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EDXRF Analysis of Some Fungal Species for the Uptake Capacity of $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb Metal Ions From Aqueous Solution

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Abstract: In this paper, Energy Dispersive X-ray Fluorescence (EDXRF) analysis of eight fungi species, namely, Aspergillus niger, Aspergillus terreus, Trichoderma longibrachiatum, Trichoderma fasciculatum, Penicillin Janthinellum, Aspergillus awamori, Phanerochaete chrysosporium, and Rhizopus arrhizus for the uptake capacity of $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb metals ions from aqueous solution have been reported. Fungal samples having superior ion removal capacity through bioaccumulation and biosorption were obtained from sites contaminated with heavy metals. The detection limit in EDXRF set up was improved considerably using selective absorbers in the path of incident photons from the X-ray tube to reduce the background in the desired energy region. It has been observed that all fungi species under present study have greater affinity for $_{82}$Pb ions as compared to $_{28}$Ni and $_{48}$Cd metal ions. The Trichoderma longibrachiatum and Trichoderma fasciculatum fungi species were identified to be more efficient for removal of heavy metal ions from waste water. The measured uptake capacity of Trichoderma longibrachiatum for $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb ions from aqueous solution is 0.52 mg/g, 0.97 mg/g, and 6.4 mg/g, respectively, and for Trichoderma fasciculatum it is 0.43 mg/g, 0.79 mg/g, and 3.5 mg/g, respectively. This indicated the potential of these identified fungi species as biosorbent for removal of high metal ions from waste water and industrial effluents.

Keywords: EDXRF, bioaccumulation, biosorption, detection limit, waste water, industrial effluents.

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1. INTRODUCTION

In largely populated cities, sources of drinking water are exposed to the industrial effluents containing toxic metal ions like $_{24}$Cr, $_{48}$Cd, and $_{82}$Pb, beyond permissible limits and pose threats to all living organisms [5]. The heavy metal ions in domestic wastes can enter from various sources like; $_{82}$Pb and $_{29}$Cu metal ions from water supply metal piping, $_{30}$Zn from galvanic corrosion, $_{48}$Cd from cosmetics, fertilizers, and batteries, and $_{28}$Ni from diesel fuels. These heavy metal ions from their respective source enter nearby water sources and pollute them. Because of the strict environmental regulations, a cost effective alternate technology for treatment of the polluted waste water is highly desirable. There are various physiochemical treatment technologies available to remove toxic heavy metals from waste water such as chemical precipitation and bioremediation, *i.e.*, the use of living or nonliving microorganisms. Bioremediation is innovative low-cost and high adsorption capacity technique having strong affinity towards metal ions in aqueous solution. Bio-removal is generally a three step process, *i.e.*, biosorption of metal ions onto the surface of microorganisms, chemical transformation, and intracellular uptake of metals ions. Microorganisms may produce organic acids which are good metal chelators and help in leaching out metal ions from surfaces. Metals may also be biosorbed or complexed by carboxyl groups found in microbial polysaccharides and other polymers [26]. It is well known that extra cellular substances produced by some microorganisms in waste water can scavenge metals following interaction of carboxyl groups on acidic polysaccharides with metal ions [16]. The use of microbial cells in biosorption is more advantageous for water treatment because they make the process faster. The ion-exchange capacity of the microbial cell wall can be greater than that of a commercial ion-exchange resin.

The research interest on the use of biomass of fungi, algae, and bacteria as an adsorbent material to remove heavy metal ions has received added impetus in recent years [7, 10, 13, 4, 14, 23, 6]. The use of immobilized microorganisms in waste water treatment is under consideration in several laboratories. The common mechanism for detoxification of metal ions by microorganisms is exclusion from the cell or cytoplasm, incorporation into granules, precipitation within the cell wall, complex with extra cellular polymers and transformation of metals through oxidation-reduction. This phenomenon is of great importance in waste water treatment plants. Wilhelmi and Duncan [4] showed that immobilized cells of Saccharomyces cerevisiae had capability to adsorb $_{24}$Cr, $_{27}$Co, $_{28}$Ni, $_{29}$Cu, $_{30}$Zn, and $_{48}$Cd through eight repeated adsorption-desorption cycles in continuous flow packed bed columns. Darnall *et al*. [7] and Ross *et al*. [10] found that the fungal and algal cells display a high affinity for heavy metals. Akhtar *et al*. [13] reported that 80% of heavy metals can be removed by fungi in liquid media. Pseudomonas aeruginosa can remove $_{82}$Pb from industrial

effluent through bisorption [14, 23, 6]. Several microorganisms isolated from activated sludge, *e.g*., Zooglea ramigera, Bacillus licheniformis, produce extra cellular polymers that are able to make complexes and subsequently accumulate $_{26}$Fe, $_{28}$Ni, $_{29}$Cu, $_{48}$Cd, and $_{92}$U metals ion. Zooglea ramigera can accumulate 0.1 g of $_{29}$Cu per gram of biomass [2]. This bacterium when immobilized in alginate beads is able to accumulate $_{48}$Cd concentrations as high as 250 mg/L from solutions [22]. Fungal mycelia (e.g. Aspergillus and Penicillium) have also been considered suitable for metal removal from waste water [12]. Spirulina is another promising microorganism for removal of heavy metals from industrial effluents [20]. Ahuja *et al*. [27] have reported that under laboratory conditions Acinetobacter anitratus can remove $_{82}$Pb and $_{48}$Cd upto 97% and 85%, respectively. The ability to remove heavy metals varies greatly among microbes. The choice of organism for heavy metal removal is important due to difference in their capacity for sorption of different metals. Therefore, it is required to further identify more microbes with good efficiency for removal of heavy metals from waste water for its safe use in agriculture. The controlled and heavy metal treated fungi samples have been characterized here to identify the efficient strains of various fungi species.

Various analytical techniques for quantitative elemental analysis of different kinds of samples have been developed over the years. Some of these techniques, namely, atomic absorption spectroscopy (AAS), inductively coupled plasma-mass spectrometry (ICP-MS), Auger electron spectrometry (AES) and chemical analysis are destructive. Other analytical methods, including particle induced X-ray emission (PIXE) and energy dispersive X-ray fluorescence (EDXRF) work on the same principle of creation of inner-shell vacancies in the target element and measurements of the fluorescent X rays emitted from the sample [1, 24, 9, 17]. In PIXE technique, beam of charged ions is used to eject inner-shell electrons from atoms in a specimen target, whereas, in EDXRF X-ray photon from radioisotope sources or X-ray tube is used as incident particle [15, 18, 8, 19, 11, 25]. Due to their ability of rapid, simultaneous, and nondestructive multi-element analysis, these techniques have been well established to determine the elemental concentrations of various types of samples. These techniques cannot be used for low-Z elements (Z<12) whose low-energy characteristic X rays are not detectable. In case of biomedical samples, PIXE cannot be used because the volatile material, like $_{16}$S, $_{34}$Se, and $_{80}$Hg, may be lost, due to local high temperature. PIXE probes the surface and near-surface region of materials, whereas, EDXRF is useful to determine the bulk elements also. In the present work, EDXRF technique has been used to study the uptake capacity of various fungi species for removal of $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb metals ions from aqueous solution.

2. PREPARATIONS OF THE FUNGUS SAMPLES

The samples of efficient microbes for removal of heavy metals were prepared by Bio-technological department of Guru Jambheshwar University of Science and Technology, Haryana. The fungal species were isolated from waste water, sludge, and industrial effluents from Karnal, Panipat and Sonipat districts of Haryana state by serial dilution method using Rose Bengal agar medium. Efficient fungal isolates were grown on Potato Dextrose Agar (PDA). The fungal spores were examined using standard keys and were stained using lactophenol cotton blue stain and observed under microscope. Fungal isolates were identified at Department of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi, as Aspergillus niger, Aspergillus terreus, Trichoderma longibrachiatum, Trichoderma fasciculatum, Penicillin Janthinellum, Aspergillus awamori, Phanerochaete chrysosporium, Rhizopus arrhizus. These fungi were grown in normal Potato dextrose broth without metal, which is used as control.

***Preparation of the stoch solution*:** Potato dextrose broth (Hi-MEDIA, India) containing 15 mg/L and 25 mg/L of $_{28}Ni$, $_{48}Cd$, and $_{82}Pb$, were prepared from stock solution by dilution. The stock solution (1000 mg/L) of $_{28}Ni$, $_{48}Cd$, and $_{82}Pb$ was prepared by dissolving 4.05 g, 1.63 g, and 1.60 g of $NiCl_2.6H_2O$, $CdCl_2$, and $Pb(NO_3)_2$, respectively, in double distilled water in separate vessels. The mixed solution of $_{82}Pb$, $_{48}Cd$, and $_{28}Ni$ metal ions was prepared by mixing all the solutions in equal proportions. Prior to experiment all the glassware were treated with 0.1 M HCl before and after the experiments to avoid binding of metals to it. The spore suspension of 1 ml of efficient fungi was inoculated into 100 ml of potato broth containing 15 mg/L of $_{28}Ni$, $_{48}Cd$, and $_{82}Pb$ and 25 mg/L of $_{28}Ni$, $_{48}Cd$, and $_{82}Pb$. After 5 days, fungal growth was harvested with the help of filter paper and harvested fungal biomass was dried in oven at 80°C. The dried fungal biomass was powdered to fine particles of uniform size. To eliminate or reduce density and composition variations, the powdered samples were pressed in a die or plastically deformable caps to form pellet using a pure steel die and pressure of 20 kN/cm^2 from hydraulic press (Paul Auto Weber, Germany). The details about different kinds of fungus species along with the treatment specifications are given in Table 1.

3. EXPERIMENTAL DETAILS

The concentration of $_{28}Ni$, $_{48}Cd$, and $_{82}Pb$ metal ions in the fungi samples were measured using EDXRF spectrometer available at Department of Physics, Panjab University, Chandigarh. The essential components of an EDXRF spectrometer are X-ray source, specimen chamber, and detection system with

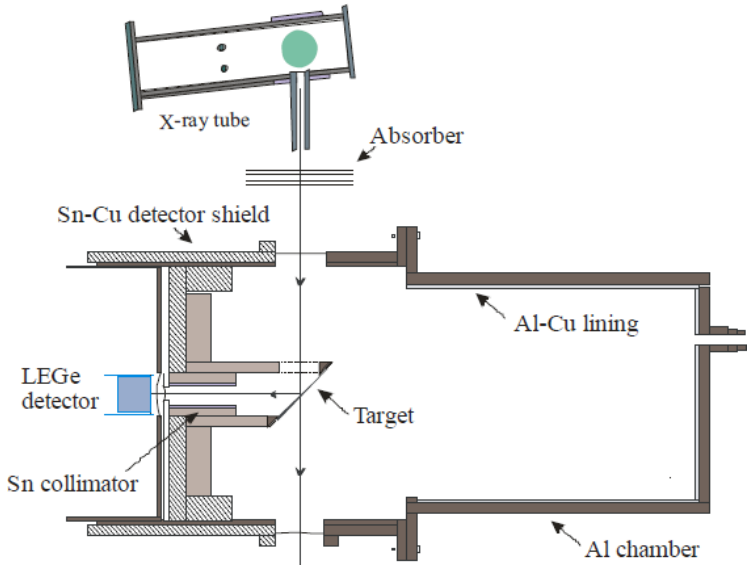

Figure 1: Reflection-mode X-ray tube based EDXRF set up used in the present measurements.

necessary electronics to process signals. A computer based electronic system with appropriate data reduction software was used for accumulation and analysis of spectra. Reflection-mode X-ray tube based EDXRF set up used in the present measurements is shown in Figure 1

EDXRF set up used in the present measurement consist of water cooled 2.4 kW $_{42}$Mo anode X-ray tube (60 kV, PW 2274/22, Pananalytic, Netherlands) as a source of excitation. The take off angle of the tube window with respect to horizontal direction is 6°. The tube emits $_{42}$Mo *K* X rays along with the bremsstrahlung radiations ranging up to the maximum applied tube voltage. The tube was equipped with $_{50}$Sn collimator of diameter 3 mm to reduce the incident flux. The background due to scattered photons was minimized by placing the X-ray tube, target sample and detector at 90° reflection mode geometrical arrangement. A low-energy germanium (LEGe) detector (size = 100 mm^2 × 10 mm, Be window = 8 μm, FWHM = 150 eV at 5.89 keV, Canberra, US) in horizontal configuration coupled with PC-based multichannel analyzer was used to collect the fluorescent X-ray spectra from the samples. The targets were mounted at 45° with the detector and X-ray tube axis. The X-ray tube and detector were kept outside the chamber. The alignment of X-ray tube collimator and chamber collimator was done using laser beam. The X-ray tube was operated at 29 kV to avoid excitation of the $_{50}$Sn-collimator (B_K = 29.20

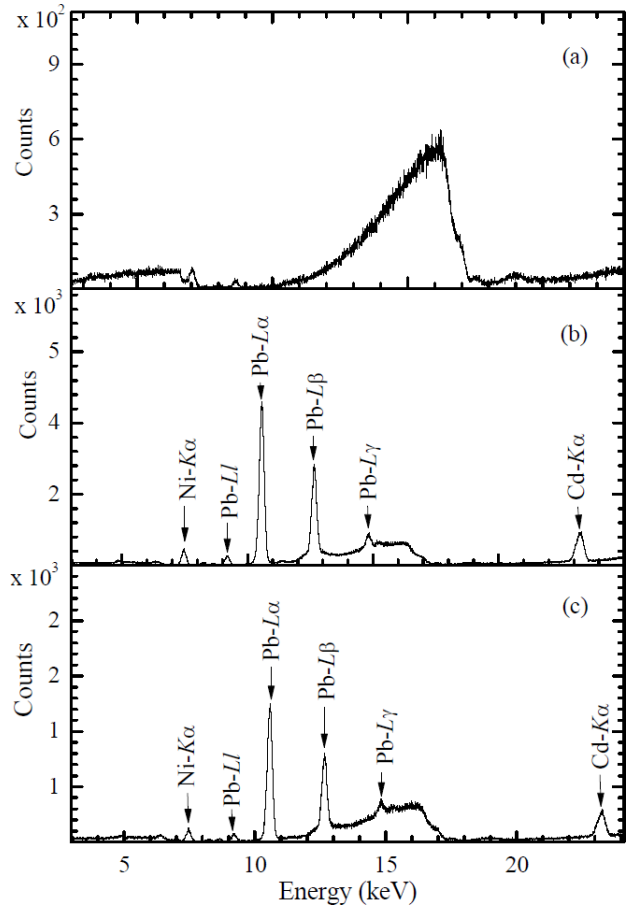

Figure 2: Typical EDXRF spectra for fungi samples, (a) controlled, (b) treated with 15 mg/L of $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb solution, and (c) treated with 25 mg/L of $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb solution.

keV). The spectrum of each sample was taken for ~ 600 seconds. The emitted photon spectrum consists of the $_{42}$Mo (19.65 keV) X rays and bremsstrahlung from the anode of X-ray tube was modified using $_{39}$Y (53 mg/cm^2, B_K = 17.038 keV, Jump ratio = 6.3) and $_{30}$Zn (50 mg/cm^2, B_K = 17.998 keV, Jump ratio = 7.4) absorbers to improve the peak-to-background ratio. The detector was also shielded using $_{50}$Sn-$_{29}$Cu-$_{13}$Al cylinder. Typical EDXRF spectra for *Trichoderma longibrachiatum* fungi samples, (a) controlled, (b) treated with 15 mg/L of $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb solution, and (c) treated with 25 mg/L of $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb solution are shown in the Figure 2.

4. EVALUATION PROCEDURE

The measured X-ray counting rate for the given element is related to its concentration (m_s) using the relation,

$$m_s = \frac{N_s}{\beta_s \sigma_s (I_o G)_s \varepsilon_s}, \tag{1}$$

where N_s is the photopeak area per unit time of the element of interest in the sample, $(I_o G)_s$ is intensity of the exciting radiation falling on area of the target visible to the detector, ε_s is the detector efficiency at the X-ray energy region of interest, m_s is the mass-thickness of the element in g/cm^2, and β_s is the self-absorption correction factor that accounts for attenuation of the incident and the emitted X rays in the target material. The values of β_s can be calculated using the expression,

$$\beta_s = \frac{1 - \exp\left[-\left(\mu_1^s / \cos\theta_{in} + \mu_2^s / \cos\theta_e\right) m_s\right]}{\left(\mu_1^s / \cos\theta_{in} + \mu_2^s / \cos\theta_e\right) m_s} \tag{2}$$

where 's' stands for different elements present in the target, μ_1^s and μ_2^s are the mass-attenuation coefficients for the incident and emitted X rays.

This method of β_s correction was possible for known matrix targets or where standard single element foils were used [21]. In case of biological samples the matrix is not known and therefore such correction cannot be estimated theoretically using available attenuation coefficients. In case of thick biological samples having unknown matrix, an experimental method for the determination of β_s value is given here. For the experimental evaluation of β_s using Equation (2) the values of μ_1^s , μ_2^s , θ_{in}, θ_e, and m_s need to be known separately. Here the quantity $\exp\left[-\left(\mu_1^s / \cos\theta_{in} + \mu_2^s / \cos\theta_e\right) m\right]$ occurring in Equation (2) can be measured experimentally. It represents the combined attenuations of the incident and fluorescent X rays in the total specimen thickness. This has been done by measuring the relative X-ray intensities with and without specimen by placing a thin standard foil at the back of specimen. It gives the value of exponential term occurring in the expression for β_s , *i.e.*,

$$\frac{I_T' - I_s}{I_T} = \exp\left\{-\left(\frac{\mu_1^s}{\cos\theta_{in}} + \frac{\mu_2^s}{\cos\theta_e}\right) m\right\} \tag{3}$$

where I_s, I_T, and I'_T are the intensities of the X rays emitted from the specimen alone, the target of standard foil alone and the specimen with target respectively. Two standard foils of $_{28}$Ni (259 μg/cm^2) and CdSe (393 μg/cm^2) were used after the samples to find the transmission of incident X rays through samples and also to find the self -absorption coefficient. All the samples were irradiated for three different sets, *i.e.*, irradiating specimen alone, standard targets alone, and specimen with standard targets to find the relative intensity. The good statics spectra were taken for all three sets of samples. The peak position was also monitored to check for any gain shift during the experiment. The background spectra has been taken separately and subtracted from the spectrum of each sample after normalization. The value of β_s is then evaluated by substituting this experimentally determined exponential factor in Equation (2) and other terms by taking negative of logarithm of this term. It has been confirmed that the method of experimental evaluation of absorption correction factor (matrix effects) and the fundamental parameter approach for determination of the trace element concentrations in thick samples of biological material can be carried out for unknown samples, with confidence. The concentration of the elements evaluated in g/cm^2 is converted into μg/g using the relation;

$$\text{Concentration } \left(\mu g/g\right) = \frac{m_j\left(g/cm^2\right)}{m_s} \times 10^6 \qquad (4)$$

Where m_s is the weight of the sample in g/cm^2.

5. RESULTS AND DISCUSSION

It has been observed from the X-ray spectra that no heavy metal was present in the controlled samples. The concentration of $_{28}$Ni and $_{48}$Cd metal ions has been evaluated using *K*a X-ray peak, whereas, in case of the $_{82}$Pb metal ions, the *L*a X-ray has been used. Peaks of $_{28}$Ni-*K*a, $_{48}$Cd-*K*a, and $_{82}$Pb-*L*a X rays are well separated in the spectrum of the treated fungi samples. The peak areas were evaluated from total counts in the peak region by subtracting the background counts interpolated from the smooth regions above and below the peak. The X-ray spectra were checked for the contributions of the escape peak while evaluating the peak areas. The concentration of metal ion uptake by different fungi species (in μg/g) computed using the weighted average of different samples is given in Table1. The error in presently measured

elemental concentrations was estimated to be between 10-15% arising from the photo electric cross-section (~ 2%), fluorescence yields (~ 3%), X-ray absorption correction term (~ 3%), detector efficiency (~ 5%) plus statistical and fitting errors of the individual X-ray lines. The EDXRF analysis of fungi species show different intake capacity for the various metal ions. It is clear from the table that Trichoderma longibrachiatum and Trichoderma fasciculatum fungus has greater affinity for the $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb ions as compared to other species in study when exposed to 15 mg/L solution and 25 mg/L of solution. Trichoderma longibrachiatum fungus has the maximum $_{82}$Pb biosorption capacity, *i.e.*,6.4 mg/g followed by Trichoderma fasciculatum, *i.e.,* 3.5 mg/g. The maximum $_{48}$Cd biosorption capacity was 0.97 mg/g for Trichoderma longibrachiatum and 0.79 mg/g for *Trichoderma*

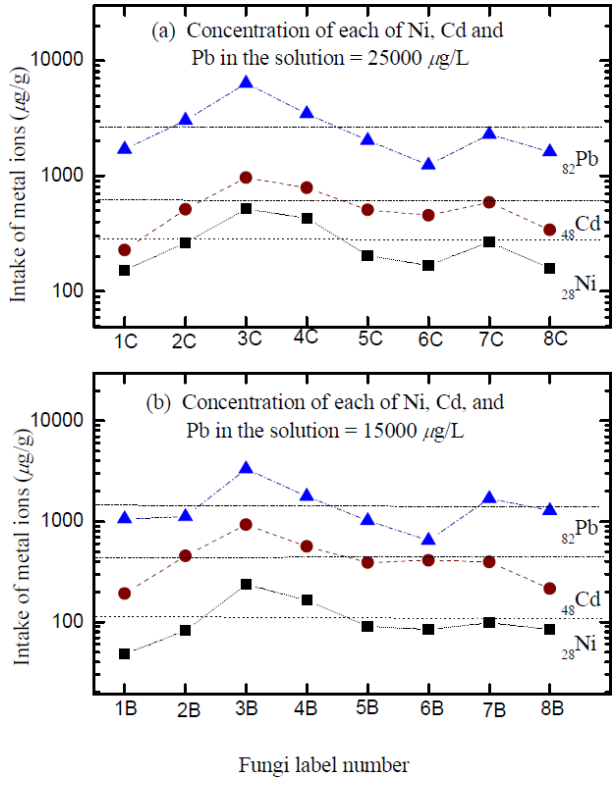

Figure 3: Plots showing the intake of the metals in (μg/g) by different fungi species, (a) intake of $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb from 25 mg/L solution, and (b) intake of $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb from 15 mg/L solution. Horizontal lines indicate the average value of the intake of metal for each element.

Table 1: Different kinds of fungus along with the dose given, mass thickness, and uptake per unit weight of fungus obtained using EDXRF technique.

Name of fungi sample	Fungi label	Concentration of each of $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb(mg/L) ions	Mass thickness (mg/cm^2) $_{8}$Ni	Uptake per unit weight (µg/g) $_{48}$Cd	$_{82}$Pb	
Aspergillus niger	1A	-	133	-	-	-
Aspergillus terreus	2A	-	85	-	-	-
Trichoderma longibrachiatum	3A	-	104	-	-	-
Trichoderma fasciculatum	4A	-	115	-	-	-
Penicillin Janthinellum	5A	-	105	-	-	-
Aspergillus awamori	6A	-	106	-	-	-
Phanerochaete chrysosporium	7A	-	100	-	-	-
Rhizopus arrhizus	8A	-	56	-	-	-
Aspergillus niger	1B	15	104	48	192	1058
Aspergillus terreus	2B	15	110	82	454	1118
Trichoderma longibrachiatum	3B	15	55	236	927	3327
Trichoderma fasciculatum	4B	15	85	165	565	1776
Penicillin Janthinellum	5B	15	110	91	391	1018
Aspergillus awamori	6B	15	131	84	412	649
Phanerochaete chrysosporium	7B	15	81	99	395	1691
Rhizopus arrhizus	8B	15	131	84	214	1282
Aspergillus niger	1C	25	131	153	229	1702
Aspergillus terreus	2C	25	95	263	516	3042
Trichoderma longibrachiatum	3C	25	58	517	966	6345
Trichoderma fasciculatum	4C	25	81	432	790	3457
Penicillin Janthinellum	5C	25	122	205	508	2033
Aspergillus awamori	6C	25	131	168	458	1244
Phanerochaete chrysosporium	7C	25	78	269	590	2295
Rhizopus arrhizus	8C	25	132	159	341	1621

fasciculatum. The maximum $_{28}$Ni biosorption capacity for Trichoderma longibrachiatum and Trichoderma fasciculatum was 0.52 mg/g and 0.43 mg/g, respectively. Aspergillus awamori fungus species has least affinity for the uptake of $_{82}$Pb ions and Aspergillus niger fungus species has least affinity for the uptake of $_{28}$Ni and $_{48}$Cd ions as compared to other species in study when exposed to 15 mg/L solution and 25 mg/L of solution. All other species in present study has moderate uptake capacity for$_{28}$Ni, $_{48}$Cd, and $_{82}$Pb metal ions from the aqueous solution. The intake of $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb metal ions (in μg/g) by different fungi species from (a) 15 mg/L solution and (b) 25 mg/L solution are also plotted in Figure 3. From plot it is clear that all fungi species in present study has strong affinity for the absorption of $_{82}$Pb ions from Potato Dextrose medium as compared to absorption of $_{28}$Ni and $_{48}$Cd ions.

The uptake and percentage removal of heavy metals from liquid medium are expected to be influenced by a number of environmental factors, *e.g., pH*, incubation time, inoculum size biomass concentration, presence of other heavy metals and temperature. With increase in *pH* values, more and more ligands having negative charge would be exposed and are expected to result in increase in attraction of positively charged metal ions. The uptake also depends upon the incubation time, *i.e.*, uptake by a typical fungi can first increase followed by decrease. Above the optimum temperatures, microbial enzyme activity in general decreases with temperature because of enzyme denaturation or the damage of active binding sites in the biomass or even microbial death [3]. The uptake of $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb metal ions from Potato Dextrose medium by Trichoderma fasciculatum and Trichoderma longibrachiatum in different environmental factors need to be investigated so as to optimize the best conditions for the maximum uptake of these metal ions.

CONCLUSION

In the present work, eight fungi species, namely, Aspergillus niger, Aspergillus terreus, Trichoderma longibrachiatum, Trichoderma fasciculatum, Penicillin Janthinellum, Aspergillus awamori, Phanerochaete chrysosporium, and Rhizopus arrhizus have been analyzed for the uptake capacity of the $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb metal ions from aqueous solution. The EDXRF technique has been used to determine the metal ion concentration in these fungi species. The detection limit in X-ray tube set up has been improved considerably using selective absorbers in the path of incident photons from the X-ray tube to reduce the background in the desired energy region. It has been observed that all fungi species under study have greater affinity for $_{82}$Pb as compared to $_{28}$Ni and $_{48}$Cd

metal ions. Trichoderma longibrachiatum and Trichoderma fasciculatum fungi species are identified to be efficient than other species. The uptake capacity of Trichoderma longibrachiatum for the $_{28}$Ni, $_{48}$Cd, and $_{82}$Pb ions is measured to be 0.52 mg/g, 0.97 mg/g, and 6.4 mg/g, respectively, and that for the Trichoderma fasciculatum it *i*s 0.43 mg/g, 0.79 mg/g, and 3.5 mg/g, respectively. Further studies of these fungi species in different environmental parameters are required for their utilization in the waste water treatments.

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