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THE OCCURRENCE AND DISTRIBUTION OF FUNGI IN A WASTE STABILIZATION POND SYSTEM

YASMEEN AKHTAR AND A. GHAFFAR

Department of Botany,
University of Karachi, Karachi-32, Pakistan.

Abstract

The occurrence, distribution and relative abundance of fungi in a small waste stabilization pond system at the Karachi University Campus, Karachi, Pakistan, was determined using dilution plate technique. Forty-six species of filamentous fungi were isolated from various sampling points located throughout the system. Among isolates, *Aspergillus flavus* and *A. niger* were the most prominent with isolation frequencies of 80% and 74% respectively. Other commonly occuring fungi were *A. terreus* (42%), *Cladosporium cladosporioides* (41%), *Fusarium solani* (49%), *Penicillium jenseni* (36%) and *P. canescens* (29%). These predominant species were isolated from all sampling points of the treatment system.

Introduction

Waste stablization ponds are frequently chosen and used for the treatment of raw waste waters because of their low initial cost, simple design, ease of operation and efficient performance (Aguirre & Gloyna, 1970). They are classified into different types according to design, depth, population diversity, and the type of metabolism (aerobic, anaerobic or both) performed by the organisms present. The stabilization ponds, a biological method for waste water treatment, provide the habitat for populations of a wide variety of organisms including mainly algae, bacteria and fungi (Cooke, 1970a, 1971).

Earlier studies have shown fungi to be abundantly present in various sewage treatment systems. In case of trickling filter type treatment plant Cooke & Hirsch (1958) and Cooke (1970b) reported fungi as important members of the system. In the activated sludge process fungi have been found effective in the biological treatment of wastes by Cooke & Pipes (1970). The presence of fungi in the waste – stabilization pond system have also been reported by Cooke & Matsuura (1969) and Crranco *et. al.*, (1984). Most of the fungi of stabilization ponds are saprobic and only a few of them may act as predactors of other organisms.

Relatively little information is available on the presence and distribution of fungi in polluted waters and sewage treatment systems in Pakistan. The only studies of this nature were conducted by Ahmed (1979) and Bhatti *et. al.*, (1979). Such information is essential for determining and understanding the role of fungi in waste – stabilization pond systems. The present report deals with the fungal populations of a waste – stabilization pond system located at the Karachi University Campus (KUC), Karachi, and provides information of their distribution, seasonal variation and relative abundance.

Material and Methods

Collection of sample: The general layout and feeding schedule of the oxidation pond treatment system studied has been described in detail by Zain & Altaf (1980). The samples of raw waste water were collected at the time of pumping the raw waste water from the influent sump into the service tank. The samples from individual ponds were collected from the surface at the out let of each pond at fortnightly interval from March to December, 1980. In January and February, 1980 only one sample of raw waste water was available as the ponds were under construction. In the month of June the effluent samples of pond number 3 and pond number 4 were also not available due to cleaning of the ponds. At each sampling point, surface samples were collected in sterilized plastic bottles (ca 200 ml) between 9.00 – 10.00 A.M. Immediately after collection the temperature of the samples were taken and pH was determined in the laboratory using a pH meter.

Mycological Analysis: The techniques used for the isolation and quantification of fungi have been described by Cooke (1963). Each sample was diluted according to the prescribed procedure and plated in replicates of four with Czapek Dox agar, pH, 5.5 (Raper & Thom, 1949), supplemented with 2.5 ml each of Pencillin and Streptomycin (1 g of antibiotic was dissolved in 150 ml of sterile distilled water) to supress bacterial growth. The plates were incubated at 25°C and after 7 days all developing colonies were counted and picked for further identifications on Czapek Dox agar slants. The counts were expressed as per gran dry wt of the samples. Colonies of fungi were identified after refrence to Thom & Raper (1945), Raper & Thom (1949), Gilman (1957), Cooke (1963), Rifai (1969) and Ellis (1971).

Results

A summary of number of fungi isolated from raw and treated waste waters throughout the study period is presented in Table 1. The average colony counts ($\log_{10}$/g dry wt) ranged from 5.95 to 4.83 indicating a declining trend of fungal populations in raw sewage and oxidation pond waters. Additionally, a significant reduction of pond effluents from that of raw waste water was observed during the sampling period (Table 1).

The distribution and relative abundance of various species of fungi by sampling points during the study period was analyzed (Table 2). A total of 46 species of fungi belonging to 20 genera were isolated from all samples. Of these 35 species were isolated

Table 1. Number of fungi isolated from the waste – stabulization pond system during January to December, 1980.

Dates	No. of Colonies (log_{10}/gm dry wt.)				
	Rs.	P-1	P-2	P-3	P-4
Jan. 22 1980	5.419	–	–	–	–
Feb. 19 1980	5.488	–	–	–	–
Mar. 08 1980	5.789	–	–	5.134	5.055
Mar. 15 1980	5.802	–	–	5.109	4.692
Apr. 02 1980	6.040	–	–	5.221	5.578
Apr. 16 1980	6.105	–	–	4.074	4.532
May. 05 1980	5.789	–	–	4.692	4.356
May. 19 1980	5.958	6.410	–	5.302	4.626
Jun. 02 1980	6.146	6.302	–	–	–
Jun. 16 1980	6.730	5.476	5.507	–	–
Jul. 02 1980	5.775	5.363	5.363	5.607	5.423
Jul. 16 1980	6.356	6.356	4.419	4.430	4.481
Aug. 02 1980	6.147	4.875	4.875	4.715	5.009
Aug. 23 1980	6.341	4.754	4.857	4.782	4.619
Sep. 02 1980	5.664	4.724	4.283	4.833	4.641
Sep. 23 1980	5.655	4.946	4.606	5.134	4.769
Oct. 07 1980	–	4.657	4.993	4.761	4.724
Oct. 14 1980	5.789	4.802	4.397	5.109	4.514
Nov. 01 1980	5.692	4.833	4.852	4.715	4.588
Nov. 25 1980	6.678	5.512	5.441	5.134	5.420
Dec. 02 1980	6.122	4.879	4.879	5.009	4.879
Dec. 16 1980	5.375	4.731	4.539	4.585	5.069
$\overline{X}$	5.945	5.228	4.847	4.908	4.831
S.E.	0.364	0.624	0.385	0.347	0.345
CV%	6.122	11.935	7.943	7.070	7.327
Reduction%	–	11.84	18.47	17.45	18.73

Rs: Raw Sewage; P-1: Oxidation Pond No. 1, (Bifiliated); P-2, Oxidation Pond No. 2, (Bifiliated); P-3, Oxidation Pond No. 3, P-4, Oxidation Pond No. 4.

from raw sewage; 24 from effluents of oxidation pond 1; 22 from pond 2 effluents, 26 species were isolated from pond 3 effluents and 32 species from pond 4 effuents.

The following species, with percent isolation freguency of the total samples, were most prominent: *Asperzillus flavus* 74%; *A. niger* 80%; *A. terreus* 42%; *Cladosporium cladosporioides* 41%; *Fusarium solani* 49%; *Penicillium jenseni* 36% and *P. canescens*

Table 2. Incidence of species of fungi isolated from the oxidation pond system at Karachi University Campus during January to December, 1980.

Fungi	Sampling points* RS	P-1	P-2	P-3	P-4	Isolation Frequency
	No. of times appearing out of total samples of:					
	21	15	13	18	18	%
Aspergillus niger van Tieghem	15	10	10	14	14	74
A. flavus Link	16	11	11	16	14	80
A. terreus Thom	5	9	8	9	5	42.3
A. sydowi (Bainier & Sartory) Thom & Church	3	2	3	2	3	15.2
A. candidus Link	–	1	–	–	–	1.2
A. hennebergi Blochwitz	1	–	1	–	1	3.5
A. tamarii Kita	2	–	–	1	–	3.5
A. sulphureus Fresenius	–	–	–	–	1	1.2
A. flavipes (Bainier and Sartory) Thom & Church	–	1	–	1	1	3.5
A. fumigatus Fresenius	3	4	3	2	2	16.4
A. caespitosus Raper and Thom	–	–	1	–	1	2.3
A. luchuensis Imai	–	1	–	1	–	2.3
A. unguis (Emile-Weil and Gaudin) Emend. Thom & Raper	1	1	1	3	3	10.5
A. panamensis Raper & Thom	–	–	–	–	1	1.2
A. nidulans (Eidam) Wint	–	–	–	–	1	1.2
A. sp.	–	–	–	–	1	1.2
Alternaria tenuis Nees	3	1	1	1	1	8.2
Cladosporium cladosporioides (Fresenius) de Vries	8	8	4	11	4	41
Cheatomium brasiliene Battista and Potual	1	–	–	–	–	1.2
Curvularia tuberculata Jain	1	–	–	–	–	1.2
Drechslera hawaiiensis (Bugnicourt) Subram. & Jain ex M.B. Ellis;	3	3	1	4	3	16.4
Fusarium solani (Martius) Appel and Wollenweber	11	9	7	6	9	49.4
F. lateritium var. *buxi.*	2	1	2	3	2	11.7
Mucor sp.	2	3	2	3	3	15.2
Neurospora crassa	–	1	–	–	–	1.2

Paecilomyces marquandii (Massee) Hughes	5	2	3	4	4	21.1
Phialophora sp	—	—	—	1	2	3.5
Phoma sp.	2	—	—	—	1	3.5
Penicillium jenseni Zaleski	9	3	6	5	8	36.5
P. canescens Sopp.	5	3	2	6	9	29.4
P. lilacinum Thom	2	2	1	—	1	7.0
P. purpurogenum Stoll	2	1	2	1	1	8.2
P. janthinellum Biourge	4	2	1	4	2	15.2
P. funiculosum Thom	1	1	1	1	1	5.8
P. fellutanum Biourge	1	1	1	1	1	5.8
Rhizopus nigricans Ehrenberg	1	—	—	1	1	3.5
Stysanus stemonitis (Persoon) Corda	1	—	—	—	—	1.2
Stibella bulbicola Hennings.	1	—	—	—	—	1.2
Stachybotrys atra Corda	1	—	—	—	—	1.2
Sepedonicum sp.	2	—	—	—	—	2.3
Syncephalastrum racemosum (Cohn) Schroeter	—	—	1	1	1	3.5
Trichoderma harzianum Rifai	5	4	2	1	5	20.0
Trichoderma sp.	1	—	—	1	1	3.5
White coloured yeast	1	—	—	—	—	1.2
Green Coloured yeast	1	—	—	—	—	1.2
White filamentous non-sporing colony	—	3	—	—	—	3.5

Rs: Raw Sewage; P-1: Oxidation Pond No. 1, (Bifiliated); P-2, Oxidation Pond No. 2, (Bifiliated); P-3, Oxidation Pond No. 3, P-4, Oxidation Pond No. 4.

29%. These most frequently isolated species of fungi were present throughout the treatment system at all sampling stations. Species of several interesting genera were isolated only once or twice and specifically from raw waste waters only. These included *Chaetonium, Curvularia, Stysanus, Stibella, Stachybotrys* and *Sepedonicum.*

The most frequently isolated species of fungi which appeared as a leading dominant (recovered in largest numbers during each sampling) at each sampling point of the oxidation pond treatment system are listed (Table 3). Among these only species of *Aspergillus* appeared as leading dominants at all sampling points and these were generally more abundent than species of *Fusarium* and *Penicillium.*

The relative abundance of populations of some commonly occuring species of fungi in oxidation pond system during January to December 1980 is shown in Fig. 1. The predominant species of both *Aspergillus* and *Penicillium* showed seasonal fluctuations,

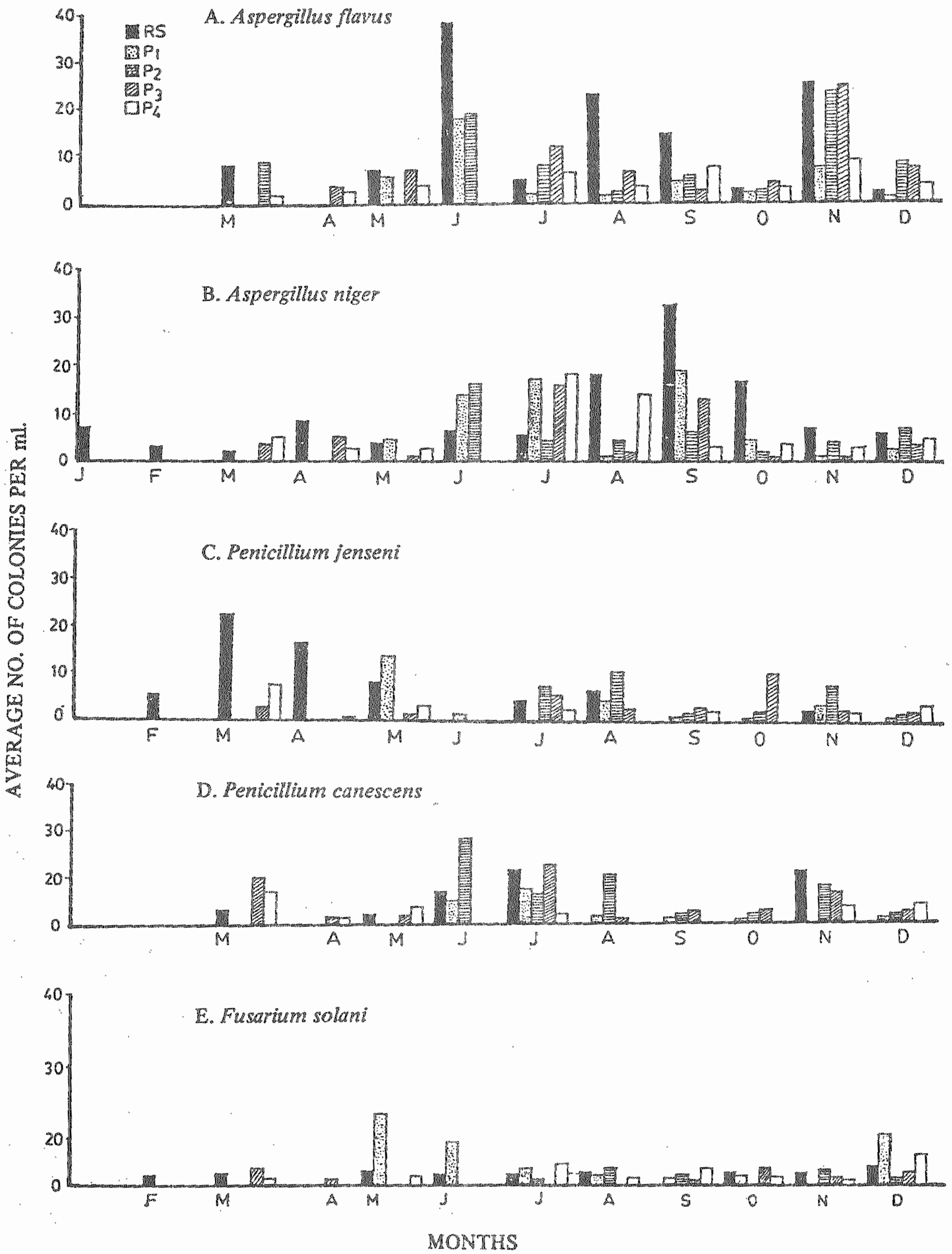

Fig. 1. Abundance of populations of some commonly occurring species of fungi in the oxidation pond system at Karachi University Campus (KUC). A. *A. flavus;* B. *A. niger;* C. *P. jenseni;* D. *P. canescens;* E. *F. solani.* Rs: Raw Sewage; P-1, Oxidation Pond No. 1, P-2, Oxidation Pond No. 2, P-3, Oxidation Pond No. 3, P-4, Oxidation Pond No. 4.

Table 3. Species of fungi which appeared as a leading dominant (recovered in highest numbers) in the oxidation pond treatment system.

Fungi	Sampling Points* RS	P-1	P-2	P-3	P-4
	No. of times appearing as leading dominants				
Aspergillus flavus Link	9	3	5	4	8
A. niger van Tieghem	4	5	3	3	8
Fusarium solani (Martius) Appel & Wollenweber	—	2	—	1	1
Penicillium jenseni Zaleski	5	—	3	1	2
P. canescens Sopp.	3	—	1	2	3

Rs: Raw Sewage; P-1: Oxidation Pond No. 1, (Bifiliated); P-2, Oxidation Pond No. 2, (Bifiliated); P-3, Oxidation Pond No. 3, P-4, Oxidation Pond No. 4.

while the incidence of *Fusarium solani* was relatively uniform throughout the study period. In general the populations of these species were lower during January to May period than June and December. Maximum numbers were recorded during June and September for most of these species.

Discussion

The waste stabilization pond system examined is specifically designed for the treatment of domestic waste of a small community. Sewage entering the system is highly organically enriched containing a variety of waste materials that exerts fairly high biological oxygen demand (BOD). During the treatment process, the BOD is decreased by 74 ~ 85% with a corresponding increase in the dissolved oxygen (DO) content (Zain & Altaf 1980).

The oxidation pond treatment system of KUC showed a significant reduction in the total colony counts/g dry weight as compared to that of raw waste water (11.84 to 18.73%, Table 1). Thus it appears that judicious maintenance of the system leads to a significant decrease in the fungal populations of the waste water. This is in agreement with the reduction in the water quality characteristies in individual pond effluents as compared to raw waste water reported by Zain & Altaf (1980). The reduction in the total counts of the pond habitats may be due to predation by other organisms, changes in the nutrient quality of the habitat and other chemical characteristics such as the presence of toxins or other antibiotic substances (Cooke, 1973).

The species recovery (incidence) data (Table 2) revealed that whereas a fairly large number of different types of fungi (representative of 20 genera) were isolated, the majority were isolated no more than 1-3 times from a particular sampling site. A total of 46 species of fungi were isolated from the system, but only 7 of these occurred predominantly with isolation frequency ranging from 29 to 80%. Most of the genera and species recovered in the present study were also isolated from waste stabilization ponds by previous investigators (Cooke & Matsuura, 1969; Carranco *et. al.*, 1984). Whereas Cooke & Matsuura (1969) reported more species from such habitats, but they used enrichment culture techniques which yielded qualitative data only. The differences in the number of fungal species may also be attributed to the quality and characteristics of waste waters as well as the type and selectivity of the medium used for isolation.

The most commonly appearing species found in the system were *A. niger, A. flavus, P. jenseni, P. canesceus* and *F. solani.* Seasonal variation occurred mainly due to fluctuations in the populations of these fungi and to a lesser extent due to changes in the number of species (Fig. 1). Thus it could be assumed that the habitat tends to select only a few successfully adaptable species whose populations increase with the type and level of pollutants present.

All fungal isolates were of filamentous type and appear to be members of geofungi (of soil origin). While most of these may be transient, the predominantly occurring species should be considered important permanent inhabitants of the pond system. The continuous presence and relative abundance of the predominant fungal species further suggest to their significance as members of a population of organisms that readily adapts to, and thrives in, apparently unfavourable environment of pollution habitats. The results of this study also support the findings of previous workers in that fungi are a vital part of community of saprobic organisms that degrade and remove organic wastes from the effluents of the waste – stabilization ponds. The fungal flora of the waste treatment pond examined was marked by species diversity, and generally low frequency of most species, predominance of only 7 species and a random or irregular seasonal distribution of majority especially of frequently isolated fungal species.

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