyses of wastewater, it was found that pH was neutral while EC and turbidity of the wastewater samples were greater than the WHO standards for the irrigation purpose. The total viable counts for all the wastewater samples exceeded the standard limit of 1.0x 10^2 cfu/ml. Three bacterial and four fungal species were detected from the wastewater samples. Preliminary information about microbial diversity in wastewater was attained through this work that is useful in future for wastewater treatment in reference to bioremediation.

Introduction

Pakistan is an agricultural country having the world's largest canal irrigation system. Owing to rapid increase in population and uncertain environmental conditions, this water is not adequate to cope with the crops water requirement and needs additional means to provide extra water for agricultural purposes. To fulfill the present demand, use of municipal sewage water, domestic liquid waste and industrial effluents are becoming a common practice (Butt et al., 2005). However the use of waste water in irrigation involves risks and negative impacts of great importance such as health risks for irrigators due to contact with contaminated water, health risks for the consumers due to contaminants incorporated in products, deterioration of surface and ground water due to non-controlled water application, deterioration of soil quality due to buildup of chemical pollutants and development of habitats for disease vectors such as mosquitoes and flies (WHO, 1989).

From the health point of view, the contaminants of greatest concern are the pathogenic micro and macro organisms. Pathogenic viruses, bacteria, fungus, protozoa and helminthes may be present in the wastewater and survive in the environment for long periods (Mara and Cairncross, 1989). Water has been traced to be one of the ways by which humans could be infected with various diseases include typhoid fever, cholera and bacillary dysentery (Tortora et al., 2002).

Developing countries are nowadays facing freshwater quality degradation by wastewater introduction through poor drainage system into the environment. The quality of water has deteriorated because of the inadequacy of treatment plants, improper drainage system and direct discharge of untreated sewage into rivers and inefficient management of the piped water distribution system (UNEP, 2001). Wastewater management at all stages of handling is inadequate in rural areas. Poorly maintained or non-existent drainage systems and runoff from animal wastes that pollute aquifers, poorly maintained greenhouses, and introduction of increasingly industrial effluents in to the wastewater. Untreated sewage in rural areas often flows freely into streets, agricultural fields, and wades, directly contaminating food and water, and directly contributing to a critical community and environmental health crisis. An increasing proportion of surface and ground water resources in the region are being polluted mainly due to inappropriate disposal of municipal wastewater and infiltration from poorly constructed and maintained on-site sanitation or drainage facilities (UNEP, 1999).

The water quality has been deteriorated due to indiscrimination over extraction of ground water complemented with polluted recharge sources like Nullah lai and other wastewater streams. Central part of the country has both chemical and microbial contamination. The major cause of bacterial contamination of ground water is the Nullah Lai which carries 120 MGD wastewater of twin cities of Islamabad and Rawalpindi. Risks increase where water supply, drainage system and sanitation coverage is smaller since contamination risks increases. So, it is crucial that sanitation systems have high levels of hygienic standards to prevent the spread of disease.

In major cities of Pakistan, use of wastewater for agriculture is common. As there is no proper planning for the drainage or disposed off wastewater so wastewater directly enters into the water bodies. Wastewater is often the only source of water for irrigation in these areas. Farmers of such cities use sewerage water because fresh water from natural resources is not easily available (Bailkey and Nasr, 2000). The use of wastewater for irrigation may affect the whole biological community including species diversity and accumulation of toxic contaminants in food chain. Aim of the study is to examine the extent of contamination in untreated wastewater of Nullah Lai as well as to know the diversity of microbes especially bacteria and fungi and at which unit (CFU) they are present in the wastewater of Nullah Lai (Islamabad and Rawalpindi).

Materials and Methods

Collection of samples

The wastewater samples were collected from the surface of Nullah Lai, Islamabad and Rawalpindi at four different sites (I-9 (Islamabad, Pindora Chungi, Rawalpindi (PCR), Gawal Mandi, Rawalpindi (GMR) and Ammar Chowk, Rawalpindi (ACR) (Fig. 1). Collected samples (three from each site) were poured

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into sterile polypropylene bottles and brought in laboratory for physical and microbial investigation.

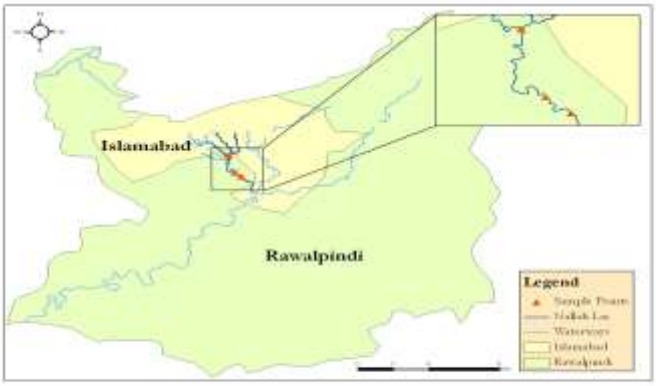

Fig. 1 Wastewater sampling sites of Nullah Lai.

Physical analyses

For the determination of physical characteristics of wastewater samples, color and odor was observed and then pH was measured by using pH meter, Electrical Conductivity (EC) was determined with the help of EC meter and turbidity was assessed by using turbidity meter.

Dilution preparation

Suspension of 1 ml of wastewater was mixed in 10 ml of sterile distilled water, serially diluted (three-folds) and exploited for the estimation of bacterial and fungal populations by standard spread plate dilution method described by Seeley and Vandemark. (1981).

Isolation of microbes

Media used for the microbiological analyses of wastewater were Potato Dextrose Agar (PDA) for fungal and Nutrient Agar (NA) for bacterial strains. A 0.5ml of an appropriate dilution was inoculated on sterile media plates. Plates were incubated at 30^{o}Cfor 24 h and 72 h respectively and then counted the colonies (Adesemoye et al., 2006). To obtain the pure cultures of bacteria, isolates from the mother plates were streaked on a freshly prepared nutrient agar plates by using sterile wire loop. Plates were inverted and incubated at 30°C for 24 h.

Identification of microbes

The fungal cultures were identified based on macroscopic (colonial morphology, color, texture, shape and appearance of colony) and microscopic characteristics (septation in mycelium, presence of specific reproductive structures, shape and structure of conidia) while identification of bacterial isolates was based on various morphological and biochemical characterization.

Results

Physical analyses of wastewater samples

The pH of samples was ranged in 7-7.55 at 25^{o}C. The PCR has the lowest pH value of 7 while I- 9 has the highest pH value of 7.55. The pH values of rest of the sampling sites were almost similar and close to each other. The observed EC of ACR has the lowest EC 960 us/cm at 29.6^{o}C whereas, PCR has the highest EC 1366 us/cm at 28.5^{o}C. The EC of remaining sites were almost close to each other. The observed turbidity of I-9 has the lowest value (1.9 NTU) and PCR has the highest turbidity value (608 NTU).

Assessment of microbial growth

Total 3 fungal species (*Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus)* and 5 bacterial types were isolated from the wastewater samples. Occurrence of fungal and bacterial species (+ for presence and – for absence) is shown in table 2 and 3.

Colony forming unit of isolated fungi

The CFU of *Aspergillus niger* of PCR and ACR was similar and higher with comparison to other sites. CFU of *Aspergillus flavus* of GMR and ACR was same and lowest while site GMR has maximum CFU and the CFU of *Aspergillus fumigatus* of I-9 and PCR was similar while gradual increase in GMR and ACR (Fig. 2).

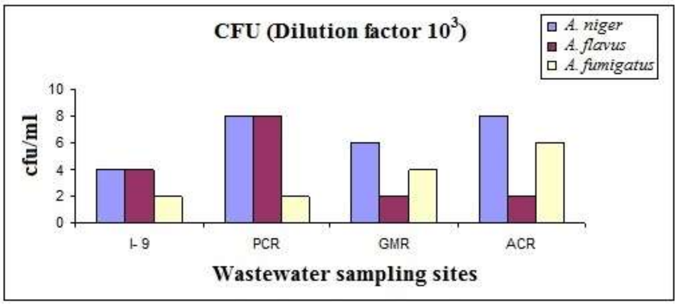

Fig. 2 CFU of fungi isolated from the wastewater samples.

Colony forming unit of isolated bacteria

After 24 and 48 h of incubation, PCR has highest CFU value while ACR has the lowest CFU (Fig. 3).

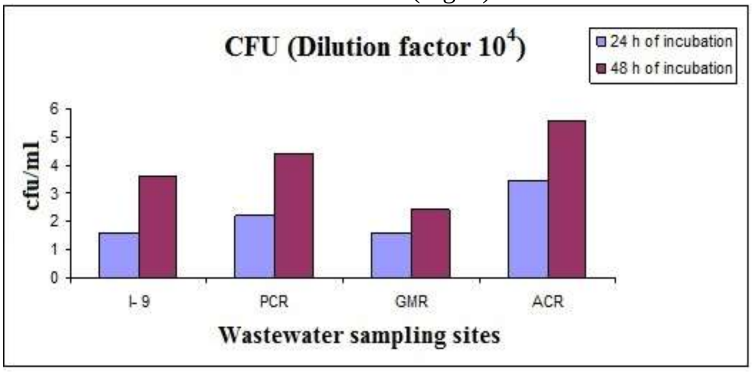

Fig. 3 CFU of bacteria isolated from the wastewater samples.

Discussion

Physical characteristics of the freshly collected water samples served as a starting point for such studies. The wastewater samples have very offensive odor. The pH (at 25^{o}C) of wastewater samples of all sites was neutral (7.0-7.55). According to Medera et al. (1982), the pH of most natural waters range in 6.5-8.5 while the deviation from the neutral (7.0) is as a result of the CO_2, bicarbonate and carbonate equilibrium and plays a part in determining both the qualitative and quantitative abundance of micro flora. Generally low pH values obtained in the river water might be due to the high levels of free CO_2 in the water samples which may consequently affect the bacterial counts. This was also reported by Edema et al. (2001). Fluctuations in optimum pH ranges may lead to an increase or decrease in the toxicity of poisons in water bodies. The pH values recorded for all the sampling sites were within the WHO pH tolerance limit of 6-9. Electrical conductivity of water is a useful and easy indicator of its salinity or total salt content. Wastewater effluents often contain high amounts of dissolved salts from domestic sewage. High salt concentrations in waste effluents however, can increase the salinity of the receiving water which may result in adverse ecological effects on aquatic biota (Ademoroti, 1996). The EC of the collected wastewater samples ranged in 690- 1366 us/cm that was higher than the WHO guideline values of 1000µs/cm with the exception of only one site. According to Akan et al. (2008), the mean conductivity values for all the samples were higher, ranges from 1021-1534 µs/cm.

Table 1. Physical characteristics of wastewater samples

Sites	Samples	Color	Odour	pH	EC (us/cm)	Turbidity (NTU)
	Sample1	Pale yellow	Pungent	7.35	1020	17.5
I-9	Sample2	Pale yellow	Pungent	7.55	1097	1.9
	Sample3	Pale yellow	Pungent	7.41	1205	72
	Sample1	Pale yellow	Pungent	7.0	1366	14.1
PCR	Sample2	Pale yellow	Pungent	7.21	1202	608
	Sample3	Pale yellow	Pungent	7.49	1029	121
	Sample1	Pale yellow	Pungent	7.12	1134	45
GMR	Sample2	Yellowish black	Pungent	7.44	1076	167
	Sample3	Pale yellow	Pungent	7.48	1018	42
	Sample1	Pale yellow	Pungent	7.28	1111	47
ACR	Sample2	Yellowish black	Pungent	7.53	1198	12.9
	Sample3	Pale yellow	Pungent	7.44	960	29

Table 2. Occurrence of fungi in wastewater samples

Site	Sample	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus fumigatus*
I- 9	Sample 1	+	+	+
	Sample 2	+	-	+
	Sample 3	+	-	+
PCR	Sample 1	+	+	+
	Sample 2	+	-	+
	Sample 3	+	-	+
GMR	Sample 1	+	+	+
	Sample 2	+	-	+
	Sample 3	+	-	+
ACR	Sample 1	+	+	+
	Sample 2	+	-	+
	Sample 3	+	-	+

Table 3. Occurrence of bacteria in wastewater samples

Site	Sample	*Gram +ve Cocci (flat)*	*Gram +ve Cocci (Raised)*	*Gram +ve Bacillus*	*Gram +ve Staphylococcus*	*Gram +ve Streptobacillus*
I- 9	Sample 1	+	+	+	+	-
	Sample 2	+	-	+	-	+
	Sample 3	-	+	-	+	-
PCR	Sample 1	+	+	-	+	-
	Sample 2	+	+	-	-	+
	Sample 3	+	-	-	+	-
GMR	Sample 1	+	-	+	+	+
	Sample 2	+	+	+	-	-
	Sample 3	+	+	-	+	-
ACR	Sample 1	+	+	-	+	+
	Sample 2	-	+	-	-	+
	Sample 3	+	-	+	-	-

Table 4. Biochemical characterization of bacterial isolates.

Test	*Cocci*(flat)	*Cocci*(raised)	*Bacillus* sp.	*Staphylococcus*	*Streptobacillus*
Starch hydrolysis	-	-	-	-	-
Gelatin liquefaction	+	-	-	-	-

Table 5. Response of bacteria towards differential media

Isolate ID	Test	Result	Interpretation
NA	Mannitol salt agar	+	*Staphlococcus aureus*

The turbidity of the wastewater samples ranged in1.9-608 NTU which were higher than WHO standard of 5 NTU with the exception of one site only. Turbidity was observed to increase if the color of the water changes from white to light-yellow, reddish or grayish and from there to greenish or brown (Omezuruike et al., 2008). According to one the study, the mean turbidity values of the wastewater ranging in 33- 42 NTU which was higher than the WHO guidelines (Akan et al. 2008).

The total viable count (TVC) indicates that the wastewater of Nullah Lai has the highest microbial load after 24 and 48 h of incubation having a value of 1.48 x 10^5 cfu/ml and 8.71x 10^5 cfu/ml respectively which was higher than the recommended value of 1.0x 10^2 cfu/ml for water. The TVCs for all the wastewater samples were generally high. According to Omezuruike et al. (2008), the TVCs for all the water samples were high having a value of 1.8 x10^4 cfu/ml after 24 h and 2.6 x10^4 cfu/ml after 48 h of incubation. Illegal dumping of domestic wastes, livestock management, fecal deposit and waste dumps also affect the bacterial concentration in run-off.

According to Adesemoye et al. (2006), the mean bacterial population counted from the wastewater samples was 3.32x 10^7 cfu/ml while the fungal population counted from the wastewater samples was 1.60x 10^5 cfu/ml and the mean microbial population of the wastewater contaminated soil samples was 3.36x 10^7 cfu/g, which was statistically greater than the 1.74x10^6 cfu/g counted from the uncontaminated soil. So in all the above reported studies the TVCs for all the wastewater samples and soil were high than the standard limit. Environmental stresses brought about by the contamination could be adduced for the reduction in microbial species diversity but increasing the population of few surviving species. Previous reports have proposed extensive microbial diversity with population estimated approximately 4x 10^3 to 10^4 species per gram of uncontaminated soil (Borneman et al., 1996). The isolated bacterial species were identified to be same with those commonly encountered in water and aquatic environments as reported in a study on streams surface water in U.S.A (Clark and Norris, 2000).

Current study is the preliminary findings of fungal and bacterial diversity in polluted water. High microbial load in wastewater causes negative effects on soil health and agriculture. So it is necessary to treat the wastewater to the level recommended before use for irrigation purpose.

References

Ademoroti, C. M. A. 1996. Standard method for water and effluent analysis. March prints and Consultancy. Foludex Ltd. Ibadan. 182.

Adesemoye, A. O., B. O. Opere and S. C. Makinde. 2006. Microbial content of abattoir wastewater and its contaminated soil in Lagos, Nigeria .*Afri. J. of Biotechnol.* **5** (20): 1963-1968.

Akan, J. C., F. I. Abdulrahman, G. A. Dimari and V. O. Ogugbuaja. 2008. Physicochemical Determination of Pollutants in Wastewater and Vegetable Samples along the Jakara Wastewater Channelin Kano Metropolis, Kano State, Nigeria. *Eurp. J. of Sci. Reser*. **23** (1): 122-133.

Bailkey, M. and J. Nasr. 2002. From Brownfields to Greenfields: Producing Food in North American Cities. Community Food Security News. Fall 1999/ Winter 2000: 6.

Borneman, J., P. W. Skroch, K. M. Sullivan, J. A. Palus, N. G. Rumjanek, J. I. Jansen, J. Neinhuis and E. W. Triplett. 1996. Molecular microbial diversity of an agricultural soil in Wiscosin. *Appl. Environ. Microbiol*. **62:** 1935-1943.

Butt, M. S., K. Sharif, E. Baber and A. Aziz. 2005. Management of Environmental quality: *International journal*. **16:** 338-346.

Clark, M. L. and Norris, J. R. 2000. Occurrence of fecal coliform bacteria in selected streams in Wyoming, 1990-99 USGS. Water resources investigations report 00-4198. Cheyenne. Wyoming.

Edema, M. O., A. M. Omemu and O. M. Fapetu. 2001. Microbiology and Physicochemical Analysis of different sources of drinking water in Adeokuta. Nigeria. *Nigeria. J. Microbial*. **15** (1): 57-61.

Mara, D. and S. Craincross. 1989. Guidelines for the safe use of Wastewater and Excreta in Agriculture and Aquaculture. WHO and UNEP. Geneva.

Medera, V., H. E. Allen and R. C. Minear. 1982. Non metallic constituents; Examination of Water Pollution Control. A reference handbook Physical, Chem., Radiol. Exam. **2:** 169-357.

Omezuruike, O. I., A. O. Damilola, O. T. Adeola, A. Enobong and S. B. Olufunke. 2008. Microbiology and physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos State, Nigeria. *Afri. J. of Biotechnol*. **7**(5): 617-621.

Seeley, H. W. and P. J. Vandemark. 1981. Microbes in Action. A laboratory manual of Microbiology. 3rd Ed. W.H Freeman and Company U.S.A. pp. 350.

Tortora, J. G., R. B. Funke and L. C. Case. 2002. Microbiology An introduction. Media update of 7 Edn. Including bibliography and index publisher. *Daryl Fox.* pp. 258-260.

UNEP. 1999. Global environmental outlook 2000. (London, Earthscan).

UNEP. 2001. State of the Environment Nepal**.** United Nations Environment Programs (UNEP).

WHO. 1989. Health Guidelines for the use of Wastewater in Agriculture and Aquaculture. WHO. Geneva.