



Effect of Metal on Soil Microbiota

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ABSTRACT

Present investigations were conducted to evaluate the influence of Chromium contamination on soil microbiota and to obtain some Cr-resistant strains for the management of Cr-contaminated soils and of the effluents carrying the metal. Each pot was treated with different concentrations (50 ppm, 100 ppm, 250 ppm, and 500 ppm) of chromium sulphate solution separately in triplicates. Three pots served as a control with each concentration. The soil samples collected aseptically from control and treated pots after 30, 60 and 90 days were analyzed for mycobiota using the serial dilution method. Overall dominance of *Aspergillus* sp. Was observed amongst the 43 species of fungi isolated. *A. fumigatus* was most tolerant to Cr probably due to binding of Cr by certain groups on the fungus as revealed by FT-IR spectroscopy. Chromium sulphate adversely affected the mycobiota qualitatively as well as quantitatively. Soil fungal diversity is also adversely affected by Cr contamination. *A. fumigatus* biomass with Cr binding functional groups might be utilized for in-situ management of Cr in soils and biosorption-based effluent treatment systems.

Keywords: Metal-tolerant fungi, *Aspergillus fumigatus*, Chromium sulphate, FTIR spectroscopy.

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INTRODUCTION

Soil is a vital part of the natural environment and it is as important as plants, animals, landforms, rocks and rivers. This influences the distribution of different plant species and gives a habitat for a wide range of organisms. Soil pollution is the reduction in the productivity of soil because of soil pollutants and these pollutants gave an adverse effect on physical, chemical and biological properties of soil and reduce its productivity. Many factors such as pesticides, fertilizers, organic manure chemicals, radioactive wastes, plastics, paper, bottles all contributes towards soil pollutions. These pollutants may reach the soil through water or directly and from there they reach the plants and then into the grazing animals and ultimately to the humans. These pollutants accumulate in the soil and degrade very slowly and have a very deleterious effect on plant as well as mycoflora in the soil environment¹.

Recently, industrial activity growth and increasing water usage worldwide have led to the release of various pollutants into the environment. Soil may become contaminated with heavy metals from a variety of anthropogenic sources² as well as those which are irrigated

by untreated waste water. The use of contaminated water for irrigation, can contribute to the accumulation of chemical and biological pollutants in soil which alter the physicochemical and biological properties of soils^{3,4}.

Microorganisms present in the soil play a crucial role in soil fertility and primary production through organic matter decomposition and nutrient cycling⁵. Some microorganisms such as fungi and bacteria comprise the main components of soil microbial biomass². Many studies indicate that, heavy metals accumulation in soils gives toxic effects on soil mycoflora⁶, population size and activities of the soil microbial communities^{4,7-9}.

Heavy metals released through a number of industries as major pollutants in marine, ground, industrial and treated wastewater. Heavy metal is widely used in many industries such as storage battery manufacturing, printing, pigments, fuels photographic materials and explosive manufacturing¹⁰. Heavy metals can be dangerously toxic as they damage liver, kidney, nerves and bones are also functional groups of vital enzymes. Whereas heavy metal Cr (III) essential in human nutrition (especially in glucose metabolism), while Cr (VI) compound are toxic, several can cause lung cancer. Chromium and its compounds are extensively used in leather tanning, cement, electroplating, dyeing, metal processing, paint, pigment, textile, steel and fabrication industries and these produce large amount of toxic waste water effluents¹¹.

The current observation was conducted with an aim to isolate those fungal species from soil which are able to surviving heavy metal chromium pollution and to obtain Cr



resistant fungal strains which might facilitate the management of metal level in soil and effluents.

MATERIALS AND METHODS

Forty-eight pots of 150 ml capacity, each filled with 100 gm soil sample were taken for the present investigation. Twelve pots from these forty-eight pots were treated with 25 ml of distilled water at time period of seven days for a total period of twelve weeks. These twelve pots served as control. The remaining thirty-six pots were treated with different concentration of chromium metal solution (as CrSO_4). Nine pots were taken for each concentration *i.e.* (50 ppm, 100 ppm, 250 ppm and 500 ppm) of chromium solution.

After 30 days, soil from the four pots of control was mixed completely to obtain a composite sample. Similarly, four different composite samples were prepared from the soil treated with chromium metal with each concentration. Each treated composite soil sample obtained was analyzed for mycobiota, using dilution plate method¹². 20 gm of soil sample from the composite sample were transferred to 200 ml of sterilized distilled water and stirred it well. 10 ml of this soil suspension were instantly transferred to a conical flask containing 90 ml of sterilized distilled water. From this soil suspension 1:100, 1:1000, 1:10,000 and 1:100,000 were prepared. From the suspension of each dilution 1 ml aliquots were transferred to each of a set of three Petri plates which were already pored with 20 ml of cooled and sterilized Potato Dextrose Agar medium, amended with 30 ppm streptomycin and 30 ppm Rose Bengal (per liter of medium). After inoculation, the Petri plates were incubated at $26^\circ\text{C} \pm 2$ for 3 to 4 days. The total numbers of colonies of individual fungal species growing in each Petri plates were recorded at a regular time interval. Obtained fungal strains were identified with the help of standard keys^{13,14}. The fungal strains were transferred to the Petri plates containing fresh sterilized medium for the preparation of axenic culture. Different composite samples were obtained from the chromium treated soil with different concentration were processed similarly and the procedure was repeated after 60 and 90 days.

Aspergillus fumigatus Fresenius being the dominating fungal species that could withstand the Cr toxicity was subjected to FT-IR spectroscopic analysis. For this the mycomass of *A. fumigatus* was prepared by inoculating 6 flasks each containing 100 ml MGYP medium (Malt, 3g, Glucose 10g, Yeast extract 3g and Peptone 5g; made upto 1 liter with distilled water) along with 10 ml of spore suspension of *A. fumigatus*. After 6-8 days of incubation at $26 \pm 1^\circ\text{C}$, the mycomass of *A. fumigatus* was harvested and dried in an oven at 60°C for 48 hours. Two mg of the powder was mixed with 98 mg of dry powdered KBr. The mixture was used to prepare pellets by applying pressure of 10,000 to 15000 psi using PG-Hydraulic Press. The IR spectrum was recorded on IR-affinity-1, Shimadzu spectrophotometer high resolution ($\leq 0.001/\text{cm}$).

RESULTS AND DISCUSSION

A total of forty-three fungal species were isolated from the control as well as those treated with heavy metal chromium using serial dilution method. Out of these forty-three fungal species two species belong to Zygomycota (*i.e.*, *Rhizopus* sp. and *Rhizomucor miehei*) while the remaining forty-one fungal sp. were anamorphic fungi. Nine fungal species were belonging to the genus aspergillus. The isolates of Aspergilli were largely dominated to the culture Plates. *Aspergillus fumigatus* is the more dominant species that could tolerate chromium metal at 1000 ppm concentration even at 90 days of treatment. This may be due to the capacity of *Aspergillus* sp. to produce toxins that may prevent the growth of other fungal species¹⁵. The Aspergilli were represented by nine species while the Chaetomium were represented only four species and the number of isolates of Aspergilli were also dominated to the culture Plates. It is widely accepted that Aspergilli are more plentiful in the warmer regions of the world than other species^{16,17} and some finding represent that the ability of *A. fumigatus* of surviving in very different environments, such as those with temperature up to 48°C - 60°C ^{18,19}.

The result of the present observation are in agreement with the above findings. The number of fungal species were reduced with increase in metal concentration after 30 days of treatment. The number of isolates gradually increased in control soil sample after thirty days while the number of isolates in a metal treated soil samples reduced till 60 days of treatment but number of isolates became increased during the 90 days of treatment. In the present observation an overall inhibitory effect of chromium metal was observed on soil mycoflora *i.e.*, diversity of mycoflora decreased with increasing concentration of metal pollutants with few exceptions. This is further approved with the help of diversity indices (D and 1-D).

Heavy metals block the enzyme or can interfere with some essential cellular metabolite of bacteria and protozoa²⁰. Heavy metals are toxic even in very trace amount and can cause disease in human and animals as they can cause irreversible changes in the body, especially in the Central Nervous System²¹. The contamination of the environment with chromium metal may led to liver damage and pulmonary congestion and can cause skin irritation resulting in ulcer formation²². Accumulation of chromium can also cause birth defects and the decrease in reproductive health²³.

Long-term heavy metal contamination of soil has harmful effect on soil microbial activity, especially microbial respiration²⁴. Several reports have shown large reductions in microbial activity due to short term exposure to toxic metals^{24,25}. High level of metal contamination show lower number of microbes than uncontaminated habitats^{26,27}. Heavy metals have a direct effect on soil enzyme activity, so that the spatial structure of the active group of enzyme destroyed²⁸.



Table 1: Qualitative and quantitative distribution of mycobiota in soils–control as well as treated with 50 ppm, 100 ppm, 250 ppm and 500 ppm concentrations of Chromium sulphate over a period of 30, 60 and 90 days (as obtained by dilution plate method).

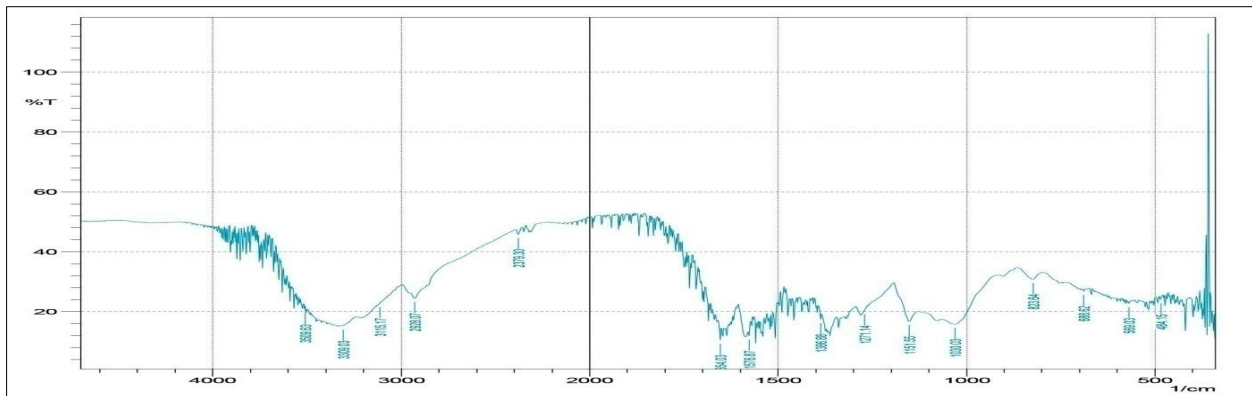
Fungal Species	30 Days										60 Days										90 Days									
	Control		50 ppm		100 ppm		250 ppm		500 ppm		Control		50 ppm		100 ppm		250 ppm		500 ppm		Control		50 ppm		100 ppm		250 ppm		500 ppm	
	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI
<i>Aspergillus fumigatus</i>	18	15.78	67	46.85	12	9.09	98	68.05	28	18.30	3	1.44	10	10.20	42	53.85	49	38.28	99	38.97	2	.56	17	5.32	289	63.10	89	32.72	22	14.96
<i>Aspergillus flavus</i>	5	4.38	-	-	11	8.33	7	4.86	7	4.37	4	1.93	2	2.04	5	6.41	10	7.81	-	-	2	.56	13	4.07	-	-	-	-	-	-
<i>Aspergillus niger</i>	18	15.78	29	20.27	13	9.84	13	9.02	25	16.33	198	95.65	78	79.59	16	20.51	13	10.15	4	1.57	27	7.58	66	20.68	4	.87	2	.73	-	-
<i>Aspergillus ustus</i>	4	3.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus nidulans</i>	-	-	16	11.18	-	-	6	4.16	2	1.30	-	-	2	2.04	1	1.28	1	.78	4	1.57	-	-	-	-	2	.43	-	-	1	.68
<i>Aspergillus terreus</i>	-	-	3	2.09	12	9.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1.10	-	-	
<i>Aspergillus giganteus</i>	-	-	-	-	18	13.63	-	-	23	15.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Aspergillus luchuensis</i>	1	.87	2	1.39	10	7.57	11	7.63	24	15.68	-	-	-	-	-	-	-	-	-	-	1	.28	-	-	1	.21	1	.36	-	-
<i>Aspergillus humicola</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	1.57	-	-	-	-	-	-	-	-	-	-
<i>Aureobasidium pullulans</i>	-	-	-	-	4	3.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Aulternaria sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	1.57	-	-	-	-	-	-	-	-	-	-
<i>Botryotrichum atrogriseum</i>	18	15.78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50	1.40	7	2.19	-	-	-	-	-	-
<i>Botryotrichum piluliferum</i>	5	4.38	-	-	9	6.81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chaetomium aureum</i>	7	6.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chaetomium bostrychodes</i>	-	-	-	-	-	-	-	-	6	3.92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chaetomium homopitatum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	4.08	
<i>Chaetomium trilaterale</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	5.44	
<i>Curvularia clavata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	10.93	-	-	-	-	-	-	2	.62	-	-	-	-	1	.68
<i>Cladosporium sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	.31	-	-	-	-	-	-
<i>Fusarium sp.</i>	10	8.77	-	-	27	20.45	-	-	18	11.76	-	-	-	4	5.12	13	10.15	-	-	-	244	68.53	205	64.26	-	-	95	34.92	2	1.36
<i>Humicola bravis</i>	9	7.89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Humicola grisea</i>	-	-	7	4.89	-	-	-	-	2	1.30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Humicola fuscoatra</i>	-	-	-	-	-	-	-	-	4	2.61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Monodictys fluctuata</i>	2	1.75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Mammaria chinobostroides</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	.56	4	1.25	1	.21	1	.36	5	3.40
<i>Penicillium sp.</i>	-	-	6	4.19	4	3.03	-	-	7	4.57	-	-	-	-	5	3.90	17	6.69	28	7.86	-	-	14	3.04	3	1.10	1	.68	-	-
<i>Penicillium vinaceum</i>	5	4.38	-	-	-	-	9	6.25	-	-	-	-	2	2.04	2	2.56	18	14.06	30	11.81	-	-	4	1.25	139	30.34	30	11.02	14	9.52
<i>Paecilomyces variotii</i>	3	2.63	4	2.79	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Periconia sp.</i>	2	1.75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Rhizopus sp.</i>	7	6.14	-	-	-	-	-	-	7	4.57	2	.96	4	4.08	4	5.12	3	2.34	4	1.57	-	-	-	-	7	1.52	6	2.20	-	-
<i>Rhizomucor miehei</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2.56	-	-	1	.39	-	-	-	-	-	-	-	-	-	-	-
<i>Trichoderma sp.</i>	-	-	9	6.29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Verticillium sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2.56	2	1.56	87	34.25	-	-	-	-	-	-	-	-	6	4.08	
<i>Verticillium terrestre</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	.43	-	-	-	-	-
<i>Verticillium glaucum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	42	15.44	81	55.10	
Number of Species	15	9	10	6	12	4	6	9	10	10	8	9	9	10	11	8	9	9	10	11	8	9	9	10	10	11	8	9	9	10
Total Isolates	114	143	120	144	153	207	98	78	128	254	356	319	459	272	147	356	319	459	272	147	356	319	459	272	147	356	319	459	272	147
Simpson's index (D)	0.0984	0.2855	0.136	0.558	0.141	0.915	0.643	0.334	0.201	0.286	0.500	0.459	0.490	0.263	0.338	0.500	0.459	0.490	0.263	0.338	0.500	0.459	0.490	0.263	0.338	0.500	0.459	0.490	0.263	0.338
Simpson's index of Diversity (1-D)	0.902	0.715	0.864	0.442	0.859	0.085	0.357	0.666	0.799	0.714	0.5	0.541	0.51	0.737	0.662	0.5	0.541	0.51	0.737	0.662	0.5	0.541	0.51	0.737	0.662	0.5	0.541	0.51	0.737	0.662



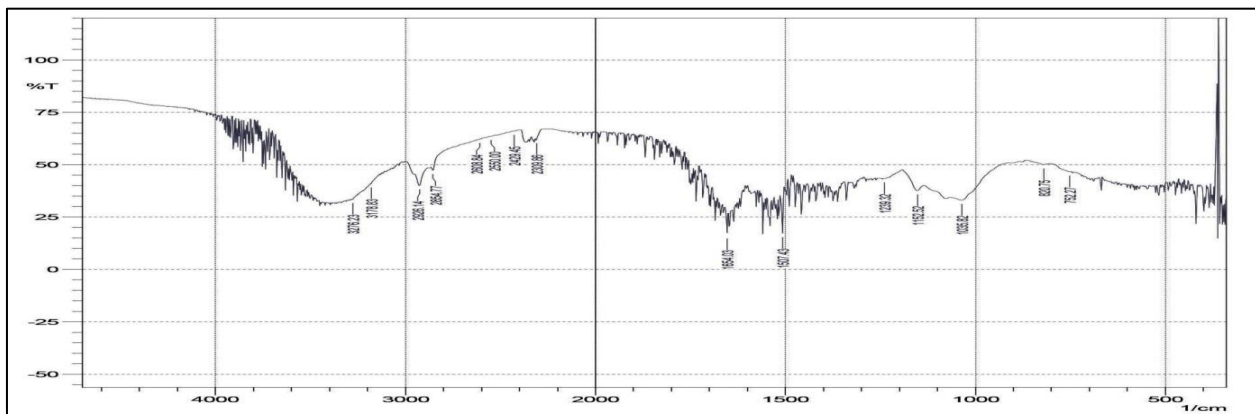
Diversely the growth and reproduction of microorganisms are inhibited, thus reducing the synthesis and metabolism of the microbial enzyme. After the soil contaminated with heavy metal, the primary impact is the amount of soil fungi, bacteria, actinomycetes and other microbial population. Metal effluents effect the microbial quantity and quality in the soil.

FT-IR spectrum of the biomass of *A. fumigatus* which appear to be most dominant fungal species in all treated metal concentration along with control. Various absorption peak values were found and 37 different functional groups of different classes compound were identified in control, 50 ppm, 100 ppm, 250 ppm and 500 ppm dry biomass of *A. fumigatus*.

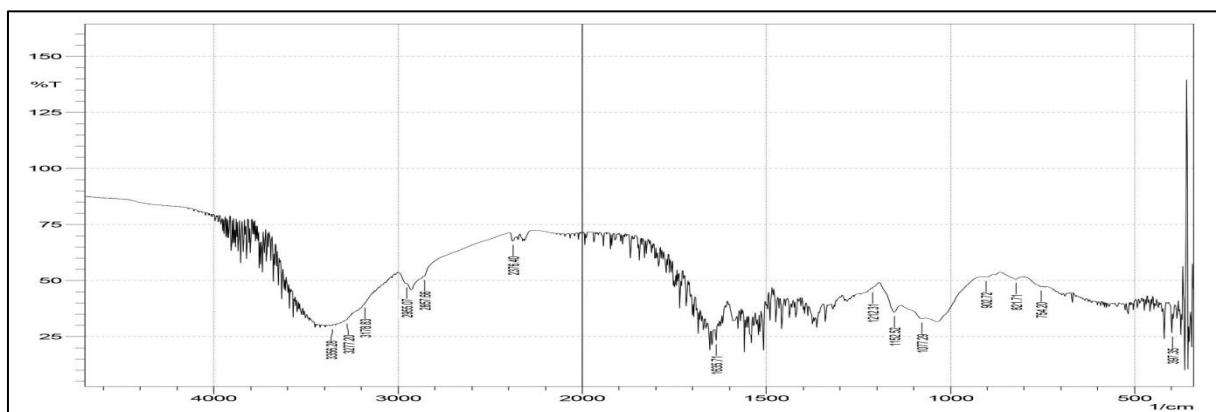
Out of 41 functional groups, only 14 groups were common in all metal concentration along with control that included (C-C aliphatic chains, C-O-C, Aromatic rings, Si-O-C, Si-O-Si, C=S, Sulfonic acid, Carboxylic acid, -O-CH₃, C-CH₃, OH, Amide, Amine and Phenol). Six different groups *i.e.*, C-Cl, C-S, Ketone, C=C, C=N and P-H were absent in higher metal concentration (500 ppm). Aromatic azo, Aliphatic azo, Aromatic/hetero rings were found in 50 ppm concentration, on the other hand Carboxylate salt was absent in all the metal concentration except control. Thiol was found only in 100 ppm metal conc. , whereas X metal-O was present in only 250 ppm conc. along with control. S-S showed their existence in 50 ppm metal conc. and control as well. In 50, 100 and 500 ppm concentration CH₂ group was recognized.



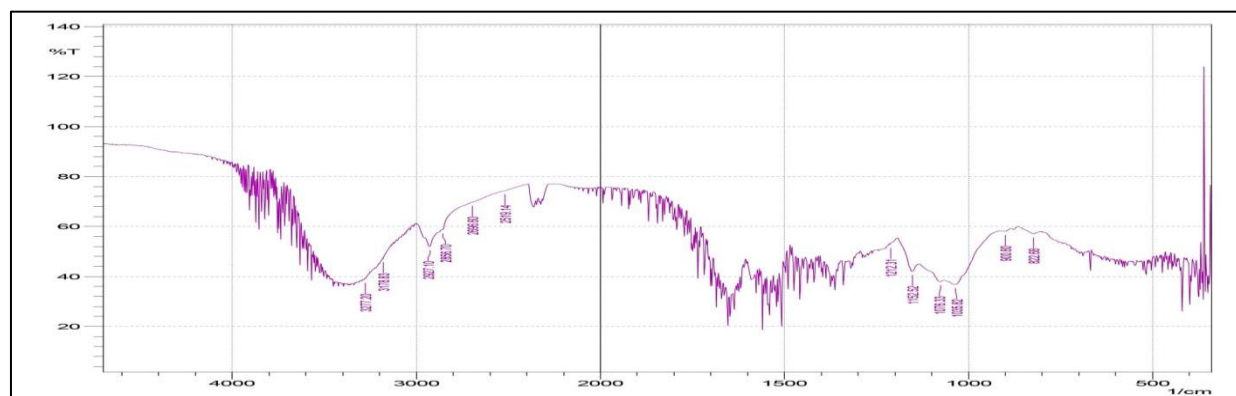
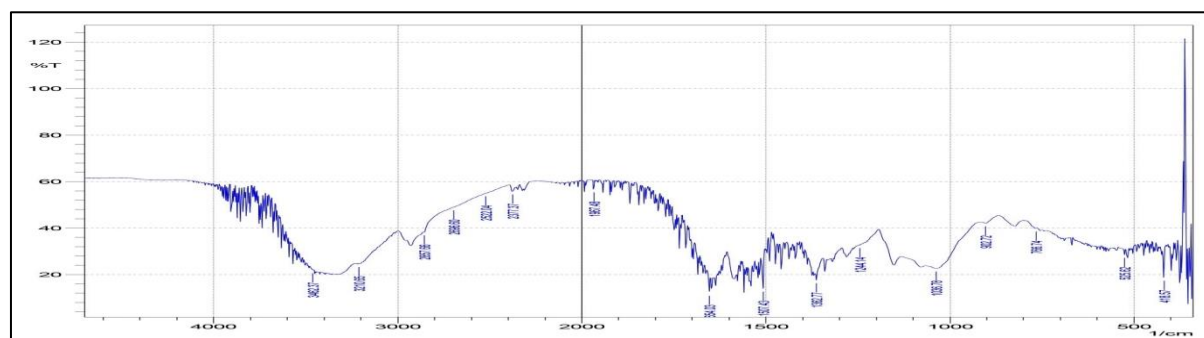
FT-IR spectra of controlled *A. fumigatus*



FT-IR spectra of *A. fumigatus* at 50 ppm metal concentration



FT-IR spectra of *A. fumigatus* at 100 ppm metal concentration

FT-IR spectra of *A. fumigatus* at 250 ppm metal concentrationFT-IR spectra of *A. fumigatus* at 500 ppm metal concentration

FTIR frequency range, intensity and functional groups of air-dried components of *A. fumigatus*.

S. N.	Functional Groups	Control	50 ppm	100 ppm	250 ppm	500 ppm	Intensity	Frequency ranges
1	C-C aliphatic chains	+	+	+	+	+	M	630-1250
2	C-O-C	+	+	+	+	+	W	800-950
3	Aromatic rings	+	+	+	+	+	S	990-1100
4	Si-O-C	+	+	+	+	+	W	1010-1095
5	Si-O-Si	+	+	+	+	+	W	1010-1095
6	C=S	+	+	+	+	+	S	1020-1225
7	Sulfonic acid	+	+	+	+	+	VW	1225-1060
8	Carboxylic acid	+	+	+	+	+	M	1610-1740
9	-O-CH ₃	+	+	+	+	+	M	1810-2850
10	C-CH ₃	+	+	+	+	+	S	2810-2960
11	OH	+	+	+	+	+	W	2880-3530
12	Amide	+	+	+	+	+	M	3150-3480
13	Amine	+	+	+	+	+	M	3150-3480
14	Phenol	+	+	+	+	+	W	3200-3400
15	C-Cl	+	+	+	+	-	S	550-790
16	C-S	+	+	+	+	-	S	670-780
17	Amide	+	+	+	+	-	S	1550-1700
18	Ketone	+	+	+	+	-	M	1600-1710
19	C=C	+	+	+	+	-	Vs	1625-1680
20	C=N	+	+	+	+	-	VS	1630-1665
21	P-H	+	+	+	+	-	VW	2290-2420
22	Sulfonamide	-	+	+	+	+	M	1050-1210
23	Sulfone	-	+	+	+	+	M	1050-1210
24	Aromatic C-H	-	+	+	+	+	S	2870-3100
25	C-I	+	+	-	+	-	S	490-660
26	C-F	+	-	+	+	-	S	720-800
27	Si-O-Si	+	+	-	-	-	S	460-550

28	X metal-O	+	-	-	+	-	S	150-430
29	S-S	+	+	-	-	-	S	425-550
30	CH ₂	-	+	+	-	+	S	2900-2940
31	C-Br	+	+	-	-	-	S	505-700
32	Aldehyde	+	-	-	-	+	W	2780-2830
33	Carboxylate salt	+	-	-	-	-	M	1315-1435
34	C-CH ₃	+	-	-	-	-	W	1355-1385
35	Aromatic azo	-	+	-	-	-	VS	1365-1450
36	Nitro	-	+	-	-	-	M	1535-1600
37	Aliphatic azo	-	+	-	-	-	M	1540-1590
38	Aromatic/hetero ring	-	+	-	-	-	S	1550-1610
39	Aromatic ring	-	-	+	-	-	M	1450-1505
40	Thiol	-	-	+	-	-	S	2530-2610
41	C-C aliphatic chain	-	-	-	+	-	S	250-400

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