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MYCOBIOTA OF SOILS POLLUTED WITH METAL-AND DYE-CONTAINING EFFLUENTS FROM INDUSTRIAL UNITS LOCATED AT MEERUT AND HAPUR (WESTERN UTTAR PRADESH)

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Abstract:

A total of nineteen species of fungi were isolated from the soil exposed to industrial effluents containing heavy metals using serial dilution plate method. *R. oryzae, M. rouxii, A. flavus* and *P. spinulosum* together comprised roughly two-third (64.59%) of the isolates obtained from metal-polluted soils. The Fusaria constituted only a very meagre fraction of 0.78% only. The mucoraceous fungi dominated the plates followed by aspergilli. A total of twenty four species of fungi were isolated from the soils polluted with effluents containing dyes. *M. rouxii, R. oryzae, A. flavus* and *P. spinulosum* together comprised majority (55.49%) of the isolates. The mucoraceous fungi constituted most prominent group followed by aspergilli and penicillia — the three groups together comprised overwhelming majority (75.24%) of the total isolates. The genus *Aspergillus* was represented by maximum number of species (8 species excluding *Emericella nidulans*) followed by *Penicillium* (4 species). Family Moniliaceae has the highest contribution to the mycoflora as 15 species (out of 35 isolated) belonged to this family. Monilliaceae was the most diverse taxonomic family. Metal pollution appears to exert greater adverse impact on soil mycodiversity than dye pollution. The potential of fungi to adsorb heavy metals must be an important factor contributing to their ability to survive and flourish in soils.

Keywords: heavy metals; dyes; pollution; soil; fungal diversity

INTRODUCTION

Water, being highly integrated component of most ecosystems, is highly prone to pollution — natural as well as anthropogenic. Discharges from the industries constitute one of the major sources of environmental pollution-including water pollution. These carry a range of inorganic as well as organic pollutants including phenols, formaldehyde, dyes and heavy metals. These pollutants pose a serious threat to the environment (Bhole *et al*., 2004; Joseph and Chinonye, 2016). The contamination of environment by "heavy metals" from natural sources as well as a result of human activities is a matter of global concern (Dushenkov *et al*., 1995; Garcia *et al*., 2016). The heavy metals like Al, Cd, Co, Cu, Cr, Fe, Hg, Mn, Ni, Pb and Zn deserve immediate attention as these are known to be regularly discharged into the environment (Guo *et al*., 2003; Son *et al*., 2004; El-Shafey, 2010; Abu Hasan *et al*., 2012). Once these reach the soil matrix, these enter the food web through ground water aquifers (Walton, 1995; Athar and Ahmad, 2002), ultimately getting biomagnified to toxic concentrations (Herawati *et al*., 2000). In the soil matrix itself, these affect soil microbial communities and consequently the biogeochemical cycle and plant growth. The effluents from industrial units involved in the manufacturing of batteries, gasoline additives, paint pigments, alloys and sheets etc. often contain high concentrations of lead (Tunali *et al*., 2012). The wastes from industrial processes such as electroplating, smelting, alloy manufacturing, paints and pigments, cadmium-nickel batteries, fertilizers and pesticides serve as routes for contamination of environment with cadmium (Grayson and Othumer, 1978; Salim *et al*., 1992; Rao *et al.,* 2010).

The dyes also constitute a major cause of water pollution. The dyes find use in a variety of industries including textile, rubber, paper and pulp, plastic, cosmetic, tannery, pharmaceutical and food–processing units. The dyestuff, especially those in current use, has been designed to resist fading as well as light-induced oxidation (Nigam *et al*., 2000; Casieri *et al.,* 2008). Consequently, these acquire persistence, recalcitrance and become resistant to microbial degradation (Kabbout and Taha, 2014). The discharge of such dyes into the environment causes damage to the latter in a variety of manners. Even at very small concentrations (as low as 1.0 ppm), the dyes impart strong colour to the water; not only this, a number of toxic degradation products may also be formed (Dutta, 1994; Asamudo *et al*., 2005; Moorthi *et al*., 2007). Such polluted water has an adverse impact on the receptor water bodies, soil as well as other abiotic and biotic components of the environment including human life. The present communication pertains to a study of mycobiota of metal- and dye-polluted soils of Meerut and Hapur an aim to obtain some fungal strains resistant to these pollutants for future use in biotechnological processes.

MATERIALS AND METHODS

Three samples of soils polluted with heavy metals were collected from the fields situated in Partapur industrial area (Meerut) while two samples were collected from the fields located at Shobhapur (Delhi-Roorkee bypass), Meerut. The effluents from electroplating, leather, paints and batteries manufacturing units are discharged in these fields. Three samples of dye-polluted soils were collected from Partapur industrial area (Meerut) and two from Pilakhuwa (Hapur) from the fields where the dye-containing effluents from the textile industries are discharged. Before taking soil sample from a given site, the upper layer of the soil was removed with the help of a sterilized trowel to remove extraneous litter / organic matter. Soil samples were then taken out with the help of a sterilized trowel and were collected in fresh sterile polythene bags aseptically. These samples were brought to the laboratory where all the five soil samples of metal-polluted sites were mixed thoroughly to obtain one composite sample. Similarly, samples collected from dye-polluted soils were combined to obtain another composite sample.

Dilution plate method (Waksman, 1917) was used to assess mycobiota of each 'composite' soil sample. 20 g of soil from a given composite sample were transferred into 200 ml of sterilized distilled water dispensed in a 500 ml Erlenmeyer (conical) flask. The contents were stirred for 30 minutes to wash fungal propagules from the soil. 10 ml of this suspension (of 1:10 dilution) were immediately transferred to a 250 ml conical flask containing 90 ml of sterilized distilled water to yield soil suspension of 1:100 dilution. The latter was used for preparing further serial dilutions (1:1000 and 1:10000) aseptically. From the suspensions of 1:100 dilution, one ml aliquot was transferred to each of a set of three Petri dishes followed by the addition of 20 ml of cooled and sterilized PDA medium (Riker and Riker, 1936) with 30 ppm of streptomycin. Similar treatment was given to the suspensions of 1:1000 and 1:10000 dilutions.

The Petri dishes containing medium and the inocula were rotated (so as to facilitate thorough mixing of broth with inoculums). These Petri plates were then incubated at 25±1°C for 6 to 8 days in a B.O.D. incubator. The inoculated Petri dishes under incubation were observed for the fungal growth from the third day itself when the fast-growing fungi started appearing in the Petri dishes. A complete record of the fungal species and their numbers (CFU_S: Colony Forming Units) in the Petri dishes was maintained. The identification of the fungal species was done on the basis of their morphology and cultural characteristics with the help of keys and manuals published by Gilman (1957), Ellis (1971, 1976), Subramanian (1971), Barnett and Hunter (1972), Domsch and Gams (1972), Domsch *et al.* (1980) and Nagamani *et al*. (2006). The total number of colonies and the number of colonies of each fungal species growing in every Petri dish were recorded to determine their frequencies. The frequency classes were expressed as mentioned by Saksena (1955).

RESULTS AND DISCUSSION

A total of nineteen species of fungi were isolated from the soil exposed to industrial effluents containing heavy metals. Their comparative distribution is presented in table 1. *Aspergillus flavus*, *Mucor rouxii* and *Rhizopus oryzae* were the most frequent species (frequency class V) followed by *Penicillium spinulosum* (frequency class IV). The fungi falling in the frequency class III were: *Aspergillus oryzae, A. terreus, Drechslera avenacea* and *Penicillium frequentans*. *Rhizopus oryzae* and *Mucor rouxii* comprised 20.87% and 15.1% of the isolates respectively, thus, together accounting for a substantial (35.97%) proportion of the total mycobiota. Another zygomycetous species *Mortierella* sp., though exhibiting 25% frequency, constituted only 1.78% of the total isolates. *Aspergillus flavus* and *Penicillium spinulosum* accounted for 15.7% and 12.92% of the total isolates respectively. Thus, *R. oryzae, M. rouxii, A. flavus* and *P. spinulosum* together comprised roughly two-third (64.59%) of the isolates obtained from metal-polluted soils. The Fusaria were meagerly represented — the two species *i.e. Fusarium moniliforme* and *Fusarium oxysporum* together constituting only a very meagre fraction *i.e* 0.78% only. From the point of view of taxonomic groupings, the mucoraceous fungi dominated the plates with 37.75% of the isolates followed by aspergilli (28.8%).

Table 1: Species of fungi isolated from soils receiving industrial effluents containing heavy metals and their comparative distribution

Fungal species	Total isolates (TI)	Percentage of isolates (PI)	Frequency	Frequency class
Aspergillus flavus	79	15.7	91.66	V
A. niger	12	2.38	25	II
A. oryzae	25	4.97	41.66	III
A. terreus	19	3.77	58.33	III
A. ustus	10	1.98	33.33	II
Alternaria alternata	13	2.58	25	II
A. citri	9	1.78	16.66	I
Botryotrichum piluliferum	7	1.39	8.33	I
Drechslera avenacea	17	3.37	41.66	III
Fusarium moniliforme	2	0.39	8.33	I
F. oxysporum	2	0.39	16.66	I
Geotrichum candidum	5	0.99	33.33	II
Helminthosporium microsporum	8	1.59	33.33	II
Mortierella sp.	9	1.78	25	II
Mucor rouxii	76	15.1	83.33	V
Penicillium frequentans	22	4.37	58.33	III
P. spinulosum	65	12.92	75	IV

Rhizopus oryzae	105	20.87	91.66	V
White sterile mycelium	18	3.57	50	III
Total isolates	**503**			
Simpson's index	**8.156**			

A total of twenty four species of fungi were isolated from the soils polluted with effluents containing dyes. Their comparative distribution is presented in the table 2. The most frequent fungal species, assignable to frequency class V, are: *A. flavus* (100%), *Humicola grisea* (83.33%), *Mucor rouxii* (100%), *Penicillium spinulosum* (91.66%) and *Rhizopus oryzae* (91.66%). *A. fumigatus* (75%) and *A. niger* (83.33%) belonged to frequency class IV; white sterile mycelium also belonged to the frequency class IV. The fungi belonging to the frequency class III were: *Cladosporium echinulatum*, *Cunninghamella echinulata*, *Fusarium moniliforme*, *Penicillium implicatum*, *Rhizoctonia* sp. and *Trichoderma lignorum*. *Mucor rouxii* and *Rhizopus oryzae* comprised 15.06% and 15.82% of the isolates respectively; thus these two together accounted for about one-third (30.88%) of the isolates. *A. flavus* and *P. spinulosum* accounted for 12.9% and 11.71% of the isolates respectively.

Table 2: Species of fungi isolated from soils receiving industrial effluents containing dyes and their comparative distribution

Fungal species	**Total isolates (TI)**	**Percentage of isolates (PI)**	**Frequency**	**Frequency class**
Aspergillus flavus	85	12.9	100	V
A. fumigatus	25	3.80	75	IV
A. niger	55	8.37	83.33	IV
A. sulphureus	2	0.30	8.33	I
A. sydowii	13	1.97	25	II
A. terreus	4	0.60	16.66	I
Chaetomium flavum	3	0.45	8.33	I
Cladosporium echinulatum	21	3.19	41.66	III
Cunninghamella echinulata	12	1.82	50	III
Curvularia lunata	3	0.45	8.33	I
C. pallescens	2	0.30	16.66	I
Emericella nidulans	14	2.13	16.66	I
Fusarium moniliforme	8	1.21	50	III
Humicola grisea	44	6.69	83.33	V
Mucor rouxii	99	15.06	100	V
Penicillium frequentans	11	1.67	33.33	II
P. implicatum	15	2.28	50	III
P. paxilli	5	0.76	25	II
P. spinulosum	77	11.71	91.66	V
Rhizoctonia sp.	8	1.21	41.66	III
Rhizopus oryzae	104	15.82	91.66	V
Stachybotrys atra	16	2.43	75	IV
Trichoderma lignorum	6	0.91	41.66	III
White sterile mycelium	25	3.8	75	IV
Total	**657**			
Simpson's index	**9.11**			

Thus, *M. rouxii, R. oryzae, A. flavus* and *P. spinulosum* together comprised majority (55.49%) of the isolates obtained from soil receiving dye-containing industrial effluents. The mucoraceous fungi constituted most prominent group with 30.88% of the isolates, followed by aspergilla

(27.94%) and penicillia (16.42%) — the three groups together comprised overwhelming majority (75.24%) of the total isolates.

Out of a total of thirty five fungal species isolated from the soil samples in the present study, thirty four species could be identified, which belonged to 21 genera. The genus *Aspergillus* was represented by maximum number of species (8 species excluding *Emericella nidulans*) followed by *Penicillium* (4 species). The observation substantiates the widely held belief that the Aspergilli are more common in warmer regions of the world while Penicillia are more abundant in the colder regions of the world (Waksman, 1917; Gochenaur and Backus, 1967; Singh and Charaya, 1975; Charaya, 2006). Family Moniliaceae has the highest contribution to the mycoflora as 15 species (out of 35 isolated) belonged to this family. Incidentally, almost similar observations regarding the contribution of aspergilli and penicillia as well as family Trichocomaceae as such, were recorded by Abdel-Azeem *et al*. (2007) while examining the effect of long-term heavy metal contamination on diversity of terricolous fungi in Egypt. They recorded species/genus ratio (S/G) per family and found that Trichocomaceae (including Moniliaceae) was the most diverse taxonomical rank (S/G=4.5). In the present study also, Moniliaceae turned out to be taxonomically most diverse family (Table 3) with S/G=3.

Table 3: Species: Genus Ratio of the Families of Mycobiota isolated in the present study

Family	No. of Genera (G)	No. of Species (S)	S/G
1. Trichocomaceae	1	1	1
2. Chaetomiaceae	1	1	1
3. Mortierellaceae	1	1	1
4. Cunninghamellaceae	1	1	1
5. Mucoraceae	2	2	1
6. Agonomycetaceae	1	1	1
7. Moniliaceae	5	15	3
8. Dematiaceae	8	10	1.25
9. Tuberculariaceae	1	2	2

As far as the species diversity is concerned, the soil samples receiving effluents from dye industries exhibited greater diversity (Simpson's index= 9.11) as compared to those receiving effluents containing heavy metals (Simpson's index= 8.156). 503 isolates belonging to 19 species were isolated from metal-polluted soils while 657 isolates belonging to 24 species could be isolated from dye-polluted soils (Simpson's index = 9.11). Mehra (2013) obtained 92 isolates belonging to 11 fungal species from metal-polluted sites as against 141 isolates (16 species) from control soils. From dye-polluted soils, he could obtain 94 isolates (12 species). Obviously, metal pollution appears to exert greater adverse impact on soil mycodiversity. The results of the present study, thus, confirm the findings of Mehra (2013).

In the present study, *Rhizopus oryzae*, *Aspergillus flavus*, *Mucor rouxii* and *Penicillium spinulosum* dominated the soils polluted by heavy metals. These four species together accounted for 64.59% of the total isolates. Amongst these four species again, the mucoraceous fungi (*R. oryzae* and *M. rouxii*) contributed 35.97% of the isolates – slightly greater than by *A. flavus* and *P. spinulosum* together. Ahmad *et al.* (2005) reported the predominance of *Rhizopus* sp. and *Aspergillus* sp. in the agricultural field soil of Aligarh city (U.P.) receiving long-term application of sewage and industrial effluents. Zafar *et al.* (2007) reported *Aspergillus* spp. and *Rhizopus* sp. followed by *Penicillium* sp. to be most frequently encountered isolates from soil samples which were collected from agricultural fields receiving long-term application of effluents containing heavy metals at Aligarh. Iram *et al.* (2009) analysed the mycobiota of agricultural fields of Faisalabad at Rawalpindi (Pakistan) where heavy metals and other pollutants had been emitted in industrial effluents for several years. They found *Aspergillus flavus, A. niger, Fusarium* sp., *Penicillium* sp. and *Rhizopus* sp. to be dominant in all the samples. Joshi *et al*. (2011) also found *Aspergillus flavus* to be amongst the most dominant fungi isolated from soils treated with sewage sludge and industrial effluents containing heavy metals at Karnal, Panipat and Sonepat (Haryana). Iram *et al.* (2013) analysed the mycobiota of peri-urban agricultural areas of Faislabad (Pakistan) contaminated by heavy metals; *Aspergillus flavus* was most frequently isolated fungus. Dwivedi *et al.* (2012) isolated fungi from soils contaminated with heavy metals at Noida (U.P.). They found *Aspergillus niger* and *A. flavus* to be most prevalent. Rasool and Irum (2014) found *Aspergillus fumigatus, A. flavus* and *Penicillium* sp. from heavy metal-contaminated areas in Multan and Gujrawala (Pakistan). Abdel-Azeem *et al*. (2007) studied the occurrence and diversity of mycobiota in heavy metal-contaminated sediments of Mediterranean coastal lagoons in Egypt. *A. niger* and *A. flavus* were recovered from all the sites. These two species comprised 31.9% and 27.3% of all the isolates.

There is a dearth of studies concerning long term impact of dye-containing effluents on soil mycobiota. Kousar and Charya (2002) reported *Aspergillus niger* and *Mucor mucedo* from dye-containing soils. Raju *et al*. (2007) isolated *Aspergillus flavus, Fusarium oxysporum, F. moniliforme* and *Trichoderma* sp. from soil samples around the textile industries of Hanjanjand (Karnataka). Moturi and Singara Charya (2009) found *Mucor mucedo* to be predominant fungus in the soils around textile industries in Mangalagiri. Laxmi and Nikam (2015) isolated *Aspergillus flavus, A. Niger, Fusarium oxysporum* and *Penicillium notatum* from textile dye effluents in Maharashtra. In a short term study, Kumar and Charaya (2012) found that soil treatment with malachite green (MG) solution caused marked decrease in the total number of isolates. However, *Aspergillus flavus, A. fumigatus* and *A. niger* could tolerate MG to a certain extent, and their populations were able to survive throughout the study. Kumar (2013), again in short term experiments, found that *Aspergillus flavus* and *A. fumigatus* could survive high concentrations of basic fuchsin. Thus, a reasonably good body of evidence favours the findings of the present study that *A. flavus, M. rouxii, R. oryzae* and *P. spinulosum* are able to flourish in soils polluted by heavy metals / dyes.

Bragulat *et al*. (1991) reported that basic fuchsin (500 ppm) could inhibit Zygomycetes to some extent but caused greater inhibition of Deuteromycetes. Ahmed *et al*. (2005) reported that 850 µg, 500 µg and 500 µg per ml concentrations of Ni, Cd and Cr were minimum inhibitory concentration for *Rhizopus* sp. while for *Aspergillus niger* these were only 400 µg, 150 µg and 350 µg. Zafar *et al.* (2007) calculated tolerance indices of a number of a species with respect to CO, Cr, Cu, Cd and Ni. *Rhizopus* sp. and *Penicillium* sp. were found to possess highest tolerance index — much higher than other fungal species. Joshi *et al*. (2011) found *A. niger* and *A. flavus* to be most resistant to Pb and Cr. Iram *et al*. (2013) worked out the tolerance indices of fungi to Pb and Cr. *Aspergillus flavus, A. niger and Humicola grisea* exhibited high level of resistance in the following order – *Aspergillus flavus* > *A. niger* > *Humicola grisea*. Rasool and Irum (2014) worked out the heavy metal-resistant fungi from Multan and Gujrawala (Pakistan). *A. versicolor, Penicillium* sp. and *A. flavus* were predominant fungi and were highly tolerant to Cu / Pb, Cr and Cd respectively. Dixit and Singh (2014) found *A. flavus, Mucor globosus* and *Penicillium* sp. were found to be most tolerant to heavy metals. On the basis of foregoing account, it may be prudent to assume that the dominance of *Rhizopus oryzae, Mucor rouxii, Aspergillus flavus* and *Penicillium spinulosum* in the present study may be attributed to their ability to tolerate heavy metals and dyes.

Extracellular sequestration (including biosorption) is also believed to be a factor contributing to the tolerance of fungal species to pollutants (Anahid *et al*., 2011). The maximum number of isolates from metal-polluted soils in the present study belonged to *Rhizopus oryzae.* A sample survey of existing literature concerning biosorption of metals reveals that *Rhizopus* spp., are endowed with quite high metal-adsorbing capacities as follows: (i) Cd: 30-40.5 (Tobin *et al*., 1984; Jin-Ming *et al*., 2010); (ii) Pb: 55-66 (Volesky and Holan, 1995; Fourest and Roux, 1992);

(vii) Cr: 31 (Tobin *et al*., 1984). *Aspergillus flavus* biomass is also known to be very good at biosorption of heavy metals: (i) (Pundir and Dastidar, 2010; Kumar *et al*., 2014). *Mucor rouxii* has also been found to be quite efficient at biosorption of metals: (i) Cd: 6.94-27 (Fourest and Roux, 1992; Yan and Viraraghavan, 2000); (ii) Ni: 5.24, Zn: 4.84 and Pb: 17.13 (Yan and Viraraghavan, 2000). *Penicillium spinulosum* appeared to be least efficient of the four test species in metal biosorption: (i) Cd: 0.4 (Townsley *et al*., 1966); (ii) Cu: 0.42 (Townsley *et al*., 1966). Thus, it can be speculated that the potential of a fungus to adsorb heavy metals must be an important factor contributing to their ability to survive and flourish in soils.

The maximum number of isolates from dye-polluted soils belonged to *Rhizopus oryzae* closely followed by *Mucor rouxii* (but not *A. flavus*). *Rhizopus* spp. are known to be quite efficient at biosorption of many dyes including Bromophenol blue, Brilliant green, Methylene blue, Reactive black (Aksu and Tezer, 2000; Zeroual *et al.,* 2006; Aksu *et al.,* 2010; Lang *et al.,* 2013). Tyagi (2016) reported that the dead biomass of different fungal species tested can be placed in the following order as far as biosorption of basic fuchsin is concerened: *R.oryzae* > *S. cerevisiae* > *M. rouxii* > *A. flavus* > *P. spinulosum.* In the case of malachite green, the position appears to be quite different as follows: *R. oryzae* > *M. rouxii* > *S. cerevisiae* > *A. flavus* > *P. spinulosum.*

While extracellular biosorption of pollutants is regarded by many workers to be responsible for the tolerance of microbes to pollutants, some workers including Zafar *et al.* (2007) do not agree with this suggestion. In their opinion, there is a little, if any, correlation between metal tolerance and biosorption. The results of the present study, incidentally, do not favour outright rejection of the idea that the adsorption of metals or dyes does contribute, at least partially, to microbial tolerance to pollutants. However, this aspect of the problem requires further resolution and more in-depth studies need to be undertaken to arrive at some generalisation.

Another interesting finding was that the mucoraceous fungi were quite dominant in the soils under study; and out of 34 species identified; three species belonged to this group. A number of studies in the past (Charaya, 2006) have recorded paucity of Mucorales in India, as discussed earlier in this very section. But the results of the present study do indicate their moderate presence, perhaps in the polluted habitats.

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