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# INFLUENCE OF LEAD (II) CONTAMINATION ON SOIL MYCOBIOTA

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**Abstract**

Investigations were conducted on the experimental fields of CCS University (Meerut) to evaluate the influence of Pb contamination on soil mycobiota and to obtain some Pb-resistant strains for the management of Pb 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 lead nitrate or lead 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 sixty five species of fungi isolated. *Aspergillus niger* was most tolerant to Pb probably due to binding of Pb by certain groups on the fungus as revealed by FTIR spectroscopy. Pb salts adversely affected the mycobiota qualitatively as well as quantitatively. The results indicate that though soil fungal diversity is adversely affected by Pb contamination, the surviving species flourish over a period of time leading to the partial recovery of the mycopopulation. *Aspergillus niger* biomass with Pb-binding functional groups might be utilized for *in situ* management of Pb in soils and in biosorption-based effluent treatment systems.

**Keywords:** *Aspergillus niger*, Lead nitrate, Lead sulphate, FTIR spectroscopy, Metal-tolerant fungi

## INTRODUCTION

The widespread and continuous use of heavy metals for various industrial purposes generates huge volumes of waste waters which contaminate soils, air, water and also the biosphere (Ferraz and Teixeira, 1999). Metals play an important role in the life processes of living organisms but excessive doses of these can become toxic (O' Connell *et al.,* 2008; Smith *et al.,* 2015; Vijayaraghavan and Balasubramanian, 2015). Some of these elements with no biological role can enter the system and may disturb normal processes (Maestri *et al.,* 2010). Lead is one such heavy metal with no beneficial effect on human body. No case of lead deficiency has ever been noted in the medical literature (Duda-Chodak, 2012). On the contrary, like other heavy metals, lead is capable of entering the food chains ultimately challenging the security and safety of human food. Lead may enter the natural resources from a wide variety of sources including battery manufacturing, electroplating, pigments and ammunition, paint industries, dumped electronic waste etc. (Ramasamy *et al.,* 2011; Wani *et al.,* 2015). Both water and soil can get contaminated with lead released from the breakdown of lead-based paint on buildings and park tools. Soils near roads may have higher levels of lead from years of exhaust, vapor and pollution from vehicles (Pagotto *et al*., 2001; Aslam *et al*., 2013; Radziemska and Fronczyk, 2015). Lead sulphate and its oxides are used as glue in tyre industry and in rubber compounding (Hathaway and Proctor, 2004) whereas lead nitrate is used in the manufacture of paints and fireworks; as a stabilizer in nylon, polyester and other plastics; as a coating for photothermographic paper; and in gold mining (Deschenes *et al*., 2000; Sayiner, 2014). Therefore, the effect of Pb(II) compounds on soil mycobiota needs to be evaluated because of their multi-dimensional impact on biogeochemical cycles as well as soil fertility and agricultural productivity. It has been observed that fungal populations isolated from metal-polluted environments adapt to toxic concentrations of heavy metals and are more efficient at biosorption (Prasenjit and Sumathi, 2005; Hemambika *et al*., 2011; Fazli *et al.,* 2015). Therefore, the biosorptive property of fungal biomass can be exploited for *in situ* management of soil contaminants including Pb(II). The present study deals with the effect of two compounds of Pb(II) *i.e.* lead(II) sulphate and lead(II) nitrate on soil fungal diversity. It has also been attempted to explore the possibilities of obtaining Pb(II)-resistant fungal strains which might facilitate the management of Pb(II) levels in soils and effluents through biosorption.

## MATERIALS AND METHODS

Twenty one blocks of 30cm×30cm each were demarcated in a small plot laid out at the experimental fields of the Department of Botany, C.C.S.University Campus, Meerut. 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 these 21 blocks, (i) three blocks were kept as control; (ii) nine for treatment with aqueous solution of lead (II) sulphate and (iii) nine for treatment with aqueous solution of Pb(II) nitrate. Out of the nine blocks allotted for Pb(II) sulphate solution, three each were treated with 500 ppm, 1000 ppm and 2000 ppm concentrations of the solution. Similarly, nine blocks were used for amendment with Pb(II) nitrate solution (three each for 500 ppm, 1000 ppm and 2000 ppm concentrations). The allotment of blocks for receiving the treatments was subject to completely randomized design (CRD). Each block was treated with two litres of given metal solution regularly at weekly intervals for 60 days. The three control blocks were treated with equal quantities of distilled water instead of metal solution.

Soil samples 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 ($20^{th}$/$40^{th}$/$60^{th}$ day) were mixed thoroughly but aseptically to obtain a composite sample. In this way, three composite samples were obtained for blocks treated with lead(II) sulphate (one composite sample each for 500 ppm, 1000 ppm and 2000 ppm concentrations). Similarly, three composite samples were prepared for soils treated with lead(II) nitrate on each sampling day ($20^{th}$ / $40^{th}$ / $60^{th}$ day).

Two methods, namely serial dilution plate method (Waksman, 1922) and soil plate method (Warcup, 1950) were followed to isolate the mycobiota from each composite sample. In the serial dilution method, $10^{-2}$, $10^{-3}$ and $10^{-4}$ dilutions were prepared for each composite sample. Potato Dextrose Agar Medium (Raper and Thom, 1949) with 30 ppm Rose Bengal and 30 ppm Streptomycin was used. The Petri dishes with the medium and inocula were incubated at $25 \pm 1^0$C for 5 days. For soil plate method, 5 mg of a given composite sample and the Potato Dextrose Agar medium were used. The Petri dishes with the inocula were incubated at $25 \pm 1^0$C for 6 to 8 days. The different fungal strains obtained were transferred to the Petri plates containing fresh medium to facilitate their identification and for the preparation of axenic cultures.

*Aspergillus niger* van Tieghem being the dominating fungal species that could withstand the lead (II) toxicity was subjected to FTIR spectroscopic analysis. For this, the mycomass of *Aspergillus niger* van Tieghem was prepared by inoculating 5 flasks each containing 150 ml MGYP medium (Malt 3g, Glucose 10g, Yeast extract 3g and Peptone 5g; made upto 1 litre with water) alongwith 10 ml of spore suspension of *Aspergillus niger*. After 6–8 days of incubation at $25 \pm 1^0$C, the mycomass of *Apergillus niger* was harvested and dried in an oven at $60^0 \pm 1^0$C for 24 hours followed by powdering using 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 to 15,000 psi using PG-Hydraulic Press. The IR spectrum was recorded on IR-affinity-1, Shimadzu spectrophotometer high resolution ($\leq 0.001/\text{cm}$).

The data relating to the effect of lead (II) treatment on qualitative as well as quantitative alterations in the mycobiota over different periods of time were subjected to ANOVA and 't'-test. Simpson's indices of diversity (Okpiliya, 2012) were calculated for evaluating the species diversity.

## RESULTS AND DISCUSSION

A total of 65 species of fungi were isolated from the control soils as well as those treated with lead solutions (Tables 1 and 2) using dilution plate method. Out of these, only six belong to Zygomycota and one to Ascomycota while the remaining 54 species were anamorphic fungi. The results of the present study, thus (a) are in agreement with the findings of Galloway (1935), Dube *et al.* (1980) and Charaya (2006) indicating the paucity of Mucoraceous fungi in the tropical regions of the world; (b) supports the widely held view that Aspergilli are more common in the warmer regions of the world (Waksman 1917; Singh and Charaya, 1975 and Kumar and Charaya, 2012).

In the present study, the soils were given *in situ* treatments of heavy metal "lead" in the field itself and the samples collected periodically from the site itself were analysed for fungal biota; the study yielded as many as 65 different species of fungi.

**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 lead 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 candidus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **19** | **4.28** | - | - | - | - | - | - |
| *Aspergillus flavus* | 13 | 8.84 | 5 | 9.43 | - | - | - | - | 10 | 6.75 | 14 | 11.02 | 4 | 2.81 | - | - | 29 | 6.54 | 16 | 8.64 | 5 | 2.25 | 8 | 5.33 |
| *Aspergillus fumigatus* | - | - | - | - | - | - | - | - | 5 | 3.37 | 16 | 12.69 | 2 | 1.40 | - | - | 20 | 4.51 | 12 | 6.48 | 8 | 3.60 | 5 | 3.33 |
| *Aspergillus luchuensis* | 31 | 21.08 | 18 | 33.96 | 15 | 24.19 | 14 | 56 | 36 | 24.32 | 41 | 32.28 | 48 | 33.80 | 12 | 21.42 | 56 | 12.64 | 59 | 31.89 | 54 | 24.32 | 42 | 28 |
| *Aspergillus niger* | **42** | 28.57 | 26 | 49.05 | 39 | 62.90 | 11 | 44 | 56 | 37.83 | 49 | 38.5 | 74 | 52.11 | 42 | 75 | 92 | 20.76 | 89 | 48.10 | 69 | 31.08 | 77 | 51.33 |
| *Alternaria* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 6 | 1.35 | - | - | - | - | - | - |
| *Botrytis* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 52 | 11.73 | - | - | - | - | - | - |
| *Curvularia* sp. | 2 | 1.36 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Drechslera* sp. | - | - | - | - | - | | - | - | - | - | - | - | - | | 2 | 3.57 | - | - | - | - | - | - | - | - |
| *Emericella nidulans* | - | - | - | - | - | - | - | - | 9 | 6.08 | - | - | - | - | - | - | 15 | 3.38 | - | - | - | - | - | - |
| *Fusarium oxysporum* | 11 | 7.48 | 2 | 3.77 | 1 | 1.61 | - | - | - | - | - | - | - | - | - | - | 26 | 5.86 | 9 | 4.86 | - | - | 1 | 0.66 |
| *Fusarium* sp. 1 | - | - | - | - | - | - | - | - | 16 | 10.81 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Fusarium* sp. 2 | - | - | - | - | - | - | - | - | - | - | - | - | 12 | 0.45 | - | - | - | - | - | - | - | - | - | - |
| *Fusarium* sp. 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 7 | 3.15 | 2 | 1.33 |
| *Helminthosporium* sp. 1 | - | - | - | - | - | - | - | - | 15 | 10.13 | - | - | 2 | 1.40 | - | - | - | - | - | - | - | - | - | - |
| *Helminthosporium* sp. 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 19 | 4.28 | - | - | - | - | - | - |
| *Hormiscium* sp. | 4 | 2.72 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Mucor* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 10 | 2.25 | - | - | 21 | 9.45 | - | - |
| *Penicillium oxalicum* | 4 | 2.72 | - | - | 4 | 6.45 | - | - | - | - | 2 | 1.57 | - | - | - | - | - | - | - | - | - | - | - | - |
| *Penicillium* sp.1 | 5 | 3.40 | 2 | 3.77 | 2 | 3.22 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Penicillium* sp.2 | - | - | - | - | - | - | - | - | 1 | 0.67 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Penicillium* sp.3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 9 | 2.03 | - | - | - | - | - | - |
| *Pithomyces* sp. | - | - | - | - | - | - | - | - | - | - | 5 | 3.93 | - | - | - | - | - | - | - | - | - | - | - | - |
| *Rhizoctonia* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 7 | 1.58 | - | - | - | - | - | - |
| *Rhizopus* sp.1 | 19 | 12.92 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Rhizopus* sp.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 69 | 15.57 | - | - | 33 | 14.86 | - | - |
| *Sporotrichum chlorinum* | 10 | 6.80 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Stemphylium* sp.1 | 6 | 4.08 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Stemphylium* sp.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 14 | 3.16 | | - | - | - | - | - |
| *Trichoderma* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 25 | 11.26 | 15 | 10 |
| **Number of Species** | **11** | | **05** | | **06** | | **02** | | **08** | | **06** | | **06** | | **03** | | **15** | | **05** | | **08** | | **07** | |
| **Total Isolates** | **147** | | **53** | | **62** | | **25** | | **148** | | **127** | | **142** | | **56** | | **443** | | **185** | | **222** | | **150** | |
| **Simpson's index (D)** | **0.1596** | | **0.3555** | | **0.5034** | | **0.4866** | | **0.2285** | | **0.2772** | | **0.4694** | | **0.6025** | | **0.1120** | | **0.3436** | | **0.1986** | | **0.3517** | |
| **Simpson's index of Diversity (1−D)** | **0.8404** | | **0.6445** | | **0.4966** | | **0.5134** | | **0.7715** | | **0.7228** | | **0.5306** | | **0.3975** | | **0.888** | | **0.6564** | | **0.8014** | | **0.6483** | |

**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 lead sulphate over a per**iod 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 flavus* | 11 | 12.35 | 7 | 20.58 | 11 | 16.66 | 2 | 3.12 | 19 | 9.26 | 4 | 4.65 | 6 | 5.82 | - | - | 34 | 9.88 | 26 | 20.47 | 13 | 10.74 | 22 | 14.47 |
| *Aspergillus fumigatus* | 9 | 10.11 | 4 | 11.76 | 9 | 13.63 | 1 | 1.56 | 5 | 2.43 | 2 | 2.32 | 9 | 8.73 | 1 | 1.92 | 12 | 3.48 | 19 | 14.96 | 5 | 4.13 | 4 | 2.63 |
| *Aspergillus luchuensis* | 3 | 3.37 | - | - | 5 | 7.57 | 3 | 4.68 | 33 | 16.09 | 36 | 41.86 | 29 | 28.15 | 12 | 23.07 | 36 | 10.46 | 15 | 11.81 | 31 | 25.6 | 36 | 23.68 |
| *Aspergillus niger* | 22 | 24.71 | 19 | 55.88 | 28 | 42.42 | 31 | 48.43 | 42 | 20.48 | 39 | 45.34 | 45 | 43.68 | 31 | 59.61 | 89 | 25.87 | 52 | 40.94 | 50 | 41.32 | 69 | 45.39 |
| *Aspergillus ustus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2 | 0.58 | - | - | - | - | - | - |
| *Aspergillus wentii* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 5 | 1.45 | - | - | - | - | - | - |
| *Alternaria citri* | 1 | 1.12 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Alternaria* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 7 | 2.03 | 2 | 1.57 | 1 | 0.82 | - | - |
| *Botrytis* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 27 | 7.84 | - | - | 2 | 1.65 | - | - |
| *Curvularia* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 12 | 3.48 | - | - | - | - | - | - |
| *Drechslera* sp | - | - | - | - | - | - | - | - | 9 | 4.39 | 2 | 2.32 | 1 | 0.97 | - | - | - | - | - | - | - | - | - | - |
| *Fusarium incarnatum* | 2 | 2.24 | - | - | - | - | 7 | 10.93 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Fusarium nivale* | 5 | 5.61 | 1 | 2.94 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Fusarium oxysporum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 21 | 6.10 | 2 | 1.57 | 1 | 0.82 | - | - |
| *Fusarium* sp.1 | 1 | 1.12 | 1 | 2.94 | 2 | 3.03 | 3 | 4.68 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Fusarium* sp.2 | 4 | 4.49 | - | - | - | - | 2 | 3.12 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Fusarium* sp.3 | - | - | - | - | - | - | - | - | 10 | 4.87 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Fusarium* sp.4 | - | - | - | - | - | - | - | - | 6 | 2.92 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Fusarium* sp.5 | - | - | - | - | - | - | - | - | 3 | 0.48 | 1 | 1.16 | - | - | - | - | - | - | - | - | - | - | - | - |
| *Humicola brevis* | 1 | 1.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Humicola* sp. | 1 | 0.29 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Hormiscium* sp. | - | - | - | - | - | - | - | - | 18 | 0.78 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Penicillium duclauxi* | - | - | - | - | 1 | 1.51 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Penicillium frequentans* | 3 | 3.37 | - | - | 1 | 1.51 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Penicillium vinaceum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 16 | 4.65 | 11 | 8.66 | - | - | - | - |
| *Penicillium* sp.1 | - | - | - | - | - | - | - | - | 3 | 1.46 | - | - | 1 | 0.97 | - | - | - | - | - | - | - | - | - | - |
| *Penicillium* sp.2 | - | - | - | - | - | - | - | - | 2 | 0.97 | - | - | 1 | 0.97 | 1 | 1.92 | - | - | - | - | - | - | - | - |
| *Rhizopus* sp.1 | 5 | 5.61 | 2 | 5.88 | 3 | 4.54 | 7 | 10.93 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Rhizopus* sp.2 | - | - | - | - | - | - | - | - | 21 | 10.24 | - | - | 11 | 10.67 | 7 | 13.46 | - | - | - | - | - | - | - | - |
| *Rhizopus* sp.3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 32 | 9.30 | - | - | 19 | 15.70 | - | - |
| *Scopulariopsis* sp. | - | - | - | - | - | - | - | - | 10 | 4.87 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Sporotrichum pruinosum* | 7 | 7.86 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Stemphylium* sp. | - | - | - | - | - | - | - | - | 2 | 0.97 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Trichoderma* sp. 1 | - | - | - | - | 5 | 7.57 | 8 | 12.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Trichoderma* sp. 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 21 | 13.81 |
| *Verticillium* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 4 | 1.16 | - | - | - | - | - | - |
| Black sterile mycelia | 7 | 7.86 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| White sterile mycelia 1 | 8 | 8.98 | - | - | 1 | 1.51 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| White sterile mycelia 2 | - | - | - | - | - | - | - | - | 22 | 10.73 | 2 | 2.32 | - | - | - | - | - | - | - | - | - | - | - | - |
| White sterile mycelia 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 46 | 13.37 | - | - | - | - | - | - |
| **Number of Species** | **15** | | **6** | | **10** | | **9** | | **15** | | **07** | | **08** | | **05** | | **15** | | **07** | | **07** | | **05** | |
| **Total Isolates** | **89** | | **34** | | **66** | | **64** | | **205** | | **86** | | **103** | | **52** | | **344** | | **127** | | **121** | | **152** | |
| **Simpson's index (D)** | **0.1085** | | **0.3547** | | **0.2298** | | **0.2121** | | **0.1106** | | **0.3775** | | **0.2859** | | **0.4162** | | **0.1269** | | **0.2479** | | **0.2685** | | **0.2982** | |
| **Simpson's index of Diversity (1−D)** | **0.8915** | | **0.6453** | | **0.7702** | | **0.7879** | | **0.8894** | | **0.6225** | | **0.7141** | | **0.5838** | | **0.8731** | | **0.7521** | | **0.7315** | | **0.7018** | |

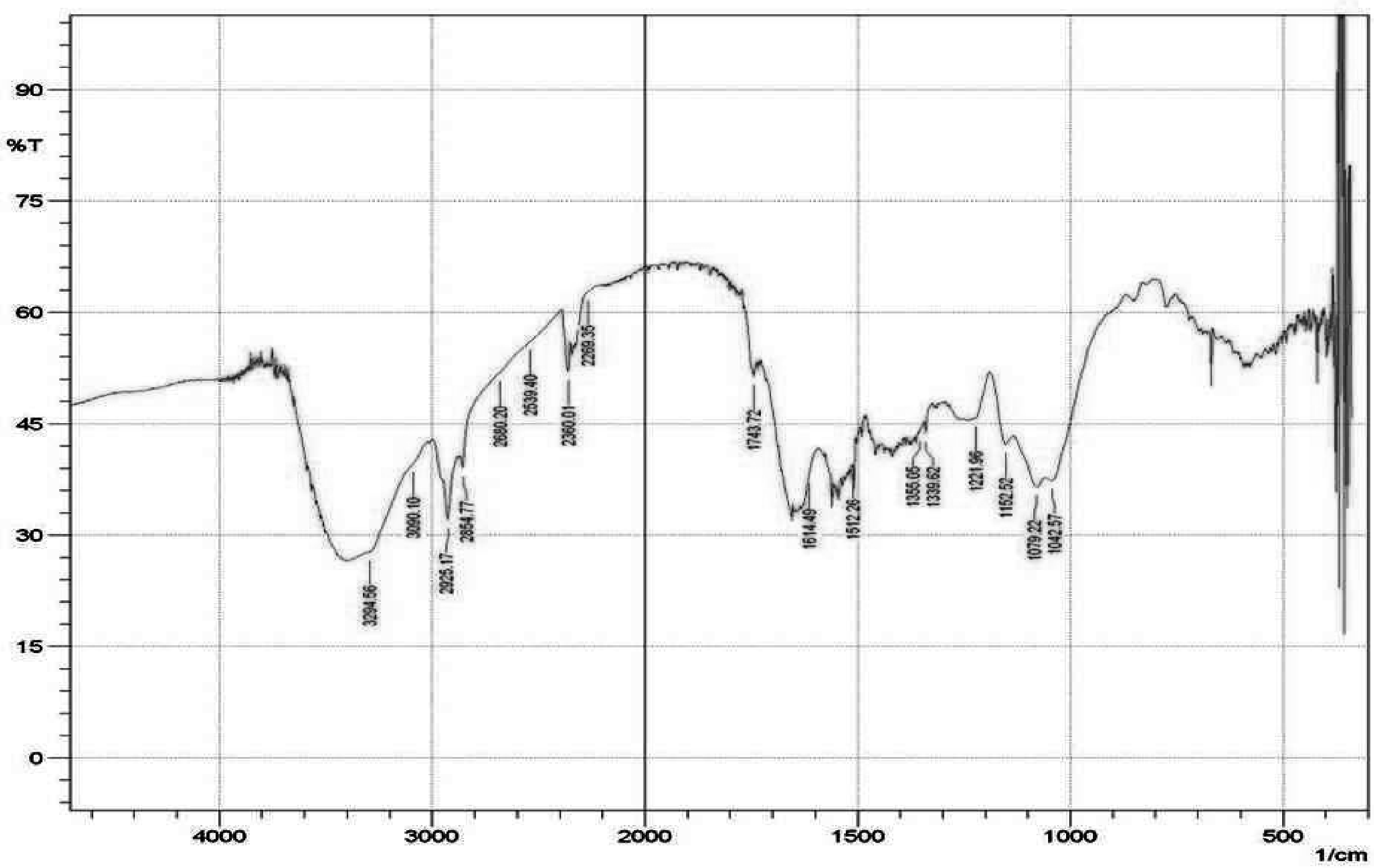


**Fig 1:** FTIR spectrum of lead tolerant *A. niger* biomass.

However, Tiwari and Charaya (2006) could isolate only 23 fungal species probably because of application of a different approach in which the "sieved soils" were filled in the pots and were subsequently given treatment with chromium sulphate. Pickett and White (1985) suggested that the soil transfer from natural conditions to pots might lead to the reduction in number of fungal species possibly due to the disturbance caused during drying, sieving and transfer to pots; and these processes limits the resource availability and system structure. Sen (2007) was able to obtain 41 fungal species, using a bit different protocol in which suspensions prepared from natural untreated soils were inoculated in the nutrient media amended with heavy metals.

As indicated by Tables 1 and 2, lead sulphate as well as lead nitrate appear to exert an inhibitory effect on the soil mycobiota as the number of species obtained from the lead-treated soils were always lesser than the number of species that were isolated from control soils. The analysis of variance revealed that the treatment with lead sulphate had significant negative effect on the qualitative as well as quantitative distribution of fungi in the soil (F= 27.13, significant at 0.01 level; and 4.96, significant at 0.05 level respectively). The results with lead nitrate also yielded significant negative effect (F= 8.89; significant at 0.05 level) in case of number of species but the values were insignificant for total number of isolates. This assertion is further confirmed by the Simpson's indices of diversity as shown in tables 1 and 2. Also, adverse effects of lead on myco-diversity became more remarkable as the duration of the treatment increased though this was found to be statistically insignificant (F= 1.42; 3.32).

**Table 3: Analysis of variance table for mycobiota in control and lead (Pb) salts treated soils.**

| **Source of variation** | **Lead sulphate** | | **Lead nitrate** | |
|---|---|---|---|---|
| | **No. of species** | **Total isolates** | **No. of species** | **Total isolates** |
| **Concentrations of treatment (500, 1000 and 2000 ppm)** | 27.13** | 4.96* | 8.89* | 4.64 |
| **Duration of treatment** | 1.42 | 6.609* | 3.32 | 10.33* |

- *Significant at 0.05 level; **Significant at 0.01 level

Taking into account the concentration of lead sulphate solution and duration of treatment, the maximum tolerance was shown by *Aspergillus niger* along with *Aspergillus luchuensis* followed by *Aspergillus flavus, Aspergillus fumigatus* and *Trichoderma* sp. which survived and dominated the soils even on 60th day in the soils treated with 2000 ppm lead sulphate solution. These four species may, therefore, be considered to be highly tolerant to lead sulphate. *Alternaria* sp. *Botrytis* sp., *Fusarium oxysporum* and a strain of *Rhizopus* marked their presence in soils treated with 1000 ppm for 60 days but their numbers were remarkably lesser than that of *Aspergillus niger.* A strain of *Penicillium* and a strain of *Rhizopus* could tolerate lead sulphate upto 2000 ppm concentration but for a shorter period of 40 days. *Alternaria citri, Fusarium incarnatum, Fusarium nivale, Fusarium* sp., *Humicola brevis, Humicola* sp., *Penicillium frequentans, Sporotrichum pruinosum,* a strain of black sterile mycelium and a strain of white sterile mycelium 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 exhibit differential response to lead sulphate.

In case of lead nitrate treated soils, *Aspergillus niger* and *Aspergillus luchuensis* were found to be most tolerant to lead nitrate. *Aspergillus flavus, Aspergillus fumigatus, Fusarium oxysporum, Fusarium* sp. and *Trichoderma lignorum* exhibited tolerance to lead nitrate solution even on the 60th day though to a limited extent. *Curvularia* sp., *Hormiscium* sp., *Rhizopus* sp., *Sporotrichum* *chlorinum* and a strain of *Stemphylium* could not withstand even the minimum concentration of lead nitrate. The results obtained through soil plate method (Table 4) also reveal that *A. niger* is most resistant to Pb (II) followed by *A. luchuensis*. Many workers have reported the dominance of *Aspergillus niger* in heavy metal contaminated soils (Iram *et al.*, 2009; Al-Soshaibani, 2011; Iram *et al.*, 2012; Iram *et al.*, 2013 and Choudhary *et al.,* 2015).

FTIR spectrum of the biomass of *Aspergillus niger*, which appears to be most resistant fungal species in the present study, was characterized by 17 peaks (Fig 1). The wavenumbers of the 17 peaks are given below with corresponding functional groups represented by the peaks following Smith and Dent (2006): (1) 1042.57 [C-C aliphatic chains (m), Aromatic rings (s), Si-O-C (w), Si-O-Si (w), C=S (s), Sulfonic acid (vw)]; (2) 1079.22 [C-C aliphatic chains (m), Aromatic rings (s), Si-O-C (w), Si-O-Si (w), C=S (s), Sulfonamide (m), Sulfone (m)]; (3) 1152.52 [C-C aliphatic chains (m), C=S (s), Sulfonamide (m), Sulfone (m), Si-O-C (w), Sulfonic acid (vw)]; (4) 1221.96 [C-C-aliphatic chains (m), C=S (s), Sulfonic acid (vw)]; (5) 1339.62 [Carboxylate salt (m), Nitro (vs)]; (6) 1355.05 [Carboxylate salt (m), C-$CH_3$ (w)]; (7) 1512.26 ; (8) 1614.49 [Amide (s), Ketone (m), Carboxylic acid (m)]; (9) 1743.72 [Ester (m), Aliphatic ester (m), Lactone (m), Anhydride (m)]; (10) 2269.35 [Diazonium salt (m), Isocyanate (vw)]; (11) 2360.01 [P-H (vw)]; (12) 2539.4 [Thiol (s)]; (13) 2680.2 [Aldehyde (w)]; (14) 2854.77 [C-$CH_3$ (s)]; (15) 2929.17 [C-$CH_3$ (s), Aromatic C-H (s), OH (w), $CH_2$ (s)]; (16) 3090.1 [Aromatic C-H (s), OH (w)]; (17) 3294.56 [OH (w), Amide (m), Amine (m), Phenol (w), Alkyne (vw)].

Ahluwaliya and Goyal (2007) in their studies on FTIR spectroscopy on *Aspergillus niger* biomass revealed the presence of amine, C=N, C=C, C-Cl and C-O functional groups which are involved in lead binding. Kurc *et al.* (2016) attributed the binding sites of lead to amine groups present on the surface of *Penicillium* sp. Rama Rao *et al.* (2005) suggested the involvement of alcohol / amine (OH / $NH_2$) and CH-OH functional groups in metal binding though using different species of *Aspergillus.* Ratnasari and Hemlatha (2015) concluded in their studies on FTIR spectroscopy of *Aspergillus* spp. that OH, NH, C-H, C=O, amide, alcohols, amines and carboxylic acid groups were present on the surface and are responsible for the biosorption of metals. In the present study also, the presence of amine, OH, amide, carboxylic acid indicate the potential of *Aspergillus niger* to bind lead. Therefore, *Aspergillus niger* seems to serve as a fit material for removal of lead (Pb) from effluents / soil through biosorption.

**Table 4: Qualitative distribution of mycobiota in soils– control as well as treated with 500 ppm, 1000 ppm and 2000 ppm concentrations of lead sulphate and lead nitrate over a period of 60 days (as obtained by soil plate method).**

| Fungi | Control (20 days) | Lead sulphate (20 days) | | | Lead nitrate (20 days) | | | Control (40 days) | Lead sulphate (40 days) | | | Lead nitrate (40 days) | | | Control (60 days) | Lead sulphate (60 days) | | | Lead 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 flauvs* | - | - | + | - | - | + | - | - | + | - | + | - | - | - | ++ | +++ | +++ | ++ | - | + | - |
| *Aspergillus fumigatus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | + | - | - | - | - |
| *Aspergillus luchuensis* | - | - | - | - | - | - | - | +++ | ++ | ++ | ++++ | ++++ | + | ++ | ++++ | +++ | +++ | ++ | ++ | +++ | ++ |
| *Aspergillus niger* | + | ++ | +++ | ++ | +++ | ++ | ++++ | +++ | +++ | ++++ | ++++ | +++ | +++ | +++ | ++++ | +++ | ++++ | ++ | +++ | ++ | +++ |
| *Choanephora* sp. | ++ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Fusarium incarnatum* | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Fusarium nivale* | - | - | - | - | - | - | - | ++ | + | + | ++ | + | + | + | - | - | - | - | - | - | - |
| *Fusarium oxysporum* | - | - | - | - | +++ | - | - | - | - | - | - | - | - | - | - | ++ | ++ | + | - | + | - |
| *Fusarium* sp. | + | - | ++ | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Mucor racemosus* | - | - | - | - | - | - | - | - | ++++ | - | - | - | +++ | - | - | - | - | - | - | - | - |
| *Mucor* sp. | ++++ | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | +++ | ++ | + | - | - | - |
| *Penicillium frequentens* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - |
| *Penicillium oxalicum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| *Penicillium* sp. | - | - | - | - | - | - | - | + | - | - | + | - | - | - | - | - | - | - | - | - | - |
| *Rhizopus arrhizus* | - | - | - | - | - | - | - | ++++ | +++++ | ++ | ++ | ++ | +++++ | - | - | - | - | - | - | - | - |
| *Rhizopus* sp. | +++ | +++ | - | + | - | - | - | - | - | - | - | - | - | - | +++ | - | ++++ | - | - | - | - |
| *Sporotrichum* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - |
| *Trichoderma lignorum* | - | - | - | - | - | - | - | ++ | - | - | - | - | - | + | +++ | + | + | + | + | ++ | + |
| White sterile mycelium | - | +++ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

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

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