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Fungi of wastewaters and stabilisation pond

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Abstract. The fungal composition of domestic wastes and waste stabilisation pond water samples from two geographically distinct localities—Dharwad and Gulbarga in Karnataka State was studied. Fungi of wastewaters and stabilisation pond at Dharwad comprised of 13 species belonging to 8 genera and 16 species belonging to 11 genera respectively and that of wastewaters at Gulbarga comprised of 19 species belonging to 12 genera. Species belonging to 9 genera were recorded commonly in both the localities. Statistical analysis of the results indicated that *Aspergillus flavus*, *Aspergillus niger*, *Penicillium oxalicum* and *Trichosporon* sp. occurred in all the samples with high percentage of occurrence and high degree of consistency. Occurrence of some of the organisms commonly in two different environments (from the point of organic loading)—wastewaters and stabilisation pond-reflects upon their lymaphilic nature.

Keywords. Fungi; wastewaters; stabilisation pond.

1. Introduction

The bacterial composition of faeces, domestic wastes and stabilisation ponds is well understood (Geldreich 1978). Yet little information is available on their fungal composition. Pioneering studies on these lines were carried out by Cooke and his coworker (Cooke 1954 a, b, c; Cooke and Matsuura 1966; 1969; Cooke 1970; 1977). However information on fungi of wastewaters and stabilisation ponds in India appears to be rather limited excepting for a few reports (Vittal Rao *et al* 1965; Rai 1981; Vittal and Aravazhi 1981). The present report aims to shed some more light on the fungal composition of domestic wastewaters and stabilisation ponds in India.

2. Materials and methods

Two cities Dharwad and Gulbarga in the northern part of Karnataka State differing in geographical as well as climatic conditions were selected. Dharwad lies between latitude 14°78′ to 15°50′N and longitude 74°48′ to 76°00′E and has a comparatively cooler climatic conditions, with an average temperature of 28·8°C (maximum in summer months being 36°C) and an annual rainfall of 148·34 cm. Gulbarga on the other hand occupies latitude 16°12′ to 17°46′N and longitude 76°04′ to 77°42′E, has a warmer climate recording temperatures of 44–46°C in May and has an annual rainfall of 71·56 cm.

Wastewaters accruing from the residential quarters and hostels in the respective University campuses in Dharwad and Gulbarga were screened for the presence of fungal populations. Karnatak University campus at Dharwad has a stabilisation pond in the Botanical Gardens. Its design and operational characteristics are thus: 45·75 m length, 13·70 m breadth and 1·22 m depth, surface area of 627 m^2, inflow of wastewater 77282 L/day, holding capacity of 764630 L and a detention period of 10 days (Rodgi and Kanbur 1971). Water samples from this stabilisation pond were studied for the presence of fungal species. However, the wastewater at Gulbarga University campus is allowed into a septic tank.

Wastewater samples were collected in a sterile glass container around 6·30 to 7·30 AM on the day of sampling, allowed to settle for 15 min and the supernatant fluid was subjected to physico-chemical and biological analyses. On a single day of sampling, five samples were collected.

Samples from the stabilization pond were collected from 5 different points: one each near the influent and the effluent chambers, and the remaining 3 equidistantly located between these two chambers. Samples were withdrawn from 1 ft below the surface. They were settled for 15 min and the supernatant was subjected to the mycological studies.

The wastewater samples as well as stabilisation pond samples were collected once in a fortnight. The wastewater samples were analyzed for physico-chemical characteristics as per APHA, AWWA and WPCF (1971) as well as for the organic fractions—total carbohydrates (Seifter *et al* 1950), total proteins (Lowry *et al* 1951) and total lipids (Folch *et al* 1957). Serial dilution technique was adopted for the mycological screening of wastewater as well as pond samples. A 0·2 ml sample from 10^{-3} dilution was inoculated by spread plate method onto potato dextrose agar (pH 6·0) containing 300 mg/L streptomycin. Five replicate plates were maintained for each sample and the inoculated plates were incubated at room temperature. Colonies on each plate were identified after sporulation, usually during the 3rd to 6th day of inoculation.

Percentage occurrence of a species was calculated on the basis of total number of colonies of a particular fungus against the total number of colonies of all fungi in a given sampling.

3. Results

The values of physico-chemical characteristics and organic fractions of wastewater and stabilisation pond effluents at Dharwad and wastewater samples at Gulbarga are presented in table 1.

3.1 *Fungi in wastewater at Dharwad*

Thirteen species belonging to 8 genera were isolated (table 2). *Aspergillus flavus, A. niger, Penicillium oxalicum, Trichosporon* sp., *A. terreus* and *Curvularia lunata* formed the major bulk of the fungal community, and occurred in the same order of percentage of occurrence. However, from the point of consistency of occurrence, *A. niger* occurred with the highest consistency, followed by *A. flavus, P. oxalicum, Trichosporon* sp., *C. lunata* and *A. terreus.* Amongst other fungal species *Cunninghamella elegans* and *A. niveus* were isolated from many samples but with low percentage of occurrence as well as consistency. Remaining were randomly isolated.

Species belonging to the genus *Aspergillus* dominated over other organisms with per cent occurrence of $58·42 \pm 8·71$, followed by the species of *Penicillium* with $19·63 \pm 4.14\%$ occurrence.

Table 1. Characteristics of wastewaters and stabilization pond effluents.

	Wastewater at Dharwad	Wastewater at Gulbarga	Stabilization pond effluents*
A. Physico-chemical characteristics:			
pH	6·9–7·6	7·9–9·0	7·9–10·2
Dissolved oxygen	0·0–0·2	0·0–0·2	4·0–15·0
Biochemical oxygen demand (B.O.D.)	250·0–370·0 (314·20)	245·0–500·0 (367·30)	35·0–100·0
Phosphates, dissolved.	3·5–4·6 (4·1)	4·7–6·2 (5·45)	0·8–3·6
Ammonia-N	35·0–49·0 (41·5)	38·0–60·0 (51·5)	5·0–10·0
B. Organic fractions:			
Total carbohydrates	12·936–25·880 (19·509)	18·600–29·440 (24·410)	NA
Total proteins	23·722–51·691 (31·256)	26·700–37·000 (31·590)	NA
Total lipids	32·00–56·00 (46·87)	36·00–47·00 (42·00)	NA

All values in mg/L, except for pH which are in units; numbers in parentheses indicate average values.
*after Patil (1979).
NA = Not analysed.

3.2 *Fungi in stabilisation pond at Dharwad*

Sixteen fungi belonging to 11 genera were isolated during the course of this study (table 2). The most commonly isolated forms were *A. flavus*, *A. niger*, *P. oxalicum* and *Trichosporon* sp in the order of per cent occurrence. Of the remaining, *A. terreus*, *C. lunata*, *C. elegans*, *Trichoderma viride* and *A. fumigatus* were isolated on most of the occasions though with low per cent occurrence and consistency.

Species of *Aspergillus* dominated over other fungi with 55·52 ± 3·29% occurrence followed by *P. oxalicum* (20·28 ± 2·41% occurrence) and *Trichosporon* sp (15·02 ± 2·74% occurrence).

3.3 *Fungi in wastewater at Gulbarga*

Nineteen species belonging to 12 genera were isolated (table 3). *A. flavus*, *A. niger*, *P. oxalicum* and *Trichosporon* sp were the most commonly isolated forms. However, *A. niger* was the most abundantly occurring species throughout this study. From the point of occurrence as well as consistency, *A. flavus*, *P. oxalicum* and *Trichosporon* sp followed next to *A. niger*.

Aspergillus species dominated over other with 59·31 ± 6·85% occurrence. Species of *Penicillium* and *Trichosporon* followed next with per cent occurrence of 18·22 ± 4·19 and 14·86 ± 4·79 respectively. Diversity of species of *Penicillium* was more in Gulbarga than in Dharwad.

Table 2. Occurrence of various fungal species in wastewaters and stabilization pond at Dharwad (numbers in the parantheses represent coefficient of variation).

Wastewaters		Stabilization pond	
Organism	Occurrence ± S.D. (%)	Organism	Occurrence ± S.D. (%)
1. *Aspergillus flavus*	28·01 ± 5·78 (20·63)	1. *Aspergillus flavus*	27·71 ± 3.51 (12·66)
2. *A. niger*	24·03 ± 4·56 (18·97)	2. *A. fumigatus*	1·02 ± 0·66 (64·70)
3. *A. niveus*	1·05 ± 0·83 (79·00)	3. *A. nidulans*	0·93 ± 0·86 (92·47)
4. *A. terreus*	5·09 ± 2·25 (44·20)	4. *A. niger*	21·59 ± 1·60 (7·41)
5. *Cunninghamella elegans*	1·95 ± 1·20 (61·53)	5. *A. niveus*	1·11 ± 1·10 (99·09)
6. *Curvularia lunata*	3·66 ± 1·46 (39·89)	6. *A. terreus*	3·16 ± 1·51 (47·78)
7. *Fusarium oxysporum*	0·51 ± 0·68 (133·33)	7. *Candida* sp.	0·67 ± 0·49 (73·13)
8. *F. solani*	0·32 ± 0·69 (215·62)	8. *Cladosporium* sp.	0·41 ± 0·47 (114·63)
9. *Mucor* sp.	0·80 ± 0·78 (97·5)	9. *Cunnighamella elegans*	1·86 ± 0·91 (48·92)
10. *Penicillium citrinum*	0·75 ± 0·70 (93·33)	10. *Curvularia lunata*	2·98 ± 1·56 (52·35)
11. *P. oxalicum*	18·88 ± 4·54 (24·05)	11. *Fusarium solani*	0·48 ± 0·41 (85·41)
12. *Rhizopus nigricans*	0·93 ± 0·91 (97·05)	12. *Geotrichum candidum*	0·93 ± 0·60 (64·51)
13. *Trichosporon* sp.	14·03 ± 5·59 (39·84)	13. *Monilia* sp.	0·33 ± 0·42 (127·27)
		14. *Penicillium oxalicum*	20·28 ± 2·41 (11·88)
		15. *Trichoderma viride*	1·61 ± 0·81 (50·31)
		16. *Trichosporon* sp.	15·02 ± 2·74 (18·24)

4. Discussion

In the present study 28 species belonging to 16 genera have been isolated from domestic wastewater and stabilisation pond water samples. Of these, species belonging to 9 genera, viz., *Aspergillus*, *Candida*, *Curvularia*, *Fusarium*, *Geotrichum*, *Mucor*, *Penicillium*, *Rhizopus* and *Trichosporon* were isolated from samples collected at Dharwad as well as Gulbarga. Species of 13 of the genera reported in the present study were earlier recorded from wastewaters and polluted waters in USA by Cooke (1970, 1977) and of 3 genera by Vittal Rao *et al* (1965), at Nagpur, 11 genera by Rai (1981) at Nagpur and 6 from samples at Madras (Vittal and Aravazhi 1981). This indicates the ubiquitous distribution of various fungi in different geographical locations.

Table 3. Occurrence of various fungal species in wastewaters at Gulbarga.

Organism	Occurrence ± S.D.(%)
1. *Acrophialophora fusispora*	0·29 ± 0·68 (234·28)
2. *Aspergillus flavus*	19·15 ± 4·07 (21·25)
3. *A. nidulans*	0·63 ± 0·74 (117·46)
4. *A. niger*	35·97 ± 5·97 (16·59)
5. *A. niveus*	1·22 ± 0·94 (77·05)
6. *A. terreus*	2·10 ± 1·80 (85·71)
7. *Candida albicans*	0·68 ± 0·57 (83·82)
8. *Curvularia lunata*	2·66 ± 1·49 (56·01)
9. *Fusarium equiseti*	0·50 ± 0·57 (114·00)
10. *Geotrichum candidum*	0·86 ± 0·56 (65·11)
11. *Mucor* sp.	0·64 ± 0·80 (125·00)
12. *Penicillium attrinum*	0·67 ± 0·75 (111·94)
13. *P. oxalicum*	17·11 ± 4·50 (26·30)
14. *P. pinophylum*	0·22 ± 0·43 (195·45)
15. *P. simplicissimum*	0·22 ± 0·32 (145·45)
16. *Phoma sorghina*	0·67 ± 0·72 (107·46)
17. *Rhizopus oryzae*	0·89 ± 0.81 (91·01)
18. *Saccharomyces cerevisiae*	0·54 ± 0·68 (125·92)
19. *Trichosporon* sp.	14·86 ± 4·79 (32·23)

Numbers in parentheses represent coefficient of variation.

Fungal composition of samples varied in different samplings. Number of species isolated per sampling ranged from 8–12 and 12–14 in wastewater and stabilization pond samples at Dharwad respectively and 7–15 in wastewater samples at Gulbarga. *A. flavus*, *A. niger*, *P. oxalicum* and *Trichosporon* sp were constantly recorded from all the samples examined. *A. flavus* was the predominant form at Dharwad whereas *A. niger* was the predominant fungus in wastewater samples at Gulbarga, which probably reflects upon the latter's tolerance to high temperature levels usually recorded in Gulbarga region. Several species have been recorded with very low percentage of occurrence, in both wastewaters and stabilization pond. Isolation of *Acrophialophora fusispora*, a common soil inhabitant, appears to be related to the mixing up of wastewaters with the nearby soil. This fungus has not been previously reported either from wastewaters or polluted waters elsewhere.

As domestic wastes with high organic load (table 1) undergo biological purification in the stabilization pond, values of Biochemical oxygen demand and nutrients are reduced considerably along with wide diurnal fluctuations in the physico-chemical characteristics (including increases in pH and dissolved oxygen during the afternoon) due to the activities of the pond community *in toto* (Patil 1979). As such, the ambient conditions offered to the inhabitants of wastewaters and to those of stabilisation pond ecosystem differ considerably. The common occurrence of several fungal species in wastewaters as well as in pond reveals their lymaphilic nature. Remaining could be considered as lymaxenes.

The occurrence of fungi in a stabilization pond indicates that they are capable of utilising nutrients from the wastewaters. Cooke and Matsuura (1969) too reported that there was an increase of 5–200 fold in the fungal and yeast populations within an year of operation of a stabilization pond, speaking thereby of the mileu of the pond as a favourable habitat for them. In a stabilization pond ecosystem fungi occupy the same functional status as bacteria, both are effective degraders. Yet in contrast to bacteria which reduce B. O. D. alone but not phosphates and ammonia-N from the domestic wastes (Patil 1979; Hiremath 1984), fungi possess the capability to reduce not only B. O. D., but phosphates and ammonia-N as well (Thanh and Simard 1973; Patil 1979; Hiremath 1984) thus leading to better quality effluents than do bacteria. As such, the presence of fungi and yeasts in waters receiving organic enrichment from one or the other sources, for that matter in any waste treatment system, can not be simply ignored as that of casual contaminants (Cooke 1973).

Studies carried out in this laboratory on the various aspects of fungal purification of domestic wastes as well as interactions of fungi with other representative coinhabitants of the stabilization pond ecosystem will be reported elsewhere.

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