

EFFECT OF TREATMENT WITH LEAD SULPHATE ON SOIL MYCOBIOTA

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Abstract: Nineteen species of fungi were isolated from control soils and that treated with lead sulphate solutions (20ppm, 40ppm, 100ppm and 250ppm) for 90 days. Treatment with lead sulphate did not result in substantial decrease in the number of species isolated. Greater number of isolates was obtained from Pb-treated soils except in general. The species which could tolerate higher concentration of lead sulphate for 90 days included *Aspergillus flavus*, *Aspergillus ustus*, *Aspergillus niger* and *Trichoderma lignorum*. *Aspergillus fumigatus* and *Botryotrichum piluliferum* exhibited remarkable resistance to lead as these dominated the soil treated with lead sulphate solution for 90 days.

Keywords: Heavy metals, Lead pollution, Metal tolerant fungi, Soil microflora.

INTRODUCTION

The pollution by heavy metals is amongst the major environmental problems (Dushenkov *et al.*, 1995; Piccardo *et al.*, 2009; Nawachukwu *et al.*, 2010). Considerable amounts of heavy metals, including lead, are introduced into aquatic systems as a result of mining, smelting, printing, battery-manufacturing, electroplating, tanning etc. Beyond a certain limit, these metals exert adverse effects on the environment and ultimately accumulate in living tissues through the food chain with human at its top. Hence, these metals need to be removed before these enter the complex ecosystem. Physico-chemical treatment procedures developed to deal with much diluted metal-containing effluents (precipitation, flocculation, coagulation, ion-exchange etc.) are very expensive (Lacina, 2003). The ability of fungi to serve as biotrap for heavy metals and biosorb them has attracted the attention of a number of workers for purification of such effluents (Gadd and Griffiths, 1978; Hemapriya *et al.*, 2010; Can and Jialong, 2010). The purification of the metal-containing water using fungal biomass is not only cheaper but also offers the following advantages: (i) fast removal, (ii) production of small residual volume, (iii) easy installation of the process, (iv) recovery of metals from the treatment columns. Soil constitutes an excellent reservoir of immense variety of fungi. It would be, therefore, pertinent to explore the possibilities of getting suitable fungal strains from soil which are capable of removing metals from the wastewater effectively. Out of many fruitful approaches for obtaining metal-resistant microbes, one is to treat the soil with given pollutant and to isolate microbes which are able to survive it (Antonovics *et al.*, 1971). The present communication deals with the effect of lead sulphate treatment on soil mycobiota with the aim to obtain the soil fungal strains capable of surviving lead sulphate pollution with an ultimate aim to utilize these for mitigating lead pollution.

MATERIAL AND METHOD

Forty five plastic pots of 150 ml capacity were filled with 100 gm soil each. These were divided into five sets of nine pots each. Nine pots of set 1 were treated with 25 ml of distilled water at regular intervals of 7 days for a total period of 12 weeks. This set served as control. The nine pots of set 2, nine pots of set 3, nine pots of set 4 and nine pots of set 5 were treated similarly but with 20 ppm, 40 ppm, 100 ppm and 250 ppm solutions of lead (as lead sulphate) respectively (instead of distilled water). A separate sub-sample was analyzed for soil mycobiota using dilution plate method (Waksman, 1927).

After 30 days, soils from three pots of set 1 were mixed thoroughly but aseptically to obtain a composite sample. Similar composite samples were obtained for set 2, 3, 4 and 5. Each composite soil sample so obtained was analyzed for mycobiota using dilution plate method. 20 grams of soil from a given composite sample were transferred to 20 ml of sterilized distilled water and stirred for 30 minutes to wash fungal propagules from the material. 10 ml of this suspension were immediately transferred to a conical flask containing 90 ml of sterilized distilled water. This suspension (1 : 100 dilution) was used for preparation of further dilution of 1 : 1000. From the suspensions of each of the 1 : 100 and 1 : 1000 dilution, one ml aliquots were transferred to each of a set of 3 Petri dishes followed by the addition of 20 ml of cooled and sterilized Potato-Dextrose Agar Medium (Raper and Thom, 1949) with 30ppm Rose Bengal and 30 mg of Streptomycin. The Petri dishes containing the medium and the inocula were incubated at 25 °C for 6–8 days. The total numbers of colonies of individual fungal species growing in each Petri dish were recorded. The fungal strains obtained were identified using standard keys (Gilman, 1957; Subramanian, 1971; Nagmani *et al.*, 2006).

RESULT AND DISCUSSION

In all, 19 species of fungi were isolated in the present study. The data concerning the total isolates (TI) and

percentage isolates (PI) of fungi obtained at the beginning of the experiment as also after 30 days, 60 days and 90 days for control as well as various treatments are presented in the tables 1 to 3. Out of the nineteen species, only one belongs to Zygomycota. The remaining eighteen species were anamorphic fungi (Deuteromycota). The dominance of Deuteromycota observed in the present study is in full agreement with the earlier reports (Hudson, 1968; Dickinson and Pugh, 1974; Hayes and Lim, 1979; Charaya, 2006; Tiwari and Charaya, 2006; Sen and Charaya, 2010). Only one member belonging to the order Mucorales was isolated in the present study, thus, supporting the findings of Galloway (1935), Singh and Charaya (1975), Dube *et al.* (1980), Singh (2004) and Charaya (2006) which indicated that there is paucity of mucorales in the tropical regions of India. Among the Deuteromycota,

the Hyphomycetes constituted the major component. Nine species belonging to Moniliaceae and seven species belonging to the Dematiaceae were obtained. Among the Moniliaceae, six species belong to the genus *Aspergillus* while only two belong to genus *Penicillium*. It is widely believed that Aspergilli are more common in the warmer regions of the world while the Penicillia are more abundant in the colder regions. The results of the present study as also those carried out by a number of workers (Waksman, 1917; Jensen, 1931; Singh and Charaya, 1975; Charaya, 2006; Sen *et al.*, 2009) support the aforementioned generalization.

Out of initial isolates, eight fungal species i.e. *Alternaria alternata*, *A. citri*, *Aspergillus luchuensis*, *Cladosporium herbarum*, *Curvularia lunata*, *Fusarium* sp., *Helminthosporium nodulosum*

Table 1. Mycobiota isolated from control soil as well as that amended with lead sulphate (20ppm, 40ppm, 100ppm and 250ppm) after 30 days of treatment

Fungal Species	Initial		Control		Treatment								
	TI	PI	TI	PI	20 ppm		40 ppm		100 ppm		250 ppm		
<i>Alternaria alternata</i>	4	2.38	-	-	-	-	-	-	-	-	-	-	-
<i>Alternaria citri</i>	2	1.19	-	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus flavus</i>	22	13.1	4	2.58	1	0.52	1	1.07	5	8.06	5	4.23	
<i>Aspergillus fumigatus</i>	53	31.5	29	18.70	16	87.83	67	72.0	26	41.93	87	73.72	
<i>Aspergillus luchuensis</i>	8	4.76	-	-	-	-	-	-	-	-	-	-	
<i>Aspergillus niger</i>	18	10.71	20	12.90	7	3.70	10	10.7	7	11.29	14	11.86	
<i>Aspergillus terreus</i>	-	-	3	1.93	2	1.05	2	2.15	-	-	1	0.84	
<i>Aspergillus ustus</i>	13	7.73	10	6.45	-	-	-	-	-	8.06	1	0.84	
<i>Botryotrichum atrogriseum</i>	-	-	-	-	2	1.05	1	1.07	1	1.61	-	-	
<i>Botryotrichum piluliferum</i>	19	11.30	85	54.83	9	4.76	11	11.8	16	25.80	9	7.62	
<i>Cladosporium herbarum</i>	3	1.78	-	-	-	-	-	-	-	-	-	-	
<i>Curvularia lunata</i>	1	0.59	-	-	-	-	-	-	-	-	-	-	
<i>Fusarium</i> sp.	3	1.78	-	-	-	-	-	-	-	-	-	-	
<i>Helminthosporium nodulosum</i>	1	0.59	-	-	-	-	-	-	-	-	-	-	
<i>Penicillium implicatum</i>	7	4.2	-	-	-	-	-	-	-	-	-	-	
<i>Penicillium</i> sp.	-	-	2	1.29	-	-	-	-	-	-	-	-	
<i>Rhizopus</i> sp.	6	3.57	-	-	1	0.52	-	-	-	-	-	-	
<i>Trichoderma lignorum</i>	-	-	1	0.64	1	0.52	1	1.07	1	1.61	-	-	

White sterile mycelia	8	4.76	2	1.35	8	3.14	4	1.63	9	3.98	3	1.84
No. of Species	15	-	7	-	7	-	6	-	7	-	6	-
Total Isolates	168	-	148	-	254	-	245	-	226	-	163	-

Konopka *et al.* (1999) reported that the populations of culturable microbes were lower in lead contaminated soils. On the contrary, McGrath (2001) suggested that lead is insoluble in soils and, therefore, it is unlikely to be bioavailable; consequently it does not have any adverse effect on soil microbiota. Sen *et al.* (2009) observed that the addition of lead as lead nitrate to the culture medium upto 200 ppm concentrations did not have much effect on the number of species isolated from the soil, though at 400 ppm lead concentrations, the number of species as well fungal population were markedly reduced. On the other hand, Tiwari (2010) used 200 ppm and 500 ppm solutions of lead as lead nitrate and recorded substantial reduction in the total number of fungal isolates as well as mycodiversity in lead nitrate-treated soils as compared to the control soils. In the present study, 20 ppm, 40 ppm, 100 ppm and 250 ppm solutions of lead sulphate were used. The results of the present study agree to a marked

extent with that of Sen *et al.* (2009) in that she also failed to record any adverse effect on mycodiversity upto 200 ppm concentrations with lead nitrate. A number of reports (Hutchinson, 1973; Smith, 1977; Henriksson and DaSilva, 1978; Sen *et al.*, 2009) exist regarding the stimulatory effect of lead pollution on microorganisms and their activity. Sen *et al.* (2009) reported that 40 ppm concentration of lead resulted in noticeable stimulatory effect on the radial growth of *Aspergillus candidus*, *A. niger*, *Penicillium implicatum*, *Sepedonium chrysospermum* and *Trichoderma lignorum*. Kumar (2011) demonstrated that the *in vitro* growth of *Aspergillus fumigatus* and *A. niger* were stimulated by lead sulphate upto 800 ppm. Increase in the fungal population with low concentrations of lead sulphate after 30 days of treatments, in the present study, is an agreement with the observations of above cited workers to a considerable extent.

Table 3. Mycobiota isolated from control soil as well as that amended with lead sulphate (20ppm, 40ppm, 100ppm and 250ppm) after 90 days of treatment.

Fungal Species	Initial		Control		Treatment							
	TI	PI	TI	PI	20 ppm		40 ppm		100 ppm		250 ppm	
	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI
<i>Alternaria alternata</i>	4	2.38	-	-	-	-	-	-	-	-	-	-
<i>Alternaria citri</i>	2	1.19	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus flavus</i>	22	13.1	11	8.66	16	8.98	1	0.40	59	22.77	7	4.29
<i>Aspergillus fumigatus</i>	53	31.5	37	29.13	59	33.14	56	22.95	104	40.15	53	32.51
<i>Aspergillus luchuensis</i>	8	4.76	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus niger</i>	18	10.71	3	2.36	6	3.37	5	2.04	10	3.86	4	2.45
<i>Aspergillus terreus</i>	-	-	-	-	2	1.12	-	-	-	-	-	-
<i>Aspergillus ustus</i>	13	7.73	6	4.72	3	1.68	11	4.50		1.54	3	1.84
<i>Botryotrichum atrogriseum</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Botryotrichum piluliferum</i>	19	11.30	65	51.18	87	48.87	161	65.98	71	27.41	79	48.46
<i>Cladosporium herbarum</i>	3	1.78	-	-	-	-	-	-	-	-	-	-
<i>Curvularia lunata</i>	1	0.59	-	-	-	-	-	-	-	-	-	-
<i>Fusarium sp.</i>	3	1.78	-	-	-	-	-	-	-	-	-	-
<i>Helminthosporium nodulosum</i>	1	0.59	-	-	-	-	-	-	-	-	-	-
<i>Penicillium implicatum</i>	7	4.2	-	-	-	-	-	-	-	-	-	-
<i>Penicillium sp.</i>	-	-	2	1.57	-	-	-	-	-	-	-	-
<i>Rhizopus sp.</i>	6	3.57	-	-	-	-	-	-	-	-	-	-
<i>Trichoderma</i>	-	-	3	2.36	-	-	-	-	-	-	1	0.61

<i>lignorum</i>												
White sterile mycelia	8	4.76	-	-	5	2.80	10	4.09	11	4.24	16	9.81
No. of Species	15	-	7	-	7	-	6	-	6	-	7	-
Total Isolates	168	-	127	-	178	-	244	-	259	-	163	-

Another interesting result obtained in the present study is that as the number of days increased (Tables 2 and 3), the fungal populations in the soils treated with higher concentrations of lead sulphate were more than that in control soils. Babich and Stotzky (1982) suggested that the levels of a pollutant which are lethal to a majority of microbes may only cause mutation in some and thereby increase the selection of the strains which can tolerate the higher concentrations of the pollutant. The subsequent survival and multiplication of these strains might have lead to an increase in the populations of such strains resulting in a total positive effect on the fungal population.

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