MATERIALS AND METHODS
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In the present study concentration of lead was studied from some industries of Raipur city located in industrial area. Different fungi were then isolated from these industrial waste water and their lead tolerance and biosorption capacity was studied. In this chapter methodology of waste water sampling, isolation of fungi, lead tolerance and biosorption has been described.

Study Site

The concentration of lead in industrial effluent was studied from coal based iron industries, iron casting industry and a petrochemical industry of Raipur (21.15°N 81.41°E) Chhattisgarh. The industries are located in Urla and Birgaon industrial area of Raipur (Table 1 – Page 57, Plate I, II and III – Page 96, 97 and 98). The industrial sites are near about 06 km from laboratory. Urla and Birgaon are among the most polluted sites of Raipur. These industrial sites includes iron based manufacturing industries, Rice mills, Paper mills, Plastic mills, Mineral casting industries, Power industries, Chemical industries, Petrochemical industries, Dye industries, Oil industries, Charcoal industries etc. Most of the industries have appropriate drainage system for proper disposal of effluents but few of them releases waste water to the ground surface.

Sample collection

Samples were collected in sterile air tight plastic bottles. Samples (effluent) were collected from drainage area of industries generally in the second week of month, in the morning between 06 to 08 am for the period of one year (May 2010 to April 2011). Sample bottles were stored at 4°C in refrigerator for further study.

Study of Lead concentration from different samples of Industrial effluent

After the collection of sample, lead concentration was determined by Spectroquant Nova60 within 06 hr from sample collection.
Isolation of fungi from industrial effluent

- After determination of lead concentration, fungi were isolated from industrial samples having more than 01 mg/l of lead concentration.
- For isolation of fungi protocol described by Ezzuri et al. (2009) was used with some modifications.
- Five Petri dishes were used to isolate fungi for different dilution of waste water sample of $10^{-0}$ (original sample) to $10^{-4}$ (diluted sample of $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$).
- After pouring Petri dishes were incubated at 26±1°C for 5 to 7 days. After incubation period all fungal types were pure culture in PDA medium for further study.

Identification of isolated fungi

Preliminary, identification of fungi was done at research centre (School of Studies in Biotechnology, Pt. Ravishankar Shukla University Raipur) with the help of available literature. The common fungi were identified by macroscopic examination based on cultural characters observed on PDA medium and microscopic examination by lactophenol cotton blue slide. Further identification of fungi up to species level was done from National Centre of Fungal Taxonomy, New Delhi. One fungus was identified at molecular level by sequencing of D1 and D2 region of 28S rRNA. The sequenced nucleotides were BLAST to find organism. This identification was done from Xcelris laboratories Pvt. Ltd. Ahmadabad.

Study of Tolerance of Lead by some Fungal Species

For lead tolerance five flasks were prepared with one control (without lead nitrate) and four test flasks with different concentration of lead nitrate in 50 ml PDB medium. All concentrations were taken in triplicate with lead concentration of 50, 100, 500 and 1,000 mg/l (Modified from Atuanya and Oseghu, 2006).
• After preparation of flasks, dominant fungi were inoculated in same amount (1 ml inoculums cultured in PDB medium). Flasks were then incubated to 26±1°C for 7 days.

• After incubation period, mycelium were filtered by Whatman filter Paper No. 1 and dried in oven at 80°C for 10 hours.

• Tolerance of lead by fungi was calculated in Tolerance index (Ti) by measuring the dry weight (Dw) of fungi (Fomina et al. 2005)

\[
Ti \text{ of Fungi} = \frac{Dw \text{ of Test mycelium}}{Dw \text{ of control mycelium}} \times 100
\]

**Screening of fungi for Biosorption study**

Among some dominant fungi *Aspergillus flavus* var. *Scherotorium*, *A. fumigatus*, *a. niger*, *A. niger* var. *scherotorium*, *A. tamari*, *Betrniella* sp., *Chetomium globosum*, *Cladosporium oxysporium*, *C. spherospermum*, *Cunninghamella elegans* TUFC 20022, *Fusarium clamydosporium*, *Penicillium chrysogenum*, *P. digitatum* and *P. oxalicum* were selected for biosorption screening study. These fungi were grown in PDB medium containing known amount of lead. After 7 days of incubation at 26±1°C mycelium were filtered and broth were analyzed for lead concentration. Spectroquant Nova60 was used to analyze lead in filtered broth. Fungi absorbing more than 60% of lead were selected for biosorption study.

**Biosorption of lead by living biomass of some fungal species**

After screening, some fungal species were selected for biosorption study. The methodology of biosorption of lead by living biomass of fungi has been adopted from Patil *et al.* (2007) with some modifications. Testing with living biomass, fungi were grown in PDB medium containing known concentration of
lead. Test flasks were prepared by taking 50 ml of PDB in 150 ml conical flasks. Known concentration of lead was added to the medium and autoclaved at 15 lbs for 15 minute. After cooling, 1 ml of fungal inoculums was added to medium and incubated according to parameter taken. After the incubation period fungi were filtered through Whatman filter paper no. 1 and the filtered broth were analysed for lead concentration using Spectroquant Nova60. The percent of biosorption of lead by living biomass of fungi was calculated with formula –

\[ x = \frac{C - T}{C} \times 100 \]

Where: \( x \) = Biosorption percent
\( C \) = Concentration of lead in control
\( T \) = Concentration of lead in test

**Study of different parameters for biosorption by living fungal biomass**

Parameters including incubation period, temperature and pH were analyzed for biosorption of lead by living biomass of fungi. Study with incubation period, biosorption of lead by fungi was analyzed every day after inoculation up to 9th day. pH was adjusted from 3.0 to 8.0, similarly temperature was adjusted from 20 to 44°C. During the study of pH and temperature, incubation period was taken 5 or 7 days according to their performance during test of incubation period.

**Biosorption of lead by Physically and Chemically Pretreated dead biomass of Fungi**

The fungi used for study of biosorption of lead by living biomass were also studied for their ability of biosorption with physical and chemical modification. For pretreatment study, fungi were grown in Peptone dextrose liquid medium containing Dextrose 20 g, Peptone 10 g, NaCl 0.2 g, CaCl\(_2\).2H\(_2\)O 0.1 g, KCl 0.1 g, KH\(_2\)PO\(_4\) 0.5 g, NaHCO\(_3\) 0.05 g, MgSO\(_4\) 0.25 g, FeSO\(_4\). 7H\(_2\)O 0.005 g in 1,000 ml distilled water (Kapoor and Viraraghavan 1998). pH of the medium for particular
fungi was adjusted according to their performance in biosorption with living biomass. 72 hrs old fungi grown in same medium was taken as inoculums. 1,000 ml flasks with 300 ml medium were used to grow the fungi. The medium was sterilized with autoclaving and after cooling 2 ml of fungal inoculums were transferred to medium aseptically. Flasks were kept in shaker incubator with 125 rpm speed. Temperature was maintained according to the performance of fungi during biosorption with living biomass. Fungal culture obtained spherical ball shaped due to fragmentation of mycelium.

After 7 days of incubation, fungi were filtered and washed twice with distilled water. The harvested biomass was referred as Control biomass. 30 g wet control biomass was then treated with physical and chemical methods.

Physical pretreatment was done by autoclaving of Control biomass for 30 minutes at 15 lbs. Chemical modification of biomass was done as follows –

1. Treatment with sodium hydroxide – Control biomass was boiled for 15 minutes in 500 ml of 0.5 N sodium hydroxide solutions.
2. Treatment with formaldehyde – Control biomass was boiled for 15 minutes in 500 ml of 15 percent (vol/vol) formaldehyde solution.
3. Treatment with detergent – Control biomass was boiled for 15 minutes in 500 ml of water in which 2.5g of commercial laundry detergent was dissolved.

After chemical pretreatment, biomass was washed twice with distilled water. Pretreated biomass with sodium hydroxide was washed with distilled water until the pH of washed water was found 7.0 ± 0.2. Then all the biomass including control, autoclaved and chemically modified biomass were dried in oven at 60 °C for 24 hr. Dried biomass were then grinded with mortar and pestle to get powder of fine particles. 0.1g of this powder was dissolved with known amount of lead solution in 100 ml distilled water. After 15 hr of contact time lead solution was
filtered and analyzed with Spectroquant Nova60. Biosorption capacity i.e. amount of lead (mg) absorbed by per gram of biomass was calculated with formula -

\[ Q = \frac{C_i - C_f}{M} \times V \]

Where -
- \( Q \) = mg of lead ion absorbed by per g of biomass
- \( C_i \) = initial metal ion concentration (mg/l)
- \( C_f \) = final metal ion concentration (mg/l)
- \( M \) = Weight of biomass used in reaction mixture
- \( V \) = Volume of the reaction mixture in liter