Environmental Technology & Innovation 9 (2018) 91–99

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Screening and selection of indigenous metal tolerant fungal isolates for heavy metal removal

Karishma Gururajan, Prasanna D. Belur *

Department of Chemical Engineering, NITK Surathkal, Mangalore-575 025, Karnataka, India

HIGHLIGHTS

- Indigenous fungal isolates were studied for efficient heavy metal removal.
- Live biomass of the selected isolates was used for the study.
- Selected isolate exhibited 90% efficiency in lead removal in synthetic solution.
- SEM analysis of the fungal biomass shows bio-adsorption as the primary mechanism.
- FTIR confirms the participation of several organic groups associated with cell wall.

ARTICLE INFO

Article history:
Received 3 June 2017
Received in revised form 6 October 2017
Accepted 7 November 2017
Available online 22 November 2017

Keywords:
Heavy metal removal
Indigenous
Fungi
Live biomass
Lead (II)

ABSTRACT

Heavy metal removal efficiency of indigenously present metal tolerant fungal isolates obtained from a scrap dumpsite was assessed in this study. Four fungal isolates were selected based on their ability to grow in multi-metal supplemented media. Minimum inhibition concentrations of these four isolates were determined against individual metals; lead (II) (50–400 mgL^{-1}), cadmium (II) (50–400 mgL^{-1}), arsenic (III) (10–100 mgL^{-1}) and mercury (II) (10–100 mgL^{-1}). Their ability to remove metals from synthetic aqueous medium was tested and the heavy metal–fungi combination which showed the highest removal efficiency was selected. Live biomass of the selected isolate dispensed in lead solution with concentrations of 50 mgL^{-1}, 100 mgL^{-1} and 150 mgL^{-1} showed a removal of 92.27%, 92.73% and 89.36% respectively at the end of the 40th h. Scanning Electron Microscopy with Electron Dispersive X-ray (SEM-EDX) of the treated biomass confirmed the biosorptive ability of the isolate for lead when compared with the control biomass. Fourier Transforms Infra-red (FTIR) Spectroscopy showed the probable involvement of amide, carboxylic acid, hydroxyl and isocyanate groups in the adsorption of lead from the synthetic metal solution.

1. Introduction

Heavy metal pollution is continuing to be a subject of great concern in the environmental arena. Rapid industrialization and anthropogenic mishandling have extensively contributed to the release of these heavy metals into the surroundings, posing a significant threat to the environment and public health because of their toxic nature, accumulative and persistent properties and immutable quality (Kapoor and Viraraghavan, 1995; Desai et al., 2016). Out of more than 20 toxic heavy metals present; lead, arsenic, mercury and cadmium are considered to be of utmost priority according to the Agency for

* Corresponding author.
E-mail address: prsn@nitk.edu.in (P.D. Belur).

https://doi.org/10.1016/j.eti.2017.11.001
2352-1864/

Toxic Substances and Disease Registry (Public health agency of the U.S. Department of Health and Human Services). Several conventional methods have been employed for the removal of heavy metals such as chemical precipitation, ultrafiltration, ion exchange, reverse osmosis, electro winning, solvent extraction and carbon adsorption. Most of these conventional methods for heavy metal elimination are considered to be less effective, expensive and environmentally challenging due to the generation of toxic by-products with other limitations (Fu and Wang, 2011; Gunatilake, 2015). A rise in awareness on environmental issues coupled with the emergence of stringent environmental laws on industries is shifting the balance to using more economically viable alternative strategies (Kapoor and Viraraghavan, 1995; Dhankhar and Hooda, 2011).

Metal remediation using microorganisms has been widely exploited in recent years and has shown efficient progress. Most importantly, the fungal community is receiving widespread attention as an adsorbent in the field of remediation because of the high percentage of cell wall material. This increases the presence of variety of functional groups for metal binding, in turn increasing the metal sequestration efficiency of the fungi. The cell wall sorption of metal ions usually occurs by physicochemical interactions with the functional groups present on the cell surface. The mechanism of metal removal is a complex process influenced quantitatively and qualitatively by the nature and type of biomass, chemistry of metal in question and different environmental parameters (Dhankhar and Hooda, 2011; Abbas et al., 2014; Siddiquee et al., 2015). Studies have been extensively performed on the utilization of fungal biomass in the remediation and transformation of several heavy metals. Live, dead, pre-treated and immobilized forms of *Aspergillus* sp, *Penicillium* sp, *Botrytis* sp, *Trichoderma* sp, *Saprolegnia* sp, *Neurospora* sp isolated from different environment samples have been used to remove heavy and toxic metals with appreciable results (Akar et al., 2005; Ali and Hashem, 2007; Sun et al., 2010; Shazia et al., 2013; Aytar et al., 2014; Kurniati et al., 2014; Mohammadian Fazli et al., 2015; Desai et al., 2016).

A lot of attention is being diverted in the application of live fungal biomass for remediation because they are ubiquitous in nature and are dominantly present in certain soils (Malik, 2004). Continued exposure of heavy metal in any contaminated area tends to exert a selective pressure on microorganisms in soil due to long term accumulation (Zafar et al., 2007; Joshi et al., 2011). Hence, it is advantageous to discover fungi from such environmental places and identify the ones that are metal resistant to obtain a higher efficiency of metal removal (Zafar et al., 2007; Iram et al., 2013).

In the present study, fungal cultures were isolated from the soil taken from a scrap disposal unit. They were explored and checked for their degree of metal tolerance against lead, cadmium, arsenic and mercury. Their efficiency of metal removal from synthetic solutions was also assessed. The heavy metal treated biomass along with a control was subjected to SEM-EDX analysis to examine any morphological or structural changes and FTIR spectroscopy to identify the functional groups that may have been involved in metal ion interaction.

2. Materials and methods

2.1. Screening and selection of metal tolerant fungal isolates

Soil samples from a scrapyard dumpsite present amidst an industrial area (Mangalore, India) was collected to find and select multi-metal tolerant fungal isolates by the standardized serial dilution and spread plate method using potato dextrose agar (PDA) medium (Hi-Media Lab Ltd, Mumbai) which was supplemented with 10 mgL^{-1} of heavy metal mixture containing arsenic (III), mercury (II), lead (II) and cadmium (II).

The soil sample (1g) was suspended in 10 mL of sterile distilled water and 1mL of this mixture was successively pipetted to 9 mL of sterile distilled water to obtain dilutions upto 10^{-5}. 100 μL of these dilutions were spread on PDA plates supplemented with streptomycin to prevent bacterial growth. A control plate (without any metal) was also prepared. These plates were incubated at 28–30 °C for 5–7 days. The fungal isolates were selected depending on their frequency of occurrence by comparing the metal plates with the control. Similar isolates were selected and maintained on PDA plates/slants at 4 °C.

2.2. Heavy metal study of the soil sample

The heavy metal content in the soil sample was estimated by subjecting it to acid digestion using modified USEPA 3050 B method (U.S Environment Protection Agency, 1996) followed by Atomic Absorption Spectrophotometer (AAS) analysis. 1g of the dried soil sample was treated with hydrochloric acid mixture (25 mL of concentrated HCl + 25 mL of distilled water). This mixture was treated with 5 mL of concentrated nitric acid. This combination was digested overnight on a heating mantle until the dense fumes were released and a clear mixture was obtained. The digested mixture was filtered using Whatman paper (No.1) till the filter paper turned colourless. The filtrate was used for the confirmation of selective heavy metals lead, cadmium, arsenic and mercury using GBC-932 plus Atomic Absorption Spectrophotometer.

2.3. Measuring the minimum inhibitory concentration

The heavy metal tolerance of selected fungal isolates towards individual metal was determined in terms of minimum inhibitory concentration. PDA medium was amended with the desired concentrations of heavy metals ranging from 50 $mgL^{-1}$to 400 mgL^{-1} (lead and cadmium) and 10 mgL^{-1} to 100 mgL^{-1} (arsenic and mercury). The selected fungal isolates were placed in the centre of the plate using sterile filter paper discs. These plates were incubated at 28–30 °C for 3–6 days to observe the growth of fungi in the placed region. The growth was visually inspected and recorded as good, moderate, poor growth (visible) or no growth by comparing with the control plates (without any metal).

2.4. Heavy metal removal from liquid media

The selected isolates were grown on DP (Dextrose Peptone) minimal media used by Kapoor and Viraraghavan (1998) containing inorganic salts. The constituents were measured accordingly and made upto 50 mL using distilled water. The pH of the medium was found to be around 6. The fungal cultures were then inoculated into the sterilized medium and incubated on a shaker at 150 rpm at 28–30 °C for 5–6 days and were used immediately.

The metal removal ability of these isolates was evaluated by incorporating different concentrations of individual heavy metals in DP media. 50 mgL^{-1} and 100 mgL^{-1} of lead and cadmium as well as 10 mgL^{-1} and 50 mgL^{-1} of arsenic and mercury were suspended separately into flasks containing DP medium and sterilized. These flasks were then inoculated with twice the amount of prepared biomass suspension with respect to the volume of heavy metal solution added (2:1) and incubated on a shaker at 150 rpm at 28–30 °C. Un-inoculated flasks with heavy metals were used as control. The fungal biomass was harvested after 96 h by filtration using Whatman paper. The biomass obtained was dried at 70 °C for 18 h and the average dry weight was recorded. The reduction in heavy metal concentration in the filtrate (filtered broth) was estimated using AAS (GBC-932 Plus) with air–acetylene flame. The metal removal in terms of percentage was determined form the following equation (Singh et al., 2010; Li et al., 2011):

$$\%\,\text{Removal} = [(Co - C)/Co] * 100 \tag{1}$$

Where, Co = initial concentration of the metal, mgL^{-1}; C = final concentration of metal after the process, mgL^{-1}. The single best heavy metal–fungal isolate combination providing maximum removal efficiency was used for subsequent studies.

2.5. Ability of the live biomass to remove lead

The selected fungal isolate was grown in DP minimal medium. The biomass was harvested by centrifuging at 10,000 rpm for 20 min. This biomass was washed thoroughly (3–4 times) with sterile distilled water to remove any traces of residual media. It was immediately assessed for its ability to remove lead (II).

100 mL of lead metal solution prepared in distilled water with concentration ranges of 50 mgL^{-1},100 mgL^{-1} and 150 mgL^{-1} were sterilized. To the above flasks, 5g (wet weight) of live biomass were added and incubated on a shaker at 30 °C and 150 rpm. The flasks with only metal served as control. Sampling was done at 16th, 24th and 40th h and centrifuged at 10,000 rpm for 10 min to separate the biomass. The supernatant was analysed to check for the change in concentration of lead using AAS (GBC-932 Plus). The metal removal in terms of percentage was determined from Eq. (1). The bio-adsorption capacity of the biomass can be calculated using the following equation (Yan and Viraraghavan, 2000; Zafar et al., 2007):

$$Q = \left(\frac{Ci - Cf}{m}\right) V \tag{2}$$

Where, Q = mg of metal ion biosorbed per g of biomass, Ci = initial metal concentration, mgL-1; Cf = final metal concentration, mgL-1; m = mass of the biomass used, g; V = volume of the reaction mixture, L.

2.6. SEM-EDX and FTIR Analysis

The biomass treated with 50 mgL^{-1}, 100 mgL^{-1} and 150 mgL^{-1} of lead and control biomass (without any metal) was subjected to Scanning Electron Microscopy (SEM) coupled with Electron Dispersive X-ray (EDX) analysis. The samples were prepared by fixing them first with 10% gluteraldehyde for 90–120 min at 4 °C. The biomass was centrifuged at 10,000 rpm for 10 min and dehydrated using 50% and 100% ethanol to remove any possible traces of water which would otherwise hinder the process. The dehydrated samples were then dried at 60 °C to ensure complete removal of moisture (Moharrer et al., 2012). These dried samples were first coated on carbon tapes and then sputtered twice with gold particles to make them electrically conducting. The samples were then analysed for their topological and morphological characteristics using SEM (JOEL JED 230 instrument) analysis and for their chemical characteristics using EDX.

The same samples were subjected to FTIR Spectroscopy (JASCO FTIR 4200) to find any variation in the functional group peaks that may be involved in the removal of lead.

3. Results

3.1. Heavy metal analysis of soil and MIC study

The fungal cultures were isolated from a small scrap dumpsite. The soil sample was analysed for the presence of four toxic heavy metals lead (II), cadmium (II), arsenic (III) and mercury (II). The metals arsenic and mercury were found to be present below detectable limits, cadmium at a level of 37.5 μg/g of soil and the lead (II) level was found to be 13.18 mg/g of soil.

The tolerant fungal isolates from the soil were obtained using PDA media supplemented with multi-metals. A total of four isolates were selected (Isolate-1, Isolate-2, Isolate-3 and Isolate-4) to check their tolerance towards lead (II), cadmium (II), arsenic (III) and mercury (II) individually. Out of the four heavy metals, all four isolates showed maximum tolerance to

Table 1
Minimum inhibition concentration by tolerant fungal isolates for Arsenic and Mercury at 10 mgL^{-1}, 50 mgL^{-1} and 100 mgL^{-1}.

Metals	Fungi	Minimum inhibitory concentration		
		10 mgL^{-1}	50 mgL^{-1}	100 mgL^{-1}
Arsenic	Isolate-1	++	++	+-
	Isolate-2	++	++	+-
	Isolate-3	+-	+-	NG
	Isolate-4	+-	+-	NG
Mercury	Isolate-1	+-	--	NG
	Isolate-2	+-	--	NG
	Isolate-3	--	NG	NG
	Isolate-4	+-	--	NG

(++ good growth, +- moderate growth, –poor growth, NG-no growth).

Table 2
Minimum inhibition concentration by tolerant fungal isolates for Lead and Cadmium at 50 mgL^{-1},100 mgL^{-1},200 mgL^{-1} and 400 mgL^{-1}.

Metals	Fungi	Minimum inhibitory concentration			
		50 mgL^{-1}	100 mgL^{-1}	200 mgL^{-1}	400 mgL^{-1}
Cadmium	Isolate-1	--	NG	NG	NG
	Isolate-2	--	--	--	NG
	Isolate-3	--	NG	NG	NG
	Isolate-4	--	--	NG	NG
Lead	Isolate-1	++	++	++	+-
	Isolate-2	++	++	++	+-
	Isolate-3	+-	+-	+-	--
	Isolate-4	++	++	+-	+-

(++ good growth, +- moderate growth, –poor growth, NG-no growth).

lead on PDA supplemented with 50–400 mgL^{-1}. For cadmium, the isolates were found to be tolerant upto 100–200 mgL^{-1}. For arsenic, the growth of isolates was visible upto 50–100 mgL^{-1} and for mercury visible growth was recorded on 10–50 mgL^{-1} media. The isolates showed least tolerance towards mercury and cadmium. The MIC data chart is listed in Tables 1 and 2.

3.2. Heavy metal removal from liquid medium

The fungal isolates were assessed for their ability to remove heavy metals form synthetically prepared DP minimal media (pH 6) supplemented individually with 50 and 100 mgL^{-1} of lead and cadmium and 10 and 50 mgL^{-1} of arsenic and mercury after 96 h of incubation. The results are presented in Figs. 1–2. Very little removal efficiency was seen in the case of mercury and cadmium by all the four isolates (about 10–20% average). In the case of arsenic, at a concentration 10 mgL^{-1}, isolate-4 showed about 62.74% followed by isolate-3 which showed 58.5% removal. The other 2 isolates showed about an average of 34% removal. For 50 mgL^{-1}, 38.1% removal was obtained by isolate-2. In this study, best results were observed for lead (II). All the four isolates showed an average of more than 95% efficiency. The fungal isolate-2 showed 99.78% and isolate-4 showed 99.55% removal efficiency at 50 $mgL^{-1}$of lead (II). For 100 mgL^{-1}, isolate-2 showed about 81.21% and the remaining 3 isolates showed an average removal efficiency of 75%.

3.3. Removal of lead using live fungal biomass

The best metal ion-fungal biomass interaction was observed in the case of lead (II) and fungal isolate-2. The live biomass of isolate-2 was assessed for its efficiency to remove 50,100 and 150 mgL^{-1} of lead (II) dispensed in distilled water and the results are shown in Fig. 3. The samples were withdrawn at 16th, 24th and 40th h to analyse the residual lead. At the end of 40 h about 92% removal was observed at levels of 50 mgL^{-1} and 100 mgL^{-1}. For the concentration of 150 mgL^{-1}, at the end of 40th h, the removal was found to be 89%. The bio-adsorption capacity of isolate-2 for 50,100 and 150 $mgL^{-1}$of lead was 1.16 mg/g, 1.98 mg/g and 2.45 mg/g respectively.

3.4. SEM-EDX analysis and FTIR spectra before and after exposure to lead

SEM-EDX analysis was performed on the control biomass of isolate-2 and compared with the biomass treated with 50 mgL^{-1},100 mgL^{-1} and 150 mgL^{-1} of lead to visualize any change in morphological characteristics (Fig. 4).

Visible damage to the structure of the fungal biomass treated with lead when compared to the control was minimal and not much structural variations were seen indicating that the interaction may have occurred at the interphase of the

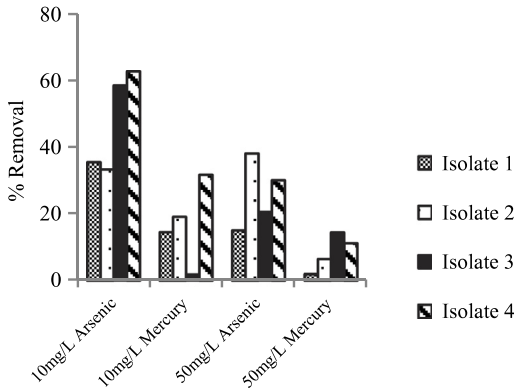

Fig. 1. Percentage removal of arsenic and mercury (10 and 50 mgL^{-1}) by the four isolates.

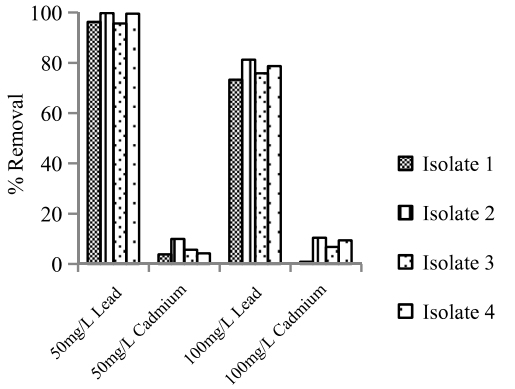

Fig. 2. Percentage removal of cadmium and lead (50 and 100 mgL^{-1}) by the four isolates.

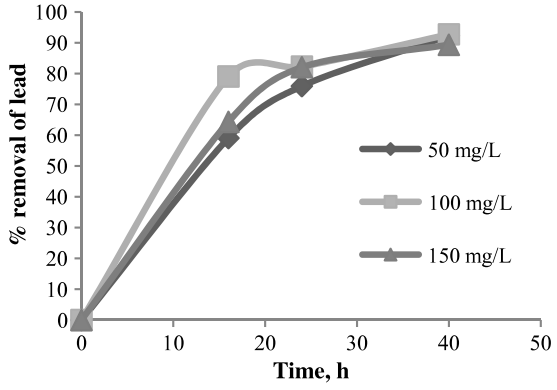

Fig. 3. Percentage removal of lead (II) ion by live fungal biomass as a function of time (h).

bio-adsorbent. Furthermore, the EDX spectra of control biomass did not show any signal for lead whereas the EDX spectra of biomass treated with 50 mgL^{-1}, 100 mgL^{-1} and 150 mgL^{-1} showed presence of lead in increasing concentrations, indicative of the biosorptive ability of the isolate-2.

Fourier transform infra-red spectroscopy (FTIR) analysis was performed for the same samples to identify the functional group that might be involved in metal ion interaction. The result of the spectrum is shown in Fig. 5.

The isolate-2 showed a broad spectrum band at 3272.61 cm^{-1} (3500–3000 cm^{-1}) representing the overlapping of N–H stretching/O–H stretching; 2923.56 and 2853.65 cm^{-1} spectrum represented C-H stretching which indicated the involvement of methylene and methyl group attributed to the fungal membrane phospholipids . The band at 1745.26 cm^{-1} could

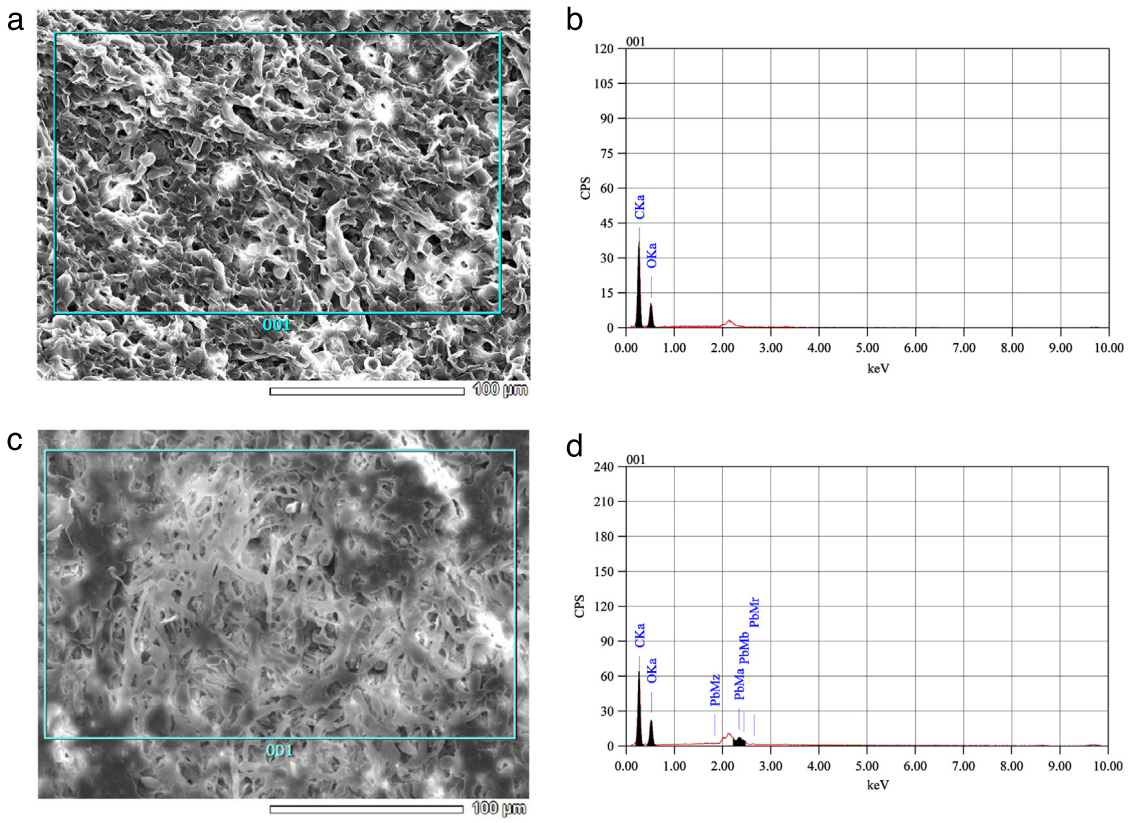

Fig. 4. (a) and (c) are SEM images of Control biomass and biomass treated with 150 mgL^{-1} lead respectively, (b) and (d) are the respective EDX spectrum.

be attributed to C=O stretching of carboxyl or amide group of esters. The vibration of peaks 1649.32 cm^{-1} and 1544.22 cm^{-1} corresponds to the C=C, C=O or C=O of conjugated ketones of primary and secondary amides and 1235.18 cm^{-1} attributes to the phosphate functional group. 1026 cm^{-1} vibration stretch belonged to the OH group of the phenolics. There was a strong variation in stretching at 2361 cm^{-1} which indicated the involvement of N=C=O (isocyanate group).

In the spectrum analysis of live biomass treated with 50 mgL^{-1}, 100 mgL^{-1} and 150 mgL^{-1} of lead, slight variation in the stretching intensities and shifting of certain peaks were observed. There was a strong stretching seen at peak 2361(isocyanate group) and 2334 cm^{-1} in 50 mgL^{-1} and 100 mgL^{-1} treated biomass but was not that prominent in biomass treated with 150 mgL^{-1}. It could be assumed that lead ions might have bonded loosely to the isocyanate functional group but further evaluation needs to be done. Similar stretching variation was observed at peak 1745 cm^{-1} indicating the involvement of carboxyl or amide group. Differences in stretching vibrations were observed across 1600 cm^{-1} to 800 cm^{-1} which indicated the probable involvement of amide I (C=O) stretch, amide II (N–H bend and C–N stretch), methyl bend, phosphate stretch, C–O–C stretch of polysaccharides.

4. Discussion

Long term exposure of toxic metals to a certain extent can modify and reduce the species diversity of the soil microbiota but enhance the selection of a dominant tolerant population (Gadd, 1993; Iram et al., 2013). The present study involves the isolation of fungal cultures from the soil of a scrapyard showing tolerance towards multi-metal solution. The microbiota in the environment have created a distinctive and in some cases peculiar ways of interacting with the abundantly present toxic and unwanted metals. Their survival is based on their inherent biochemical and anatomical trait and adaptation at a more physiological or genetic level. Some show exceptional sequestration and immobilization properties whereas some boost the solubility of metals (Gadd, 1993; Pumpel and Paknikar, 2001).

In this study, increasing the metal concentration beyond certain levels increased the sensitivity of these isolates. The change in tolerance towards different metals could be because of the altered endurance approach or resistance mechanisms exhibited by different fungi (Zafar et al., 2007). At lower concentration of metals, the isolates showed resistance and good growth which subsided as the concentrations increased. Maximum tolerance was observed for lead upto 400 mgL^{-1}. This is understandable, since the isolates were obtained from soil sample rich in lead. Similar studies have also been reported

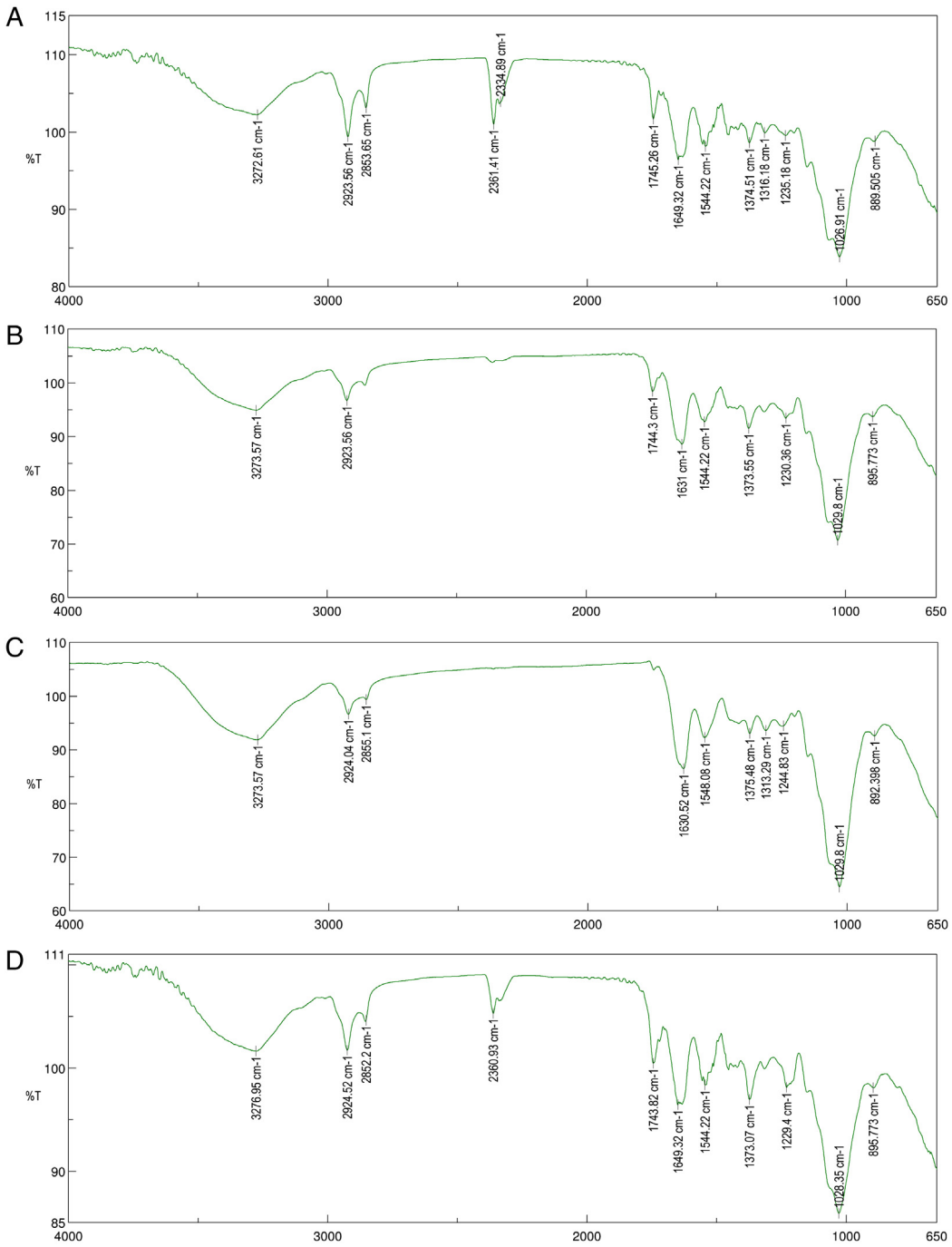

Fig. 5. FTIR spectrum of A- control biomass, B- treated with 50 mgL^{-1}, C- treated with 100 mgL^{-1}, D- 150 mgL^{-1} of lead.

(Joo and Hussein, 2012; Kumar et al., 2014). Some degree of tolerance was seen towards arsenic (50 mgL^{-1}) and mercury (10 mgL^{-1}) which were found below detectable limits in the soil. The resistance offered to these toxic metals could be dependent mostly on the isolates themselves. Their tolerance abilities could be attributed to the ability of the isolate to adapt and survive under stressed conditions (Iram et al., 2013; Mohammadian Fazli et al., 2015).

The highest removal efficiency in this study was observed for lead by all four isolates with an average of 95% efficiency. The isolate-2 showed an efficiency of 99.78% at 50 mgL^{-1} and 81.21% efficiency at 100 $mgL^{-1}$of lead. In the studies performed

by Ali and Hashem (2007), the maximum uptake of lead was around 90% by *Saprolegnia delica* and 66.53% by *T.viridae* at pH 6. Fungal isolates obtained from sewage, sludge and electroplating effluent were checked for their ability to uptake lead, cadmium, chromium and nickel where efficient performance was observed by tolerant isolates. Maximum uptake of lead (59.67 mg/g dry weight) was observed by lead tolerant isolate *Aspergillus terreus* and *T. viridae* showed a maximum removal (16.25 mg/g) for cadmium (Joshi et al., 2011). Tolerant fungi *A. flavus* isolated from sewage and industrial effluents showed maximum uptake of lead (17.35 mg/g) (Dwivedi et al., 2012). Reactor studies performed using tolerant *A. flavus* obtained from electroplating soil showed maximum removal of lead (98.77%) on the 10th day (Yadav et al., 2012). Shazia et al. (2013) indicated the influence of metal concentration on biosorption capacity where the fungal isolate *Aspergillus fumigatus* exhibited maximum biosorption rate of 76.07 at 800 $mgL^{-1}$of lead and exhibited a linear pattern. *A. niger* and *A. flavus* showed accumulation of 75% and 82% of lead respectively (Shivakumar et al., 2014). One of the most prominent features that enable the fungal biomass to initiate maximum metal removal is the cell wall material which is mainly composed of chitin, chitosan, glucans and other heteropolyssacharides.

Live biomass has been found capable of adsorbing a range of toxic metals (Kapoor and Viraraghavan, 1998). Recent research has been focussed on isolating and exploiting the strains obtained from contaminated soil and other industrial effluents which help in removing significant amounts of metals (Malik, 2004; Iram et al., 2013). Live biomass of isolate −2 in this study showed a biosorptive capacity of 1.16 mg/g (50 mgL^{-1}), 1.98 mg/g (100 mgL^{-1}) and 2.45 mg/g (150 mgL^{-1}). Live biomass of *Mucor rouxii* exhibited better lead (10 mgL^{-1}) biosorptive ability (17.13 mg/g) in comparison with pre-treated biomass as well as compared to other metals after 15 h of contact time (Yan and Viraraghavan, 2000). Live biomass of *A. niger* exhibited substantially less biosorptive capacity (2.25 mg/g) in case of lead (10 mgL^{-1}) when compared with pre-treated biomass but showed highest biosorptive capacity compared to other metals after 12 h of contact time (Kapoor and Viraraghavan, 1998). The difference in bioadsorption capacity could be attributed to the change in surface area of the biomass and the quantity of biomass used and other environmental parameters. Living or active cells may be employed to achieve efficient metal removal because of proficiency in self-replenishment, continued metabolic uptake even after physical adsorption and the potential for optimization by developing resistant species and undergoing cell surface modification (Malik, 2004). Live biomass can be subjected to different pretreatment using physical or chemical methods in order to increase their metal biosorption capacity (Kapoor and Viraraghavan, 1998; Yan and Viraraghavan, 2000). The living biomass could thus be used as an effective resource for metal remediation because of its direct use or the ease at which it can be altered to serve the desired outcome.

The cell surface and spatial structure of the cell wall of the biomass mainly influence metal biosorption (Wang and Chen, 2009). The fungal cell surface is considered to be a variegation of several functional groups mainly carboxyl (-COOH), thiol (-SH), phosphate (PO_4^{3-}), amide (-NH_2-) and hydroxide (-OH-) that participate in metal binding. Infra-red analysis, EDX assay among some others could be useful in the identification of the metal binding sites (Wang and Chen, 2009; Gupta et al., 2015). In this study, SEM-EDX analysis substantiated the use of isolate-2 as a good bioadsorbent in lead removal. The surface of the biomass on analysis showed percentage of lead in comparison with control.

The FTIR spectrum of isolate-2 showed the involvement of isocyanate group, amide I and II groups, hydroxyl group, methyl and methylene groups, phosphate group, carboxyl and carbonyl groups. Lead removal using *Botrytis* sp showed the involvement of –C-H group, carboxylate ions, aromatic amino acids and N-containing bioligands (Akar et al., 2005). Lead biosorption by *Ttrichoderma viride* also indicated the involvement of isocyanate group which was attributed to the peak 2359.76 cm^{-1} (Singh et al., 2010). In this study, the shifting of peaks was observed at 2853.65 cm^{-1}, 1649.32 cm^{-1} and 1235.18 cm^{-1}. The peak stretching intensities of lead loaded biomass were comparatively different than control biomass. There was also a slight shift and variation in intensity observed in peaks at 3272.61 cm^{-1}, 1745.26 cm^{-1} and 1026 cm^{-1} which indicates that N-H stretching and C=O stretch of carboxylic group along with hydroxyl group may have played some role in adsorption in this case. Biosorption of lead using immobilized organisms have suggested the involvement of amine, hydroxyl and carboxylate groups in the adsorption process further signifying that complexation, ion exchange and electrostatic interaction may be involved in the adsorption process (Li et al., 2011). FTIR spectrum of the pretreated *Rhizopus* sp for lead removal showed the involvement of hydroxyl groups, C–O and C–O–C groups (Abdoun-Ouallouche et al., 2014). Removal of lead using *Penicillium* sp indicated the involvement imono substituted amide, disubstituted amides, unsubstituted amides, alkyl aromatic amides (Aydin Kurç et al., 2016). The shifting of the peaks and change in its vibration intensities under the metal ion influence, propose the interaction of these functional groups. The main functional groups involved in metal removal by *Drechslera hawaiiensis* were isocyanate (–N=C=O) group, carboxyl or amide groups, hydroxyl groups, phosphate groups (El-Gendy et al., 2017) . The involvement of functional groups and its intensities may vary slightly depending on the fungal species selected and the type of cell modification performed in order to enhance its removal potential.

5. Conclusion

This preliminary study showed the selection of microorganisms from certain environmental niches, help in obtaining tolerant species with high efficiency of metal removal. Strong tolerance towards lead by all four isolates could be attributed to the presence of lead in the soil from which they were screened. Some degree of tolerance was also shown towards arsenic, indicative of their ability to survive and adapt under stressed conditions. Washed live biomass showed about an average of 90% removal efficiency for 50 mgL^{-1}, 100 mgL^{-1} and 150 mgL^{-1} lead (II) solution. The adsorbent potential of isolate-2 were verified using SEM-EDX and the phenomenon may have occurred due to their interaction with the carboxylic group, hydroxyl

group, amide group along with the isocyanate group determined from the FTIR analysis. Pre-treatment of the fungal biomass by physical or chemical means can be performed to modify the functional groups and enhance the adsorption phenomena. Further studies on the kinetics of the sorption and desorption process using the immobilized biomass and reactor studies can be performed to check its ability against industrial effluents as a final means to managing the heavy metal removal using a more alternative and economical approach.

References

Abbas, S.H., Ismail, I.M., Mostafa, T.M., Sulaymon, A.H., 2014. Biosorption of heavy metals: A review. J. Comput. Sci. Tech. 3 (4), 74–102. http://dx.doi.org/10.1021/bp00033a001.

Abdoun-Ouallouche, K., Djefal-Kerrar, A., Amrani, S., Zerrouki, S., 2014. Removal of lead and mercury from aqueous solutions by pretreated Rhizopus Stolonifer biomass. Internat. Proc. Chem. Biol. Environ. Eng. 51 (26), 139–142. http://dx.doi.org/10.7763/IPCBEE.

Akar, T., Tunali, S., Kiran, I., 2005. Botrytis cinerea as a new fungal biosorbent for removal of Pb(II) from aqueous solutions. Biochem. Eng. J. 25 (3), 227–235. http://dx.doi.org/10.1016/j.bej.2005.05.006.

Ali, E.H., Hashem, M., 2007. Removal efficiency of the heavy metals Zn(II), Pb(II) and Cd(II) by Saprolegnia delica and trichoderma viride at different pH values and temperature degrees. Mycobiology 35 (3), 135–144. http://dx.doi.org/10.4489/MYCO.2007.35.3.135.

Aydin Kurç, M., Güven, K., Korcan, E., Malkoç, S., Güven, A., 2016. Lead biosorption by a moderately Halophile Penicillium sp. Isolated from Çamalti Saltern in Turkey. Anadolu Univ. J. Sci. Technol. –C –Life. Sci. Biotech. 5 (1), 13–21. http://dx.doi.org/10.18036/btdc.01000.

Aytar, P., Gedikli, S., Buruk, Y., Cabuk, A., Burnak, N., 2014. Lead and nickel biosorption with a fungal biomass isolated from metal mine drainage: Box-Behnken experimental design. Int. J. Environ. Sci. Technol. 11 (6), 1631–1640. http://dx.doi.org/10.1007/s13762-013-0354-5.

Desai, H., Patel, D., Joshi, B., 2016. Screening and characterization of heavy metal resistant bacteria for its prospects in bioremediation of contaminated soil. Int. J. Curr. Microbiol. Appl. Sci. 5 (4), 652–658.

Dhankhar, R., Hooda, A., 2011. Fungal biosorption –an alternative to meet the challenges of heavy metal pollution in aqueous solutions. Environ. Technol. 32 (5), 467–491.

Dwivedi, S., Mishra, A., Saini, D., 2012. Removal of heavy metals in liquid media through fungi isolated from waste water. IJSR 1 (3), 181–185.

El-Gendy, M., Ahmed, A., Hassanein, N.M., Ibrahim, H.A.E., El-baky, D.H.A., 2017. Heavy metals biosorption from aqueous solution by endophytic drechslera hawaiiensis of morus alba L. derived from heavy metals habitats. Mycobiology 45 (2), 73–83.

Fu, F., Wang, Q., 2011. Removal of heavy metal ions from wastewaters: A review. J. Environ. Manag. 92 (3), 407–418. http://dx.doi.org/10.1016/j.jenvman.2010.11.011.

Gadd, G.M., 1993. Interactions of fungi with toxic metals. New Phytol. 124 (47), 25–60. http://dx.doi.org/10.2307/2558069.

Gunatilake, S.K., 2015. Methods of removing heavy metals from industrial wastewater. JMEST 1 (1), 12–18.

Gupta, V.K., Nayak, A., Agarwal, S., 2015. Bioadsorbents for remediation of heavy metals: Current status and their future prospects. Environ. Eng. Res. 20 (1), 1–18. http://dx.doi.org/10.4491/eer.2014.018.

Iram, S., Zaman, A., Iqbal, Z., Shabbir, R., 2013. Heavy metal tolerance of fungus isolated from soil contaminated with sewage and industrial wastewater. Pol. J. Environ. Stud. 22 (3), 691–697.

Joo, J.H., Hussein, K.A., 2012. Heavy metal tolerance of fungi isolated from contaminated soil. KJSSF 45 (4), 565–571. http://dx.doi.org/10.7745/KJSSF.2012.45.4.565.

Joshi, P.K., Swarup, A., Maheshwari, S., Kumar, R., Singh, N., 2011. Bioremediation of heavy metals in liquid media through fungi isolated from contaminated sources. Indian J. Microbiol. 51 (4), 482–487.

Kapoor, A., Viraraghavan, T., 1995. Fungal biosorption—an alternative treatment option for heavy metal bearing wastewaters: A review. Bioresour. Technol. 53, 195–206.

Kapoor, A., Viraraghavan, T., 1998. Biosorption of heavy metals on Aspergillus niger : Effect of pretreatment. Bioresour. Technol. 63, 109–113.

Kumar, R., Singh, P., Dhir, B., Sharma, A.K., Mehta, D., 2014. Potential of some fungal and bacterial species in bioremediation of heavy metals. J. Nucl. Phys. Mater. Sci. Rad. Appl. 1 (2), 213–223. http://dx.doi.org/10.15415/jnp.2014.12018.

Kurniati, E., Arfarita, N., Imai, T., 2014. Potential use of aspergillus flavus strain {KRP1} in utilization of mercury contaminant. Proc. Environ Sci. 20, 254–260. http://dx.doi.org/10.1016/j.proenv.2014.03.032.

Li, X., Wang, Y., Li, Y., Zhou, L., Jia, X., 2011. Biosorption behaviours of biosorbents based on microorganisms immobilized by ca-alginate for removing lead (II) from aqueous solution. Biotechnol. Bioprocess. Eng. 16, 808–820.

Malik, A., 2004. Microbial bioremediation through growing cells. Environ. Int. 30, 261–278.

Mohammadian Fazli, M., Soleimani, N., Mehrasbi, M., Darabian, S., Mohammadi, J., Ramazani, A., 2015. Highly cadmium tolerant fungi: their tolerance and removal potential. J. Environ. Health Sci. Eng. 13 (07), 19. http://dx.doi.org/10.1186/s40201-015-0176-0.

Moharrer, S., Mohammadi, B., Gharamohammadi, R.A., Yargoli, M., 2012. Biological synthesis of silver nanoparticles by aspergillus flavus, isolated from soil of ahar copper mine. Indian J. Sci. Technol. 5, 2443–2444.

Pumpel, T., Paknikar, K., 2001. Bioremediation technologies for wastewaters using metabolically active microorganisms. Adv. Appl. Microbiol. 48, 134–169.

Shazia, I., Uzma, G., Talat, A., 2013. Bioremediation of heavy metals using isolates of filamentous fungus aspergillus fumigatus collected from polluted soil of Kasur, Pakistan. Internat. Res. J. Biol. Sci. 2 (12), 66–73.

Shivakumar, C., Thippeswamy, B., Krishnappa, M., 2014. Optimization of heavy metals bioaccumulation in aspergillus niger and aspergillus flavus. Internat. J. Environ. Biol. 4 (2), 188–195.

Siddiquee, S., Rovina, K., Azad, S. Al, Naher, L., Suryani, S., Chaikaew, P., 2015. Heavy metal contaminants removal from wastewater using the potential filamentous fungi biomass: A review. J. Microb. Biochem. Technol. 7, 384–393. http://dx.doi.org/10.4172/1948-5948.100024.

Singh, R., Chadetrik, R., Kumar, R., Bishnoi, K., Bhatia, D., Kumar, A., 2010. Biosorptoin optimization of lead (II), cadmium (II) and copper (II) using response surface methodology and applicability in isotherms and thermodynamic modeling. J. Hazard. Mater. 174, 623–634.

Sun, Y.M., Horng, C.Y., Chang, F.L., Cheng, L.C., Tian, W.X., 2010. Biosorption of lead, mercury, and cadmium ions by Aspergillus terreus immobilized in a natural matrix. Pol. J. Microbiol. 59 (1), 37–44.

U.S. Environment Protection Agency, 1996. Method 3050B: Acid Digestion of Sediments, Sludges and Soils, second ed.. US Gov. Print. Office, Washington, DC.

Wang, J., Chen, C., 2009. Biosorbents for heavy metals removal and their future. Biotechnol. Adv. 27 (2), 195–226. http://dx.doi.org/10.1016/j.biotechadv.2008.11.002.

Yadav, A., Bishnoi, N., Singh, R., 2012. Role of live fungi in removing toxic metals from electroplating effluent. IJSER 7 (11), 0973–4562.

Yan, G., Viraraghavan, T., 2000. Effect of pretreatment on the bioadsorption of heavy metals on mucor rouxii. Water SA 26 (1), 119–124.

Zafar, S., Aqil, F., Ahmad, I., 2007. Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bioresour. Technol. 98, 2557–2561.