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Biodiversity of Heavy Metal-tolerant Terrestrial Mycobiota in Drainage Water Resources

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Abstract: This work was initiated to study the biodiversity of filamentous fungi in drainage water channels and their correlation with heavy metal pollution. Results showed that all water samples collected from different drainage channels were contaminated by Cd, Co, Cu, Mn, Pb and Zn but in various and relatively low concentrations. *Aspergillus, Penicillium, Mucor, Trichoderma* and *Rhizopus* were the most prevalent genera. The dominance of these genera varied in different sites. There was a basic similarity in prevalence and fluctuation of fungi isolated from both the large and the small channels.

Tolerance of *A. flavus* var. *flavus, A. fumigatus, A. terreus* var. *africanus, A. niger, A. oryzae, M. racemosus, P. citrinum, R. stolonifer* and *T. viride* to Zn, Pb and Cd was studied. Results proved that all of these fungi could resist presence of heavy metal but to various limits. *T. viride* seemed to be the most tolerant fungus against Zn and Pb whereas *M. racemosus* was the most tolerant one to Cd. This study recommends that application of multi-species system could play an effective role in biosorption of heavy metals from waste waters rather than using of individual species.

Key words: *Aspergillus,* biosorption, heavy metals, *Mucor,* mycobiota, *Penicillium,* pollution, *Rhizopus, Trichoderma.*

Introduction

Pollution of the environment by heavy metals arises as a result of many human activities, such as mining and milling industries, agricultural practices and sewage disposal. The pollutants are discharged or transported into the atmosphere and aquatic and terrestrial environments mainly as solutes or particulates and may reach high concentrations. As a result, heavy metals pose a potential threat to terrestrial biota (Hsu et al., 2006)

Aqueous heavy metal pollution represents an important environmental problem due to its toxic effects and accumulation throughout the food chain. The most common heavy metals discharged into aquatic environments are Cd, Cu, Zn and Pb (Moore and Ramamoorthy, 1984). The existence of these pollutants in wastewater bodies is of a major concern because their toxicity threatens humans and other life forms (Namasivayam and Yamuna, 1995).

While the removal of toxic heavy metals from industrial wastewaters has been practiced for several years, the effectiveness, and particularly the cost effectiveness, of the most common physical-chemical processes is limited. Biological materials have shown potential for heavy metal removal, but only low-cost biological materials with sufficiently high metal-binding capacity and selectivity for heavy metals are suitable for use in a full-scale biosorption process (Kratochvil and Volesky, 1998). Many microbial cells are known to adsorb metal ions on their cell walls or to accumulate metals in their cytoplasm. The biosorption method is recognized as having excellent selectiveness compared to the chemical treatment method; moreover, it does not produce toxic materials (Park et al., 2003).

Recent studies show that the strains isolated from contaminated soils and electroplating effluent-contaminated sludge have excellent capability of removing significant amounts of metals from both

aqueous solution as well as electroplating effluents (Malik, 2004). There has been a vast range of metals and strain combinations explored in various studies; therefore exploring the biodiversity of tolerant microorganisms and resistant strains in polluted water bodies is of a real interest. Good understanding of microbial sensitivity to heavy metals could enhance the application of microorganisms as indicators to the degree of pollution with heavy metals in a specific area. There is also a need to improve understanding of the mechanisms involved in transfer and mobilization of trace elements by microbiota and to conduct research on selection of microbial isolates growing on heavy metal contaminated sources to improve the chances of successful bioremediation.

The main objective of this study was to investigate the biodiversity of mycobiota in heavy metal polluted water bodies. Also to evaluate the tolerance of the common fungi isolated from the investigated sources to enhance their use in heavy metal bioremediation.

Materials and Methods

Site Description and Sample Collection

The main drainage channel in the western part of Delta region of Egypt starting at El-Gharbya and passing through Kafr-El Sheikh governorate to follow into the Mediterranean sea was selected as the large drainage channel in the Delta region. From this channel, sites 1-10 were studied. Other 16 small channels in the middle and eastern parts of the Delta region distributed in five governorates (Damietta, El-Dakahlia, El-Kalyobia, El-Monofeyia and El-Sharkya) were investigated (Figure 1). Water samples (500 ml/sample) were collected in sterilized glass bottles and were kept in a refrigerator until used. From each site, five separate samples were collected and were analyzed separately and the mean of the five samples was considered.

Estimation of Heavy Metal Concentration in Water Samples

Concentrations of six elements (Cd, Cu, Co, Mn, Pb and Zn) were determined from the water samples using Inductively Coupled Plasma-Mass Spectrometry (*Varian*, Model AA 55 at the Chemistry Department of Assiut University). All concentrations are expressed in ppm. All specimens were run in patches that included known standards, method blanks, and spiked specimens.

Isolation, Enumeration and Identification of Filamentous Fungi

Five ml of the water samples were filtered through sterile 0.45 µm membrane (47 mm diameter) cellulose nitrate filters. These filters were aseptically transformed onto

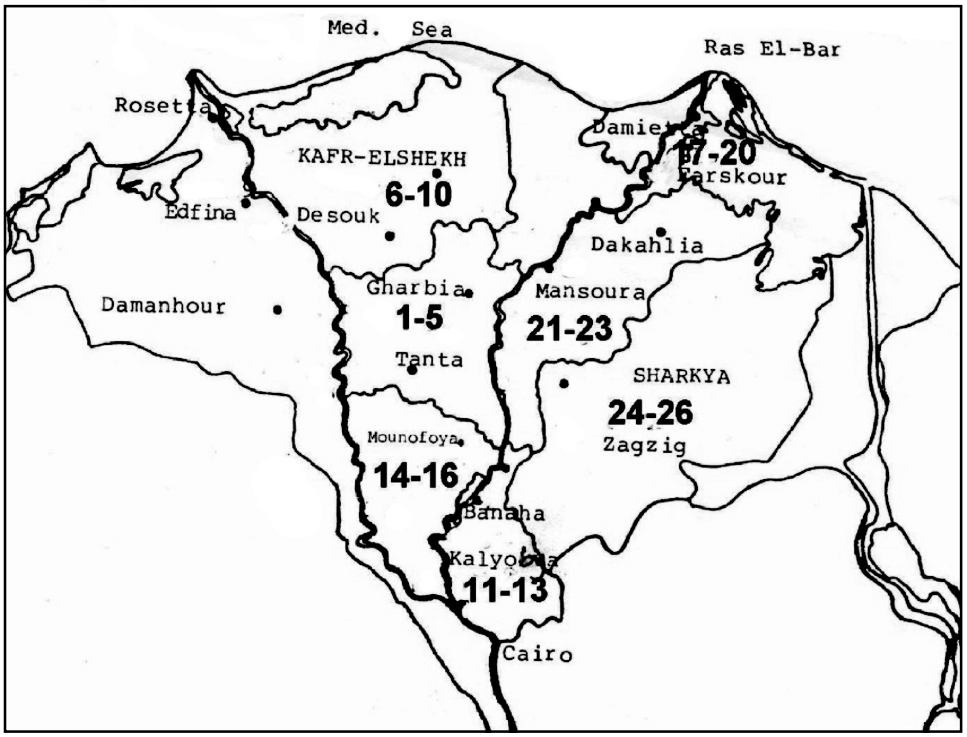

Figure 1: A map of Delta region of Egypt showing the number of selected sites where the water samples were collected from drainage water resources.

sterilized Perti plates to make the surface of the filter containing the spores upward and five replicates for each sample. Ten ml of Czapek yeast extract agar media which consisted of one litre distilled water, 1.0 g/l K_2HPO_4, 3.0 g/l $NaNO_3$, 0.5 g/l $MgSO_4.7H_2O$, 0.5 g/l KCl, 0.01 g/l $FeSO_4.7H_2O$, 20.0 g/l glucose, 2.0 g/l yeast extract, 3.0 g/l peptone and 15.0 g/l agar was added into each plate and then incubated at 28±1°C for seven days. During and after the incubation period the grown fungi were examined and identified.

Fungi were identified on the basis of macroscopic and microscopic features (Ellis, 1971; Moubasher, 1993; Raper and Fennell, 1965; Pitt, 1979; Pitt and Hocking, 1997).

Heavy Metal Tolerance of the Common Fungi

Fungal strains including *Aspergillus flavus* var. *flavus*, *A. fumigatus*, *A. niger*, *A. oryzae*, *A. terreus* var. *africanus*, *Mucor racemosus*, *Penicillium citrinum*, *Rhizopus stolonifer* and *Trichoderma viride* were tested for their resistance and growth in the presence of various concentrations (0, 50, 100, 150, 200, 250, and 300 ppm) of heavy metals (Zn, Pb and Cd). The growth rate of these organisms was determined by measuring the mycelial biomass according to the method described by Errasquín and Vázquez (2003) with some modifications. The fungus was grown in 250 ml Erlenmeyer flasks containing 50 ml of Sabouraud liquid medium (Scharlau) was used because of its comparatively low metal binding (García-Toledo et al., 1985). This medium consisted of 1% peptone and 2% dextrose, pH 5.8. Peptone, rather than other nitrogen-containing organic substrates. The medium was amended with progressively increasing concentrations (in steps of 50 ppm) of zinc, lead or cadmium until a lethal dose was achieved for each metal. The cultures were inoculated with 1 ml of spore suspension of 7-d-old colonies and incubated at 28±1°C in an orbital shaker at 150 rpm for five days. The mycelia were harvested by filtration through a nitrocellulose filter with a pore size of 0.45 µm (Millipore) and were dried overnight at 65°C to determine the dry weight. Triplicates of all assays were performed. This experiment was performed twice and the mean was taken in account.

Results and Discussion

Data in Table 1 reveal that all water samples collected from either the large or the small drainage channels were contaminated with heavy metals except for samples 7-26 which were devoid of Cu. Samples 2-4, 11-15 and 17-23 did not contain Co. The other four determined

Table 1: Heavy metals concentrations from the large drainage channel (1-10) and small drainage channels (11-26)

Site No.	*Concentration of heavy metal (ppm)*					
	Cd	*Mn*	*Zn*	*Pb*	*Cu*	*Co*
Large drainage channels						
1	0.014	0.665	0.002	0.104	0.008	0.015
2	0.015	0.047	0.053	0.050	0.015	0.000
3	0.019	0.060	0.016	0.143	0.016	0.000
4	0.015	0.076	0.012	0.303	0.014	0.000
5	0.025	0.231	0.033	0.462	0.018	0.090
6	0.017	0.114	0.037	0.528	0.007	0.035
7	0.015	0.219	0.036	0.803	0.000	0.051
8	0.064	1.135	0.034	1.075	0.000	0.079
9	0.043	0.202	0.038	1.278	0.000	0.042
10	0.026	0.193	0.055	1.496	0.000	0.225
Small drainage channels						
11	0.030	0.395	0.047	2.882	0.000	0.000
12	0.044	0.388	0.040	2.927	0.000	0.000
13	0.034	0.473	0.037	3.121	0.000	0.000
14	0.039	0.460	0.043	3.175	0.000	0.000
15	0.030	0.405	0.042	3.299	0.000	0.000
16	0.038	0.487	0.047	3.385	0.000	0.073
17	0.044	0.482	0.046	0.146	0.000	0.000
18	0.036	0.432	0.039	0.221	0.000	0.000
19	0.054	0.505	0.042	0.296	0.000	0.001
20	0.060	0.485	0.043	0.537	0.000	0.000
21	0.044	0.505	0.046	0.585	0.000	0.000
22	0.041	0.516	0.053	0.624	0.000	0.002
23	0.038	0.509	0.046	0.665	0.000	0.000
24	0.045	0.599	0.045	0.843	0.000	0.015
25	0.053	0.525	0.179	1.017	0.000	0.046
26	0.052	0.524	0.058	1.065	0.000	0.023

heavy metals (Cd, Mn, Zn and Pb) were detected but in various amounts in all samples. The highest values were detected in case of Pb in all samples and ranged from 0.221 up to 3.385 ppm. It was noticed that the large drainage channel contained relatively lower amount of Pb than small drainages. The concentration of Pb as well as the total concentration of all heavy metals in the large drainage channel gradually increased towards its outlet (Figure 2).

Mn was detected in considerable amounts in the majority of samples. Zn and Cd were determined in low values in both the large and small drainages. Regarding Cd, there was no obvious trend either to increase or to decrease among all sites. The main source of these heavy metals are various agricultural activities such as agrochemicals usage and long-term application of urban sewage sludge in agricultural soil, industrial activities

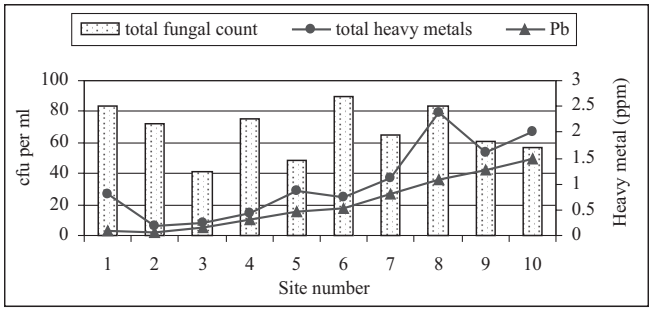

Figure 2: Total fungal count (cfu/5 ml), total heavy metals and Pb concentration (ppm) in the large drainage channel.

such as water disposal, waste incineration and vehicle exhaust (Ayres, 1992; Khan, 2005; Pal et al., 2006). Results indicated that Pb noticeably increased towards the outlet of the large drainage channel. This could be due to the intensive disposal of many factories' wastes directly into the channel's watercourse. This assumption was supported by Hong et al. (1994) who mentioned that lead has been used in large amounts for 2500 years and recently as a fuel additive.

Mycological analysis of water samples showed that 10 fungal genera comprising 25 species in addition to four species varieties were isolated and identified from 10 studied sites collected from the large drainage channel (Table 2). The total count of fungi irregularly fluctuated among all sites. There was no correlation between the total count of fungi and the level of either specific metal or total heavy metals determined in the large drainage channel (Figure 2). The highest total count was detected in site no. 6 (90 cful/5 ml) while the lowest total count was detected in site no. 3 (41 cfu/5 ml). *Aspergillus* was the most frequent genus. It was represented by 10 species and four species varieties and comprised 40% and 58% of the number of species and total count of fungi, respectively (Figure 3). Among the aspergilli, *A. flavus* var. *flavus*, *A. fumigatus*, *A. niger*, *A. tamarii*, *A. terreus* var. *africanus* and *A. terreus* var. *terreus* emerged in high remarkable occurrence (more than 50% of sites) (Table 2). *Penicillium* was the second prevalent genus and represented by six species. Only *P. chrysogenum* among all penicilli was found in high occurrence and recorded 34 cfu as a total count. *P. corylophilum* emerged in 50% of the sites but gave the highest total count of penicilli. *Mucor racemosus*, *Rhizopus stolonifer* and *Trichoderma viride* were among the prevalent genera where they were obtained from more than 50% of the sites. The genus *Fennellia* was represented by *F. flavipes* and *F. nivea* and they were encountered in moderate occurrence remark. *Gliocladium virens* was detected only in two sites whereas *Cladosporium cladosporioides* was isolated from only one site. Our findings are in agreement with those obtained by many authors, who reported the presence of these genera and species in water recourses in many latitudes (Khallil and Abdel-Sater, 1993; Steiman et al., 1995; Klich, 2001). Arvanitidou et al. (2002) reported that among the most prevailing filamentous fungi isolated from coastal water samples from northern Greek were *Penicillium, Aspergillus* and *Alternaria* spp.

From Table 3, it is clear that 35 fungal species and four species varieties belonging to 12 genera were isolated from 16 different sites from the small drainage channels. *Aspergillus* seemed to be the most prevailing genus. It was represented by 15 species and three species varieties.

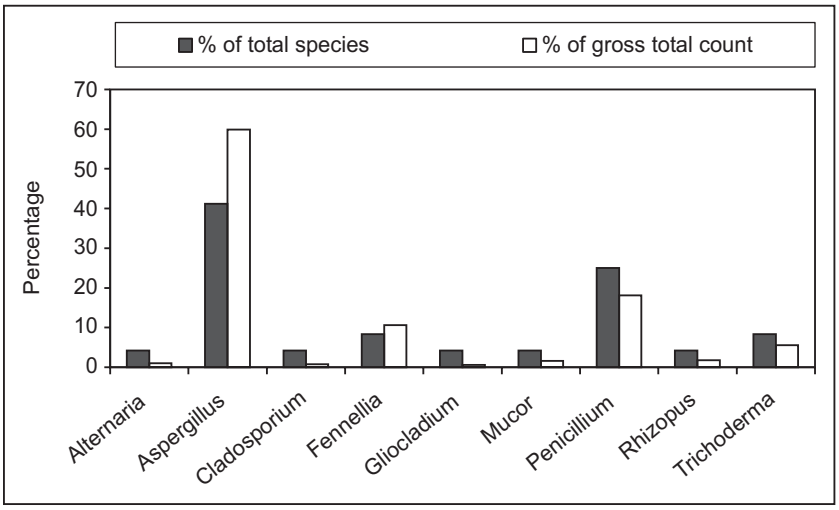

Figure 3: Percentage of species composition (%) and percentage of gross total count of genera isolated from the large drainage channel.

Table 2: Biodiversity, total count (TC in cfu/5 ml), number of cases of isolation (NCI) and occurrence remark (OR) of fungi isolated from the large drainage water channels affected by heavy metal pollution

Fungal species	*Site number*										*TC*	*NCI*	*OR*
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*			
Alternaria alternata (Fr.) Keissler	0	0	0	0	0	0	3	2	0	2	7	3	L
Aspergillus alutaceus Berkeley & Curtis	15	10	5	0	10	0	0	0	0	0	40	4	M
A. *flavus* var. *flavus* Link	30	30	0	2	15	20	10	0	10	5	122	8	H
A. flavus var. *columnaris* Raper & Fennell	0	0	0	2	0	5	0	0	3	0	10	3	L
A. fumigatus Fresenius	7	5	2	0	0	0	2	0	5	3	24	6	H
A. niger van Tieghem	10	3	2	7	5	5	5	5	5	5	52	10	H
A. oryzae (Ahlburg) Cohn	0	0	0	0	0	0	0	10	5	0	15	2	L
A. subsessilis Raper & Fennell	2	0	0	0	2	0	0	0	0	0	4	2	L
A. sydowii (Bainier & Sartory) Thom & Church	0	0	0	0	0	0	2	2	0	0	4	2	L
A. tamarii Kita	0	2	10	5	0	5	5	0	0	5	32	6	H
A. terreus var. *africanus* Fennell & Raper	0	0	0	5	0	5	9	5	5	5	34	6	H
A. terreus var. *terreus* Thom	10	5	5	10	5	5	6	10	5	5	66	10	H
A. terricola Marchal	0	0	2	1	0	0	0	0	0	0	3	2	L
Cladosporium cladosporioides (Fresen) De Vries	0	0	0	5	0	0	0	0	0	0	5	1	R
Fennellia flavipes Wiley & Simmons	0	0	0	5	3	0	5	5	0	3	21	5	M
F. nivea (Wiley & Sim.) Samson	0	0	0	0	0	20	0	15	10	6	51	4	M
Gliocladium virens Miller, Gidden & Foster	0	0	0	0	0	0	2	0	2	0	4	2	L
Mucor racemosus Fressenius	0	1	2	2	0	1	0	3	0	2	11	6	H
Penicillium chrysogenum Thom	5	5	8	3	3	0	5	0	0	5	34	7	H
P. corylophilum Diercks	0	0	0	10	0	20	5	10	5	0	50	5	M
P. funiculosum Thom	0	0	0	5	0	0	0	0	0	0	5	1	R
P. jensenii Zaleski	0	0	0	3	3	0	0	0	0	3	9	3	L
P. steckii Zaleski	0	0	0	0	0	0	0	5	0	5	10	2	L
P. waksmanii Zaleski	0	0	0	0	0	0	0	10	5	0	15	2	L
Rhizopus stolonifer (Ehrenb.) Lind	3	3	0	0	2	0	1	1	1	1	12	7	H
Trichoderma harzianum Rifai	0	8	5	10	0	0	0	0	0	0	23	3	L
T. viride Pers. ex Gray	2	0	0	0	0	4	5	0	2	2	15	5	H
Total count	84	72	41	75	48	90	65	83	61	57	678		
Number of species and varieties	9	10	9	15	9	10	14	13	13	14			
Number of genera	4	5	4	6	4	4	7	6	6	7			

* Occurrence remark (R=1, L = 2-3, M=4-5 and H=6-10 of number of cases of isolation)

Table 3: Biodiversity, total count (TC, cfu/5 ml), number of cases of isolation (NCI) and occurrence remark (OR) of fungi isolated from small drainage channels polluted with heavy metal

Fungal species	*Site number*																*TC*	*NCI*	*OR*
	11	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*			
Alternaria alternata (Fr.) Keissler	0	0	0	2	0	0	1	2	0	0	2	0	0	0	0	0	7	4	L**
Aspergillus alutaceus Berkeley & Curtis	0	0	0	0	0	7	0	0	0	0	0	0	2	2	0	0	11	3	L
A. candidus Link	0	0	0	0	0	0	1	0	0	0	0	2	0	0	9	0	12	3	L
A. carbonarius (Bainier) Thom	9	0	0	0	0	0	2	0	3	0	0	2	0	0	0	0	16	4	L
A. cervinus Massee	0	0	5	0	0	0	5	0	0	6	0	0	15	0	0	0	31	4	L
A. flavus var. *columnaris* Raper & Fennell	0	3	0	0	0	4	0	0	0	0	0	2	0	2	0	0	11	4	L
A. flavus var. *flavus* Link	3	7	0	4	7	6	5	5	12	5	8	13	7	10	3	2	97	15	H
A. fumigatus Fresenius	0	0	4	0	0	3	0	0	0	0	2	0	0	1	0	0	10	4	L
A. melleus Yukawa	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	3	2	R
A. niger van Tieghem	1	1	5	7	7	0	3	5	5	10	5	12	5	10	1	0	77	14	H
A. subsessilis Raper & Fennell	0	0	0	0	8	0	0	0	0	0	10	0	0	0	0	0	18	2	R
A. sulphureus (Fresenius)Thom & Church	0	0	0	0	0	1	0	1	0	0	2	0	0	2	0	0	6	4	L
A. terreus var. *terreus* Thom	0	0	0	0	0	0	0	0	8	0	0	0	0	0	5	0	13	2	R
A. terricola Marchal	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	3	2	R
A. ustus (Bainier)Thom & Church	0	0	0	0	0	1	0	0	0	0	0	2	0	3	5	0	11	4	L
A. versicolor (Vuillemin) Tiraboschi	0	0	0	0	0	0	0	5	0	0	0	0	2	0	0	0	7	2	R
A. wentii Wehmer	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	3	2	R
Botryotrichum piluliferum Sacc & Marchal	0	7		1	0	0	4	2	0	2	0	4	5	0	0		25	7	M
Clad. cladosporioides (Fresen) De Vries*	1	6	0	3	0	7	0	3	2	8	0	11	0	5	5	0	51	10	H
Cochliobolus hawaiiensis Alcorn	2	0	3	0	0	0	0	0	0	0	0	0	0	0	0	5	10	3	R
Fennellia flavipes Wiley & Simmons	0	1	0	0	4	0	4	6	0	10	3	10	0	1	0	0	39	8	M
Fus. chlamydosporum Wollenweber &Reinking*	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	3	2	R
F. dimerum Penzig	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	3	2	R
F. moniliforme Sheldon	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	R
Mucor racemosus Fressenius	2	3	0	5	2	2	0	0	3	2	5	1	5	0	3	1	34	12	H
Penicillium brevicompactum	15	0	0	6	16	10	0	0	0	0	0	11	22	0	10	2	92	8	M
P. chrysogenum Thom	2	0	2	0	0	1	0	0	5	0	0	0	0	0	0	0	10	4	L
P. citrinum Thom	12	0	10	10	0	10	12	13	12	12	12	14	6	0	10	2	135	13	H
P. islandicum Sopp	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2	1	R
P. steckii Zaleski	0	0	0	14	8	5	12	10	0	0	0	5	0	10	10	8	82	9	H
P. variabile Sopp	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	3	2	R
P. vinaceum Gilman & Abbott	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	R
Rhizopus stolonifer (Ehrenb.) Lind	0	2	0	3	0	0	2	1	4	1	0	2	0	0	4	2	21	9	H
Scopulariopsis brumptii Salvanet-Duval	0	0	2	0	0	0	0	2	0	0	0	0	1	0	5	0	10	4	L
Trichoderma harzianum Rifai	0	0	2	0	0	2	0	0	0	0	0	0	0	1	0	1	6	4	L
T. viride Pers. ex Gray	2	0	5	1	1	3	3	2	2	0	0	1	1	3	1	0	25	12	H
Total count	49	31	30	55	54	58	47	55	56	58	47	82	61	42	57	15	797		
Number of species and varieties	11	9	10	12	9	14	13	13	12	10	11	16	12	12	14	8			
Number of genera	6	6	6	8	6	5	7	9	6	7	5	9	6	5	7	6			

**Clad.* = *Cladosporium, Fus.* = *Fusarium* ** Occurrence remark (R=1-2, L = 3-5, M=6-8 and H=9-16 of number of cases of isolation).

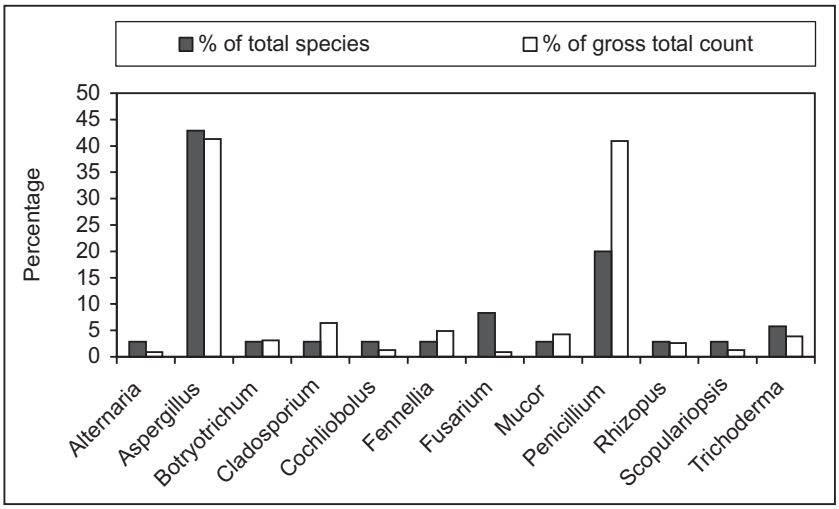

Figure 4: Percentage of species composition (%) and percentage of gross total count of genera isolated from the small drainage channels.

This genus comprised 40% of the total isolated species and 37% of the total count (Figure 4). The second prevalent genus was *Penicillium*. It was represented by six species and comprised 21% of the total species and 37% of the total count (Figure 4). *Cladosporium, Mucor, Rhizopus* and *Trichoderma* were among the prevalent genera that were recorded in high occurrence remarks. Each of these genera comprised 3% of the total species except for *Trichoderma* which comprised 6% of the total species. *Botryotrichum piluliferum* and *Fennellia flavipes* were isolated in moderate frequency and they had 25 and 39 cfu as total counts, respectively. *Alternaria alternata* and *Scopulariopsis brumptii* were isolated in low occurrence remark (25% of the sites). The other two genera *Cochliobulus* and *Fusarium* were rarely recovered (Table 3).

The similarity in biodiversity and fluctuation of the genera and species obtained from both the large and the small drainage channels greatly supported the presence of these organisms in these water bodies. These results are nearly similar with findings of other researchers from either in Egypt or many other countries (Bettucci and Roquebert, 1995; El-Nagdy, 2000). West (1986) demonstrated that the fungi isolated from potable water were dematiaceous (63%) and more specifically *Cladosporium* (27%), *Phoma* (9%), *Alternaria* and *Exophiala* (each 7%). Arvanitidou et al., (1999) indicated the prevailing genera encountered throughout the drinking water system and constituted a residual flora were *Penicillium, Aspergillus* and *Candida*. Some species of these genera were isolated from soft deposits in pipelines (Zacheus et al., 2001). El-Hissy et al. (1990) reported that the fungal population in water samples was mainly *Fusarium*, *Aspergillus* and *Penicillium*. Nasar and Munchi (1980) mentioned that the fungal population in freshwater pond of Bhagalpur (India) is mainly composed of the *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Epicoccum* and *Mucor*. We could assume that the basic similarity in prevalence and fluctuation of the obtained fungi isolated from both the large and the small channels ensure the presence of these fungi, predominantly waste water inhabitants.

Study of the tolerance of nine common fungal species to Cd, Pb and Zn indicated that all of these fungi could resist the presence of heavy metals but to various limits. Regarding their resistance, the tested fungi could be categorized into two groups (Figure 5a-i). The first group included *A. terreus* var. *africanus*, *A. fumigatus*, *A. flavus* var. *flavus*, *P. citrinum* and *M. racemosus*. The first species was the most sensitive to the three heavy metals (Figure 5a). A dramatic decrease in its growth rate was observed since the lower dose was applied. *A. fumigatus* showed some resistance against the heavy metals. Its growth gradually decreased until the lethal dose was reached (250 ppm) (Figure 5c). *A. flavus* var. *flavus* was more resistant comparable to the other two previous species especially to Zn (Figure 5d). Both *P. citrinum* and *M. racemosus* showed a similar response to the lower doses of the heavy metals (50 and 100 ppm), whereas *P. citrinum* was sharply affected. However *M. racemosus* resisted the higher doses of the heavy metal up to 250 or 300 ppm (Figure 5f and g). In this context, efficient Zn uptake by growing cells of *Aspergillus* spp. isolated from industrial waste has been reported (Sharma et al., 2000, 2002). Massaccesi et al. (2002) isolated *A. terreus* from industrially polluted sediments and applied it as Cd-

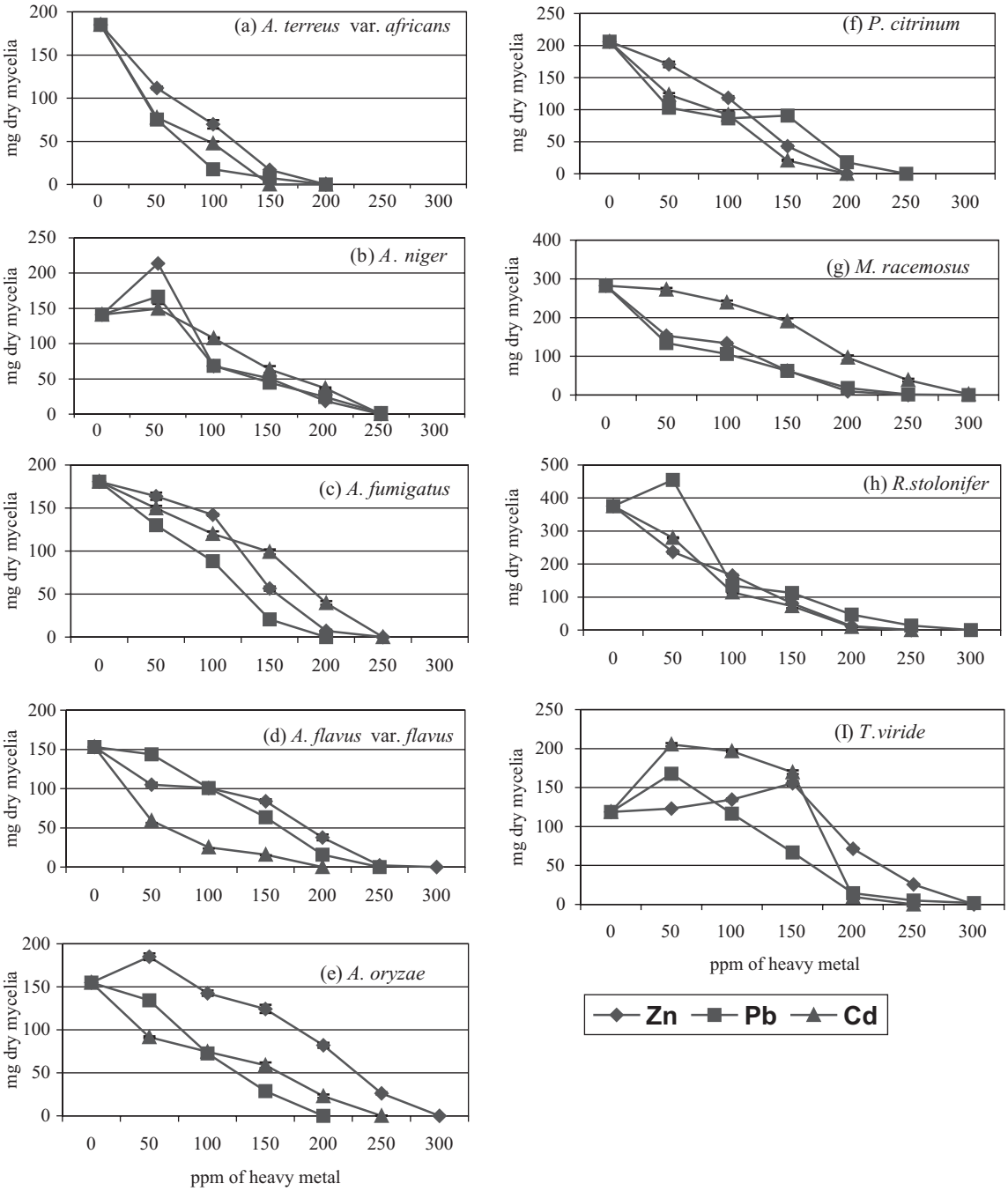

Figure 5a-i: Heavy metal tolerance of the common species isolated from the drainage channels.

resistant isolate which had 122 (ppm/g) as uptake efficiency of Cd in addition to *C. cladosporioides*, *Penicillium* spp and *Trichoderma koningii* which had efficiency to uptake Cd to 21, 37 and 82 (ppm/g), respectively.

The second group of the tested organisms included *A. niger*, *A. oryzae*, *R. stolonifer* and *T. viride*. The growth of these organisms was activated by lower dose(s) especially in case of Zn. The activation of the growth by many metals such as Zn could be attributed to the presence of Zn as a component in a variety of enzymes and DNA-binding proteins (Chou et al., 1988) or it may be complexed by various cellular components (Daniels et al., 1998). The growth of *A. niger* was accelerated by the low dose of all tested heavy metals (Figure 5b). However *T. viride* was activated by the first three doses (50-150 ppm) of both Zn and Cd. Growth of the organism was gradually declined to reach their death point (250-

300 ppm) (Figure 5i). Pal et al. (2006) isolated fungi belonging to *Aspergillus*, *Mortierella*, *Paecilomyces*, *Penicillium*, *Pythium*, *Rhizopus* and *Trichoderma* from serpentine soil of Andaman (India) as cobalt-resistant fungi and screened them for cobalt-resistance. He reported that 11 out of total 38 isolated fungi tolerated >6.0 mM Co(II).

The presence of *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus* and *Trichoderma* as common fungi in the drainage water resources and their efficiency to resist the presence of heavy metals in high concentrations (up to 300 ppm) could be recommended for using these species in bioremoval of heavy metal-polluted aqueous systems. Removal of metal ions from aqueous solutions has been studied with strains of *Penicillium* (Galun et al., 1983) , *Rhizopus arrhizus* (Tsezos and Velosky, 1982; Tobin et al., 1984), *Rhizopus oryzae* and *Aspergillus oryzae* (Huang and Huang, 1996). Recently, efficient Zn uptake by growing cells of *Aspergillus* spp. isolated from industrial waste has been reported (Sharma et al., 2000, 2002).

The present study showed that *T. viride* seemed to be the most tolerant fungus against Zn and Pb whereas *M. racemosus* was the most tolerant one to Cd. It could be supposed that both species may play an effective role in biosorption of multi metals from waste waters. The presence of these fungi and other common species in the drainage water resources could explain the low concentration of the various heavy metals in such water bodies. Also, complete absence of Cu and Co in many sites could be due to co-existence of these species. This assumption is in agreement with Malik (2004) who mentioned that use of multiple species consortia has proved advantageous for higher metal scavenging and more stability against environmental fluctuations. Valix et al. (2001) showed a growth pattern for fungi exposed to various heavy metals, which appears consistent with various strains investigated. He studied the tolerance of *Penicillium funiculosum*, *Aspergillus foetidus*, and *P. simplicissimum* for heavy metals, which could be leached, from nickel laterite ores (Ni, Co, Fe, Mg and Mn). These strains were exposed to heavy metals up to 2000 ppm and the efficiency of heavy metal uptake was varied among the different strains.

The fundamental recommendation of this study concludes that isolation of new heavy metal-tolerant fungal strains especially from polluted water has promising effect in bioremoval of heavy metal. Also, application of multi-species system could have high efficiency in bioremoval of different heavy metals from waste waters rather than application of single organism.

References

Arvanitidou, M., Kanellou, K., Constantinides, T.C. and V. Katsouyannopoulos (1999). The occurrence of fungi in hospital and community potable waters. *Lett. Appl. Microbiol.*, **29:** 81-84.

Arvanitidou, M., Kanellou, K., Katsouyannopoulos, V. and A. Tsakris (2002). Occurrence and densities of fungi from northern Greek coastal bathing waters and their relation with faecal pollution indicators. *Water Research*, **36:** 5127-5131.

Ayres, R.U. (1992). Toxic heavy metals: Materials cycle optimization. *Proc. Natl. Acad. Sci.* USA, **89:** 815-820.

Bettucci, L. and M. Roquebert (1995). Study of micro-fungi from tropical rain forest litter and soil: A preliminary study. *Nova Hedwigia,* **61:** 111-118.

Chou, A.Y., Archdeacon, J. and C.I. Kado (1998). Agrobacterium transcription regulator Ros is a prokaryotic zinc finger protein that regulates the plant oncogene *ipt. Proc. Natl. Acad. Sci.* U.S.A., **95:** 5293-5298.

Daniels, M.J., Turnercavet, J.S., Selkirk, K., Sun, H.Z., Parkinson, J., Sadler, P.J. and N.J. Robinson (1998). Coordination of Zn and Cd by prokaryotic metallothionein involvement of Hisimidazole. *J. Biol. Chem*., **273:** 22957-22961.

El-Hissy, F., Khallil, A.M. and M.A. El-Nagdy (1990). Fungi associated with some aquatic plants collected from freshwater areas at Assiut (Upper Egypt). *Journal of Islamic Academy of Science*, **3:** 298-304.

Ellis, M.B. (1971). Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England, 608.

El-Nagdy, M.A. (2000). Mycobiota of well waters in Assiut, Egypt. *Mycobiology*, **28(4):** 197-201.

Errasquín, E.L. and C. Vazquez (2003). Tolerance and uptake of heavy metals by *Trichoderma atroviride* isolated from sludge. *Chemosphere*, **50:** 137-143.

Galun, M., Keller, P., Malki, D., Feldstein, H., Galun, E., Siegel, S. and B. Siegel (1983). Recovery of Uranium(VI) from solution using precultured *Penicillium* biomass. *Water Air Soil Pollut*., **20:** 221-232.

García-Toledo, A., Babich, H. and G. Stotzky (1985). Training of *Rhizopus stolonifer* and *Cunninghamella blakesleeana* to copper: Cotolerance to cadmium, cobalt, nickel and lead. *Can. J. Microbiol*., **31:** 485-492.

Hong, S.M., Candelone, J.P., Patterson, C.C. and C.F. Boutron (1994). Greenland ice evidence of hemispheric lead pollution, two millennia age by Greek and Roman civilization. *Science*, **265:** 1814-1843.

Hsu, M.J., Selvaraj, K. and G. Agoramoorthy (2006) Taiwan's industrial heavy metal pollution threatens terrestrial biota. *Environmental Pollution*, **143:** 327-334.

Huang, C. and C.P. Huang (1996). Application of Aspergillus oryzae and Rhizopus oryzae for Cu(II) removal. *Water Res*., **30:** 1985-1990.

Kapoor, A., Viraraghavan, T. and D.R. Cullimore (1999). Removal of heavy metals using the fungus *Aspergillus niger*. *Biores Technol*., **70:** 95-104.

Khallil, A.M. and M.A. Abdel-Sater (1993). Fungi from water, soil, and air polluted by industrial effluents of Manquabad superphosphate factory (Assuit, Egypt). *J. Basic Microbiol.* **31:** 83-100.

Khan, A.G. (2005). Role of soil microbes in the rhizosphere of plants growing in trace metal contaminated soils in phytoremediation. *Journal of Trace Elements in Medicine and Biology* **18:** 355-364.

Klich, M.A. (2001). Identification of common Aspergillus species, United States Department of Agriculture, Agricultural Research Service, Southern Regional Research Center, New Orleans, LA. *In:* Abrusci, C. Martín-Gonzáalez, A. Del Amo, A. Catalina, F. Collado, J., Platas, G. (2005). Isolation and identification of bacteria and fungi from cinematographic films. *International Biodeterioration & Biodegradation*, **56:** 58-68.

Kratochvil, D. and B. Volesky (1998). Advances in the biosorption of heavy metals. *Trends Biotechnol*, **16:** 291-300.

Malik, A. (2004). Metal bioremediation through growing cells. *Environment International*, **30:** 261-278.

Massaccesi, G., Romero, M.C., Cazau, M.C. and A.M. Bucsinszky (2002). Cadmium removal capacities of filamentous soil fungi isolated from industrially polluted sediments, in La Plata (Argentina). *World J. Microbiol Biotechnol*, **18:** 817-820.

Moore, J. W. and S. Ramamoorthy (1984). Heavy Metals in Natural Waters, pp. 28-57, 77-99 and 182-204. Springer-Verlag, New York.

Moubasher, A.H. (1993). Soil Fungi in Qatar and other Arab countries. The Scientific and Applied Research Centre, University of Qatar, Doha, Qatar. 566 p.

Namasivayam, C. and R.T. Yamuna (1995). Waste biogas residual slurry as an adsorbent for the removal of Pb(II) from aqueous solution and radiator manufacturing industry wastewater. *Bioresources Technol*., **52:** 125-131.

Nasar, S.A. and J.D. Munchi (1980). Studies on the seasonal variations in the fungal population of freshwater pond of Bhagalpur, India. *Imnologica* (Berlin), **12(1):** 137-139.

Pal, A., Ghosh, S. and A.K. Paul (2006). Biosorption of cobalt by fungi from serpentine soil of Andaman. *Bioresource Technology*, **97:** 1253-1258.

Park, J.K, Lee, J.W. and J.Y. Jung (2003). Cadmium uptake capacity of two strains of *Saccharomyces cerevisiae* cells. *Enzyme and Microbial Technology*, **33:** 371-378.

Pitt, J.I. and A.D. Hocking (1997). Fungi in Food Spoilage. Academic Press, London.

Pitt, J.I. (1979). The Genus Penicillium and its Teleomorphic States *Eupenicillium* and *Talatomyces*. Academic Press, London.

Raper, K.B. and D.I. Fennell (1965). The Genus Aspergillus. Williams & Wilkins, Baltimore.

Sharma, S., Dastidar, M.G. and T.R. Sreekrishnan (2002). Zinc uptake by a fungal biomass isolated from industrial wastewaters. ASCE: Practice Period Toxic Hazard Radioact *Waste Manage*, **6(4):** 256-261.

Sharma, S., Dastidar, M.G. and T.R. Sreekrishnan (2000). Zinc uptake by an isolated fungus. Proc. IIChE Cong, Calcutta, India, vol. 2. Calcutta: Indian Institute of Chemical Engineers; December, 113-115.

Steiman, R., Guiraud, P., Sage, L., Seigle-Murandi, F. and J.L. Lafond (1995). Mycoflora of soil around the Dead Sea I—Ascomycetes (including *Aspergillus* and *Penicillium*), Basidiomycetes, Zygomycetes System. *Appl Microbiol*., **18:** 310-317.

Tobin, J.M., Cooper, D.G. and R.J. Neufeld (1984). Uptake of metal ions by Rhizopus arrhizus. *Appl Environ Microbiol*., **47:** 821-824.

Tsezos, M. and B. Velosky (1982). The mechanism of uranium iosorption by Rhizopus arrhizus. *Biotechnol Bioeng*., **29:** 385-401.

Valix, M., Tang, J.Y and R. Malik (2001). Heavy metal tolerance of fungi. *Minerals Engineering*, **14:** 499-505.

West, P.R. (1986). Isolation rates and characterization of fungi in drinking water distribution systems. *In:* Proceedings of the Water Quality Technol. Conference. AWWA, Denver, CO.

Zacheus, O.M., Lehtola, M.J., Korhonen, L.K. and P.J. Martikainen (2001). Soft deposits, the key site for microbial growth in drinking water distribution networks. *Water Res*., **35:** 1757-1765.