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Influence of chromium (III) contaminated soil on soil mycobiota

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ABSTRACT

Investigation was conducted on the experimental fields of CCS University (Meerut) during 2013–2014 to evaluate the influence of Cr (III) contamination on soil mycobiota and to obtain some Cr (III)-resistant strains for the management of Cr (III)-contaminated soils and of the effluents carrying the metal. Blocks (30cm × 30cm) each were treated with different concentrations (500 ppm/1000 ppm/2000 ppm) of chromium (III) nitrate or chromium (III) sulphate solution separately in triplicates. Three blocks served as control. The soil samples collected aseptically from control and treated blocks after 20, 40 and 60 days were analysed for mycobiota using serial dilution plate and soil plate methods. Overall dominance of anamorphic fungi and paucity of mucoraceous fungi was observed amongst the fifty two species of fungi isolated. *Aspergillus niger* was most tolerant to Cr (III) probably due to binding of Cr (III) by amide, amine and C=S groups on the fungus as revealed by FTIR spectroscopy. Reciprocal relationship between pollutant concentration 'c' and time 't' (i.e. constancy of c×t) did not hold true; such a relationship might be operative for a single species *in-vitro* systems. Cr (III) salts adversely affected the mycobiota (qualitatively and quantitatively) though not to the extent reported for Cr (VI). The results indicate that though soil fungal diversity is adversely affected by Cr (III) contamination, the surviving species flourish over a period of time leading to the partial recovery of the mycopopulation. *Aspergillus niger* biomass with Cr (III)-binding functional groups might be utilized for *in situ* management of Cr (III) in soils and in biosorption-based effluent treatment systems.

Key words: *Aspergillus niger*, Cr (III) nitrate, Cr (III) sulphate, FTIR spectroscopy, Metal-tolerant fungi

The contamination of surface water, ground water as well as the soils by the heavy metals carried along with industrial effluents (Atkinson *et al.* 1998, Srinivas *et al.* 2002) ultimately lead to increase in the concentrations of heavy metals in the environment to a point where these are likely to pose serious threat to human health (Dixit *et al.* 2015). Chromium, which exists in different oxidation states, is one such heavy metal with significant toxicological properties (Joutey *et al.* 2015). Since Cr (VI) is considered to be most toxic of these states, it has drawn the attention of most of the workers, though a limited number of studies have been carried out with respect to the Cr (III) also (Sekhar *et al.* 1998, Akthar and Mohan 1995, Ebner *et al.* 2002, Mapoleno and Torto 2004). In fact, Cr (VI) in soil is also reduced to Cr (III) which is not available to plants (Wyszkowska 2002). The higher levels of Cr (III) can also be quite fatal and damaging for environment (Jeevitha and Sumathi 2012). Chromium (III) compounds, such as chromium (III) sulphate and chromium (III) nitrate are used (i) as pigments; (ii) as catalysts; (iii) as tanning agents in tanning industry; (iv) in the production of pure chromium metal and chromium (VI) compounds; (v) in the preparation of refractory bricks, and (vi) in textile printing operations (Anonymous 2008). Obviously, the effects of Cr (III) compounds on soil fungi need to be evaluated because of their multi-dimensional role in biogeochemical cycles as well as in the maintenance of soil fertility and agricultural productivity. It has also been suggested that the fungal strains obtained from the soils exposed to a given toxic metal are more efficient at biosorption as compared to those obtained from normal or untreated sites (Ezzouhri *et al.* 2009, Mukherjee *et al.* 2013). Also, during the past few decades, fungal biomass has come to be recognised as highly economic option for effluent treatment through biosorption (Butter *et al.* 1998, Addour *et al.* 1999). This biosorptive property of mycomass may possibly be utilised for *in-situ* management of soil contaminants including Cr (III). The present communication deals with the effect of two compounds of Cr (III), i.e. chromium (III) sulphate and chromium (III)nitrate on soil fungal diversity. The study also aimed at obtaining Cr (III)-resistant fungal strains which might facilitate the management of Cr (III) levels in soils and effluents.

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MATERIALS AND METHODS

The amendment of soil with different concentrations of

pollutants for evaluating their effects on soil mycobiota was performed according to the approach suggested by Babich and Stotzky (1982). Twenty one small blocks (30 cm × 30 cm each) were demarcated in a small plot located at the experimental fields of the Department of Botany, CCS University Campus, Meerut during 2013–2014. Each block was lined with a polythene sheet along the edges (upto 45 cms depth) so as to minimize the interference amongst the blocks receiving different kinds of treatments. Out of the 21 blocks (a) three blocks were reserved for the control, (b) nine for treatment with aqueous solution of chromium (III) sulphate and (c) nine for treatment with aqueous solution of chromium (III) nitrate. Out of the nine blocks allotted for chromium (III) sulphate solution, three each were treated with 500 ppm, 1000 ppm and 2000 ppm concentration of the solution. Similarly, nine blocks were used for amendment with chromium (III) nitrate solutions (three each for 500 ppm, 1000 ppm and 2000 ppm concentrations). The allotment of blocks for receiving the treatments and for control was subject to completely randomized design (CRD). Each block was treated with sufficient quantities (two litres of given metal solution) regularly at weekly intervals for 60 days. The three control blocks were treated with distilled water instead of metal solution.

The soil samples to be analysed for mycobiota were collected from each of the control as well as treated block separately and aseptically after 20, 40 and 60 days. The samples from the three control blocks collected on a given day (20th/ 40th/ 60th day) were pooled together and mixed thoroughly aseptically to obtain a composite sample. Similarly, three composite samples were prepared for blocks treated with chromium (III) sulphate (one composite sample each for 500 ppm, 1000 ppm and 2000 ppm concentrations). In the same manner, three composite samples were prepared for soils treated with chromium (III) nitrate on each sampling day (20th/ 40th/ 60th day).

Serial dilution plate method (Waksman 1922) as followed by Dube *et al*. (1980) was used to obtain a picture of mycobiota comprising each composite sample. Serial dilutions of 10^{-2}, 10^{-3} and 10^{-4} were prepared for each composite sample. Potato Dextrose Agar Medium (Raper and Thom 1949) amended with 30 ppm Rose Bengal and 30 ppm streptomycin was used. The Petri dishes with the medium and the inocula were incubated at 25 ± 1^0C for 5 days. Since serial dilution plate method, in case of the fungi, favours the strains which produce abundant spores, each composite sample was analysed by soil plate method also (Warcup 1950). For this, 5 mg of a given composite sample and the Potato Dextrose Agar medium were used. The Petri dishes were incubated at 25 ± 1^0C for 6 to 8 days. The fungal strains were transferred to the Petri plates containing fresh medium to facilitate identification and for preparing axenic cultures.

Since FTIR spectroscopic data of fungal biomass might provide an idea of its biosorptive efficiency (Charaya *et al.* 2010), the biomass of *Aspergillus niger* van Tieghem (most dominant fungal species withstanding Cr(III) toxicity in the present study) was subjected to FTIR spectroscopic analysis. The biomass of *Aspergillus niger* van Tieghem for FTIR spectroscopic analysis was prepared by inoculating 5 flasks (of 1000 ml capacities) each containing 150 ml MGYP medium (Malt 3 g, Glucose 10 g, Yeast extract 3 g and Peptone 5 g; made up to 1 litre with water) with 10 ml of spore suspension of *Aspergillus niger*. After 10 days of incubation at 25 ± 1^0C, the biomass of *Aspergillus niger* was dried in an oven at $60^0C\pm1^0C$ for 24 hours followed by powdering with the help of a mortar and pestle. Two mg of the powder was mixed with 98 mg of dry powdered KBr (IR spectroscopy grade, Himedia). The mixture was used to prepare pellets by applying pressure of 10 000 to15 000 psi using (PG- Hydraulic Press). The IR spectrum was recorded on IR-affinity-1, Shimadzu spectrophotometer high resolution (≤ 0.001/cm).

The data relating to the effect of chromium (III) treatment on qualitative and quantitative variations in the mycobiota over different periods of time were subjected to analysis of variance and 't' test. Simpson's indices of diversity (Okpiliya 2012) were calculated for evaluating the species diversity.

RESULTS AND DISCUSSION

A total of 52 species of fungi were isolated from the control soils as well as those treated with chromium solutions (Tables 1 and 2) using dilution plate method. Out of these, only 47 sporulated. Only three belong to Zygomycota and one to Ascomycota while the remaining 43 species were anamorphic fungi. The results of the present study, thus, (a) support the findings of Galloway (1935), Dube *et al.* (1980) and Charaya (2006) indicating the paucity of Mucorales in the tropical regions of the world; and (b) conform to the widely held view that Aspergilli are more common in the warmer regions of the world as compared to the Penicillia which are more common in the colder regions (Waksman 1917, Singh and Charaya 1975), as well as by Kumar and Charaya (2012). Soil plate method yielded additional five fungal species, *i.e. Botrytis* sp, *Choanephora* sp, *Mucor* sp, *Sporotrichum chlorinum* and *Scopulariopsis* sp. though these were restricted to soil samples collected up to 60 days only. *Aspergillus niger*, however, appeared to be most resistant species (Table 3).

In the present study, the soils were given *in-situ* treatments of pollutants (in the field itself) and the samples collected periodically from the site itself were analysed for fungal biota; the study yielded as many as 52 different species of fungi. However, Tiwari and Charaya (2006) could obtain only 23 fungal species probably because of a different approach been followed. In the latter case, the soils were filled in the pots and were subsequently given treatment with chromium sulphate. It was suggested that the transfer of the soil to the pots might have led to a sizeable decrease in the number of species that could be isolated since the soil gets substantially disturbed during drying, sieving and transfer to pots. This might lead to alterations in resource availability and system structure (Pickett and White 1985)

Table 1 Qualitative and quantitative distribution of mycobiota in soils control as well as treated with 500 ppm, 1000 ppm and 2000 ppm concentrations of chromium (III) sulphate over a period of 60 days (as obtained by dilution plate method)

Fungal species	20 Days								40 Days								60 Days							
	Control		500ppm		1000ppm		2000ppm		Control		500ppm		1000ppm		2000ppm		Control		500ppm		1000ppm		2000ppm	
	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI
Alternaria citri	1	0.86																						
Alternaria sp.																	4	2.53	7	6.66				
Aspergillus flavus	13	11.20	15	19.23	21	25.92	8	13.11	12	12.76	6	10.16	6	8.95	2	3.63	9	5.69	2	1.90				
Aspergillus fumigatus	11	9.48	9	11.53	6	7.40	11	18.03	7	7.44	9	15.25	11	16.41	3	5.45								
Aspergillus luchuensis	19	16.37	5	6.41	2	2.46	9	14.75	9	9.57	5	8.47	8	11.94	6	10.90	29	18.35	12	11.42	11	13.92	15	32.60
Aspergillus niger	36	31.03	37	47.43	46	56.79	22	36.06	29	30.85	32	54.23	39	58.20	41	74.54	46	29.11	42	40	41	51.89	31	67.39
Aspergillus wentii																	5	3.16						
Aspergillus sp.																	6	3.79	25	23.80				
Emericella nidulans	7	6.03	3	3.84					5	5.31	2	3.38	1	1.49										
Fusarium incarnatum							5	8.19																
Fusarium oxysporum																	24	15.18	1	0.95	2	2.53		
Fusarium sp. 1	5	4.31	1	1.28	2	2.46	2	3.27																
Fusarium sp. 2									10	10.63	4	6.77	2	2.98	3	5.45								
Fusarium sp. 3																			5	4.76				
Helminthosporium sp.									2	2.12														
Penicillium frequentans	4	3.44																						
Penicillium italicum																	2	1.26						
Penicillium vinaceum																					9	11.39		
Penicillium sp.1	2	1.72	1	1.28																				
Penicillium sp. 2									4	4.25	1	1.69												
Rhizopus sp.	10	8.62	6	7.69			4	6.55																
Sporotrichum pruinosum									6	6.38														
Sporotrichum sp.																	1	0.63						
Trichoderma sp.1	8	6.89	1	1.28	4	4.93																		
Trichoderma sp.2									10	10.63														
Black unidentified																			11	10.47				
White sterile mycelia 1																					16	20.25		
White sterile mycelia 2																	29	18.35						
Species richness	11		9		6		7		10		7		6		5		11		8		5		2	
Total isolates	116		78		81		61		94		59		67		55		158		105		79		46	
Simpson's index of diversity (1-D)	0.842		0.722		0.6087		0.7995		0.8511		0.6704		0.6201		0.433		0.8238		0.7594		0.6651		0.4493	
Percentage reduction in number of species			18.18		45.45		36.36				30		40		50				27.27		54.54		81.81	
Percentage reduction in total number of isolates			32.75		30.17		47.41				37.23		28.72		41.48				33.54		50.00		70.88	

Table 2 Qualitative and quantitative distribution of mycobiota in soils control as well as treated with 500 ppm, 1000 ppm and 2000 ppm concentrations of chromium (III) nitrate over a period of 60 days (as obtained by dilution plate method).

Fungal species	20 Days								40 Days								60 Days							
	Control		500ppm		1000ppm		2000ppm		Control		500ppm		1000ppm		2000ppm		Control		500ppm		1000ppm		2000ppm	
	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI
Aspergillus clavatus									9	3.37														
Aspergillus flavus	9	8.03	13	19.11	16	17.58	8	12.30	12	4.49	5	7.81	10	5.81	2	3.12	12	4.04			6	4.28	10	6.45
Aspergillus fumigates	41	36.60	8	11.76	19	20.87	21	32.30									6	2.02			6	4.28		
Aspergillus luchuensis	15	13.39	15	22.05	11	12.08	9	13.84	31	11.61	12	18.75	32	18.60			66	22.22	31	24.8	55	39.28	52	33.54
Aspergillus niger	26	23.21	22	32.35	27	29.67	11	16.92	36	13.48	24	37.5	54	31.39	21	32.81	82	27.60	49	39.2	53	37.85	61	39.35
Aspergillus terreus																			2	1.6				
Botryotrichum sp.																	52	17.50	19	15.2	1	0.71	9	5.80
Cladosporium herbarum																			6	4.8				
Curvularia lunata																	9	3.03					1	0.64
Fusarium incarnatum																	32	10.77						
Fusarium nivale																	2	0.67						
Fusarium oxysporum	2	1.78	1	1.47													14	4.71	16	12.8	2	1.42	6	3.87
Fusarium sp.1	1	0.89	2	2.94	2	2.19	1	1.53																
Fusarium sp. 2									39	14.60			41	23.83	12	18.75								
Fusarium sp. 3					2	2.19	3	4.61	21	7.86			12	6.97	21	32.81								
Fusarium sp. 4									19	7.11			6	3.48										
Helminthosporium sp.									11	4.11	2	3.12			1	1.56								
Mucor racemosus																	11	3.7	2	1.6				
Penicillium sp.1																	4	1.34						
Penicillium sp. 2	3	2.67	1	1.47			2	3.07																
Penicillium sp. 3									28	10.48	4	6.25	1	0.58	2	3.12								
Penicillium sp. 4									24	8.98	15	23.43												
Rhizopus sp.	4	3.57			5	5.49			10	3.74			16	9.30	15	23.43								
Spegazzinia sp. 1									7	2.62														
Spegazzinia sp. 2																	7	2.35						
Sporotrichum pruinosum	5	4.46																						
Trichoderma lignorum																					17	12.14	16	10.32
Trichoderma sp.1	6	5.35	6	8.82	1	1.09																		
Trichoderma sp. 2									15	5.61	2	3.12												
Black sterile mycelia									5	1.87														
White sterile mycelia					8	8.79	2	3.07																
Species richness	10		8		9		9		14		7		8		7		12		7		7		7	
Total isolates	112		68		91		65		267		64		172		74		297		125		140		155	
Simpson's index of diversity (1-D)	0.837		0.799		0.8201		0.826		0.9097		0.7694		0.7965		0.7059		0.8276		0.7486		0.6886		0.7175	
Percentage reduction in number of species			20		10		10				50		42.85		50				41.66		41.66		41.66	
Percentage reduction in total number of isolates			39.28		18.75		41.96				76		35.58		72.28				57.91		52.86		47.81	

Table 3 Qualitative distribution of mycobiota in soils control as well as treated with 500 ppm, 1000 ppm and 2000 ppm concentrations of chromium(III)sulphate and chromium (III) nitrate over a period of 60 days (as obtained by soil plate method)

Fungi	Control (20 days)	Chromium sulphate (20 days)			Chromium nitrate (20 days)			Control (40 days)	Chromium sulphate (40 days)			Chromium nitrate (40 days)			Control (60 days)	Chromium sulphate (60 days)			Chromium nitrate (60 days)		
		500 ppm	1000 ppm	2000 ppm	500 ppm	1000 ppm	2000 ppm		500 ppm	1000 ppm	2000 ppm	500 ppm	1000 ppm	2000 ppm		500 ppm	1000 ppm	2000 ppm	500 ppm	1000 ppm	2000 ppm
Aspergillus clavatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-
Aspergillus flavus	-	+	+	+	+	-	+	-	-	-	-	+	-	-	++	-	+	-	-	-	-
Aspergillus fumigatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Aspergillus luchuensis	-	-	-	-	-	-	-	+++	++++	++	+	++	++	+++++	++++	+	+++	++	++	+	-
Aspergillus niger	+	+	+	+	+++	+	+	+++	+++	+	-	++	+	++++	++++	++	+++	++	+	-	++
Botrytis sp.	-	++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Choanephora sp.	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Emericella nidulans	-	+	++	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium sp.	+	-	-	-	-	-	-	++	+	+	+	-	-	++	-	-	-	-	-	-	-
Mucor sp.	++++	-	-	-	-	-	-	-	-	-	-	-	-	-	++	+	-	-	-	-	-
Penicillium sp.1	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillium sp. 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Penicillium sp. 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Rhizopus sp.	+++	-	++	-	-	-	++	++++	-	-	-	-	-	+	+++	-	-	-	-	-	-
Scopulariopsis sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-
Sporotrichum chlorinum	-	-	-	-	-	-	-	-	-	-	-	+++++	-	-	-	-	-	-	-	-	-
Sporotrichum sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Trichoderma lignorum															+++	+	-	+	+	-	-
Trichoderma sp.		_	+	+	+	+++++	++++	++	+++++	+++++	+++++	++	++	++	-	-	-	-	-	-	-
White sterile mycelium	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- = Absent; + = Rare; ++ = Infrequent; +++ = Frequent; ++++ = Predominant; +++++ = Highly dominant

which, in turn, might lead to a decrease in the species richness (Zak 1992). Sen (2007), using a still another approach was able to obtain 41 fungal species–the soil suspensions prepared from natural (untreated) soil were inoculated in the nutrient medium incorporated with heavy metals. A comparison of the three approaches including that followed in the present study indicates that while performing experiments aimed at assessing the effect of metal stress (or any such factor) on soil mycobiota, it would be preferable (i) either to treat the soil *in situ* and to subsequently isolate mycobiota from these samples; or (ii) to isolate mycobiota from unamended soil using pollutant-amended media; instead of disturbing the soil.

Chromium sulphate, as well as chromium nitrate, appear to exert a negative influence on the mycobiota (Tables 1 and 2). The number of species obtained from the soils treated with chromium salts was always lesser than the number of species isolated from control soils. The analysis of variance revealed that the treatment with chromium sulphate had significant negative effect on the qualitative as well as quantitative distribution of fungi in the soil (F= 13.55, significant at 0.01 level and 18.18, significant at 0.05 level). The results with chromium nitrate were largely similar (F=7.260, 6.12; both significant at 0.05 level). This assertion is further substantiated by the Simpson's indices of diversity (Tables 1 and 2). Decrease in soil fungal diversity as a result of the chromium pollution has been suggested by many workers including Zibliske and Wagner (1982), Tiwari and Charaya (2006) and Prigione *et al.* (2009). Earlier, Ross *et al.* (1981) as also Rogers and Li (1985) had reported adverse effects of chromium (III) on respiration and dehydrogenase activities of soil mycobiota. With some exceptions, the adverse effects of chromium on myco-diversity became more prominent as the duration of the treatment increased though this was found to be statistically insignificant (F= 2.05; 0.38). Taking into consideration the concentration of the chromium sulphate solution and duration of treatment, the maximum tolerance was shown by *Aspergillus niger* along with *Aspergillus luchuensis* which survived and flourished very well; and dominated the soils even on 60th day in the soils treated with 1000 ppm and 2000 ppm chromium sulphate solution. These two species may, therefore, be considered to be highly tolerant to chromium sulphate. *Aspergillus flavus, Aspergillus fumigatus* and a strain of *Fusarium* marked their presence in soils treated with 2000 ppm but for a shorter period of 40 days; also their numbers were remarkably lesser than that of *Aspergillus niger*. *Trichoderma* sp., *Rhizopus* sp., *Penicillium* sp., *Penicillium frequentans*, *Fusarium* sp. and *Emericella nidulans* were adversely affected by even short exposure (20 days) with lowest concentration of the pollutant. The results of the present study thus indicate that different fungal species also exhibit differential response to chromium sulphate. Differential toxicity of chromium to different microbial strains have been reported by earlier workers also. Congeevaram *et al.* (2007) had isolated strains *Aspergillus* and *Penicillium* from Cr (VI) contaminated soil. They also reported that Cr-resistant isolates could tolerate chromium toxicity upto 10000 mg/l. Bennett *et al.* (2013) reported *Aspergillus niger* and *Aspergillus flavus* from the soil heavily contaminated with chromium. Wyszkowska *et al.* (2001) reported that chromium adversely affected a number of *Azotobacter* species and actinomycetes but stimulated the proliferation of oligotrophic and nitrogen-fixing bacteria.

Mycobiota appears to be more tolerant to chromium nitrate as compared to chromium sulphate. *A. niger* and *A. luchuensis* were found to be most tolerant to chromium nitrate. *Trichoderma lignorum*, *Botryotrichum* sp., *Aspergillus flavus* and *Fusarium oxysporum* also exhibited tolerance to 2000 ppm of chromium nitrate solution even on 60th day though to a limited extent. However, some species of *Fusarium* sp. and *Penicillum* sp. failed to survive chromium nitrate contamination till 60th day. *Aspergillus niger* has been reported to be a dominant member of soils heavily contaminated by metals for long term (Iram *et al.* 2009, Dwivedi *et al.* 2012, Iram *et al.* 2012, Abdel-Azeem *et al.* 2015).

Adverse effects of chromium (III) sulphate and chromium (III) nitrate, as observed in the present study, are less marked than the effects of chromium (VI) sulphate reported earlier by Tiwari and Charaya (2006). This lower toxicity of chromium (III) salts may be attributed to their limited bioavailability and the tendency of dissolved Cr (III) to be absorbed by organic carbon (Batic and Raspor 1998). The solubilities of Cr (III) sulphate and Cr (III) nitrate have been reported to be 84 to 120 g/ml and 81 g/100 ml respectively (Anger *et al.* 2005). Chromium (III) sulphate appears to exert more inhibitory effect on soil mycobiota than chromium (III) nitrate. The percentage reduction in the number of species and number of isolates observed in soil mycobiota as a result of the addition of different concentrations of chromium (III) sulphate and chromium (III) nitrate are presented in the Tables 1 and 2. A glance at the table gives an impression that chromium sulphate exerts more inhibitory effect than chromium nitrate. However, the statistical analysis of the data yielded insignificant values of 't' not only with respect to the differences in inhibition of number of species (t=1.022) but the number of isolates (t=1.083) also.

Some workers in the past have suggested a reciprocal relationship between pollution concentration 'c' and length of exposure time 't' (Watson 1942, Hibben and Stotzky 1969). Babich and Stotzky (1982) suggested that exposure for long periods of time at low concentrations elicited responses from the mycobiota which were equivalent to short exposures at high concentrations so that 'c'× 't' was a constant. In the present study also, the higher concentrations of chromium (III) nitrate and chromium (III) sulphate exerted significantly greater negative influence on soil fungal community within a short period of time while the lower concentrations of the same solutions took much more time to exhibit similar effect. But a glance of the Tables 1 and 2 reveals that a clear cut relationship (like constancy of 'c'

and 't') might not be expected in the case of fungal communities specially those harbouring a highly complex medium like soil; of course, such a relationship may hold valid for single species *in vitro* systems adopted by the aforementioned workers. It is but natural because a variety of interactions with abiotic components as well as other biotic components in the soil play a major role in determining the soil mycobiota (Dube *et al.,* 1980). The differential response of fungal species to the metal stress is another factor which precludes such a simple 'c' × 't' expression of the response of soil microbial community to metal pollution. For example, in certain cases (Table 1 and 2), increase in exposure time or increase in concentration of the pollutant did not cause increase in adverse effect; rather a decrease in adverse effect was observed. Kumar (2011) recorded lesser number of *Aspergillus niger* from soil samples treated with 20 ppm chromium sulphate but greater number of isolates from soils treated with 40 ppm and 100 ppm concentrations of chromium. Babich and Stotzky (1982) had suggested that the levels of a pollutant which are lethal to a majority of microbes may cause only mutations in some, thereby, increasing the selection of the strains which can tolerate higher metal concentration of the pollutant. The subsequent survival and multiplication of these strains might lead to an increase in the population of such strains leading to a total positive effect on the mycobiota.

FTIR spectrum of the biomass of *Aspergillus niger,* which appears to be most resistant fungal species in the present study, was characterized by 10 peaks (Fig 1). The wave numbers (cm^{-1}) of the 10 peaks are given below with corresponding functional groups represented by the peaks within brackets as: (1) 603.78 (C=S); (2) 1044.3 (C=S); (3) 1075.48 (C=S); (4). 1249.57 (Alkyl halide, Amine, Ether, Ester, Alkene, Nitro); (5) 1400.65 (CH_3, CH_2); (6) 1548.46 (Aliphatic Azo); (7) 1647.26 (Amide); (8) 2150.8 (Thiocyanate); (9) 2924.9 (CH_3, CH_2) and (10) 3368.29 (Phenol) following Silverstein *et al.* (1981) as well as Smith and Dent (2006).

Park *et al.*, (2005), Das and Guha (2007), Khambhaty *et al.* (2009) have suggested that the amide and amine functional groups are responsible for binding of chromium to a given biomass. In the present study also, the presence of amine and amide groups (wave numbers 1249.57 and 1647.26) indicate the potential of *Aspergillus niger* to bind chromium. Kumar (2011), on the basis of FTIR spectroscopic studies, proposed a role of C=S group also in the binding of chromium. In the present study also, the FTIR spectrum of *Aspergillus niger* shows three peaks for C=S at 603.78, 1044.3 and 1075.48. Thus, there do exist significant functional groups on *Aspergillus niger* for binding of chromium; and this may be a mechanism of resistance to chromium in *Aspergillus niger* as has been proposed by Pillichshammer *et al.* (1995). Extracellular sequestration (including biosorption) is believed to be a factor contributing to the tolerance of fungal species to pollutants (Anahid *et al.* 2011). *Aspergillus niger*, thus, seems to be fit fungal species for effective management of Cr (III) removal.

The soil fungal diversity is adversely affected by Cr (III) contamination though exposure to the heavy metal for longer duration might provide chances for surviving fungal

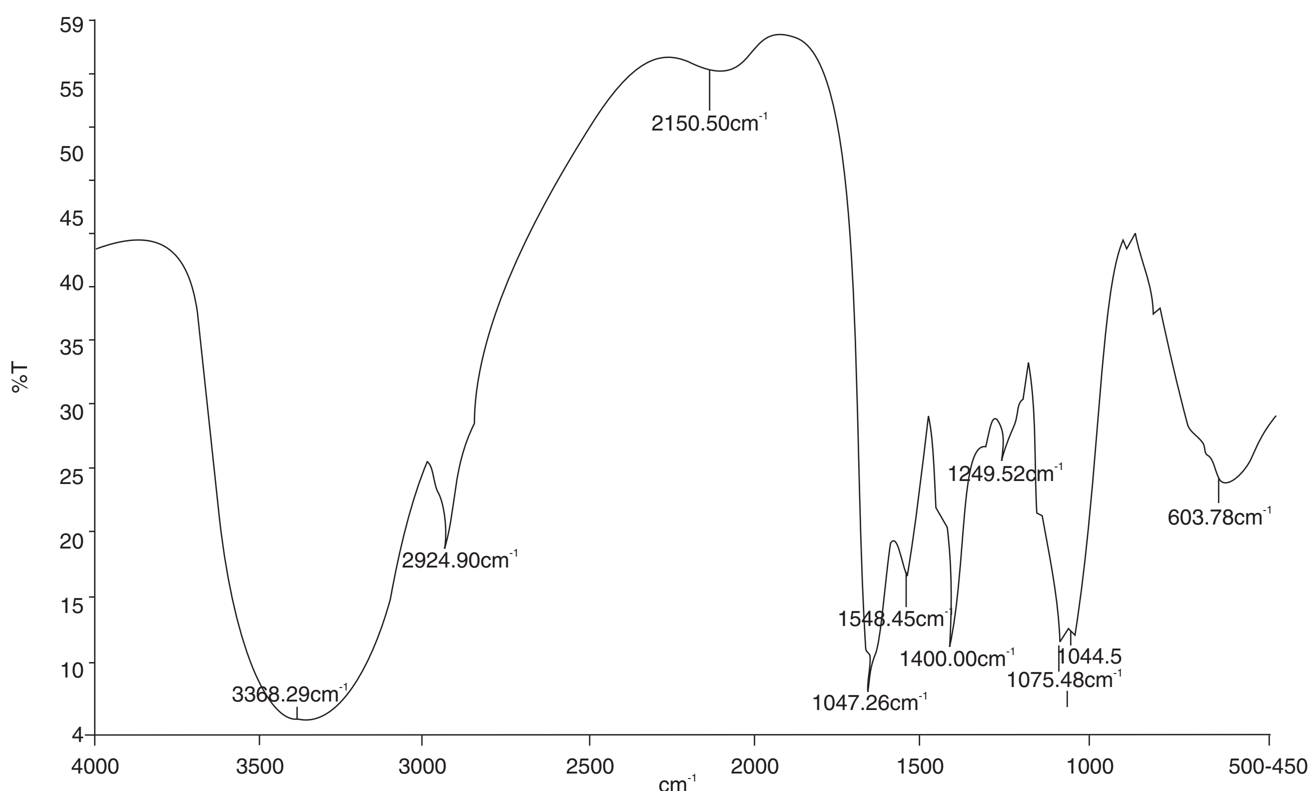

Fig 1 FT-IR spectrum of living fungal biomass *of Aspergillus niger*

species to flourish so that these are largely responsible for increase in the fungal population under such conditions. Owing to the functional group on their biomass, the surviving fungal species like *Aspergillus niger* appear to hold promise for use in microbe-based bioremediation strategies.

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