MATERIALS AND METHODS

3.1 COLLECTION OF SOIL SAMPLES

The objectives of the present study dictated the use of preferably pollutant-resistant strains of fungi in the experiments. Since polluted habitats are believed to be excellent sources of such strains (Babich and Stotzky, 1982), it was decided to screen soil samples from the sites polluted with heavy metals/dyes. Three samples of soils polluted with heavy metals were collected from the fields situated in Partapur industrial area (Meerut) while two samples were collected from the fields located at Shobhapur (Delhi-Roorkee bypass), Meerut. Effluents from electroplating, leather, paints and batteries manufacturing units are discharged in these fields. Three samples of dye-polluted soils were collected from Partapur industrial area (Meerut) and two from Pilakhuwa (Hapur) from the fields where the dye-containing effluents from the textile industries are discharged. Before taking soil sample from a given site, the upper layer of the soil was removed with the help of a sterilized trowel to remove extraneous litter/organic matter. Soil samples were then taken out with the help of a sterilized trowel and were collected in fresh sterile polythene bags aseptically. These samples were brought to the laboratory where all the five soil samples of metal-polluted sites were mixed thoroughly to obtain one composite sample. Similarly, samples collected from dye-polluted soils were combined to obtain another composite sample.

3.2 ISOLATION OF FUNGI

20 g of soil from a given composite sample were transferred into 200 ml of sterilized distilled water dispensed in a 500 ml Erlenmeyer (conical) flask. The contents were stirred for 30 minutes to wash fungal propagules from the soil. 10 ml of this suspension (of 1:10 dilution) were immediately transferred to a 250 ml conical flask containing 90 ml of sterilized distilled water to yield soil suspension of
1:100 dilution. The latter was used for preparing further serial dilutions (1:1000 and 1:10000) aseptically. From the suspensions of 1:100 dilution, one ml aliquot was transferred to each of a set of three Petri dishes followed by the addition of 20 ml of cooled and sterilized PDA medium (Riker and Riker, 1936) with 30 ppm of streptomycin. Similar treatment was given to the suspensions of 1:1000 and 1:10000 dilutions. The Petri dishes containing medium and the inocula were rotated (so as to facilitate thorough mixing of broth with inoculum). These Petri plates were then incubated at 25±1°C for 6 to 8 days in a B.O.D. incubator.

The composition of the medium (i.e. PDA) is as under:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar-Agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Potato (Peeled)</td>
<td>200.0 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.0 ml</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0.03 g</td>
</tr>
<tr>
<td>Rose Bengal</td>
<td>0.03 g</td>
</tr>
<tr>
<td>pH</td>
<td>6.2</td>
</tr>
</tbody>
</table>

200 g of peeled and sliced fresh potatoes were boiled in 500 ml of distilled water for 30 minutes. The contents were filtered through a piece of muslin cloth. The filtrate (potato broth) was made upto 500 ml with distilled water. Simultaneously, 15–20 g of agar-agar were melted in 500 ml of distilled water by heating. The 500 ml of potato broth were poured in the agar solution. 20 g of dextrose were also added to and were dissolved in the mixture. The pH of the mixture was adjusted to 6.7. To this suspension 0.03 g of Rose Bengal were also added. The total volume of the medium was made upto 1000 ml (one litre) using distilled water.

The medium was then sterilized in an autoclave at 15 lb. pressure (121°C) for 15 minutes. The Petri dishes and other glasswares used in the experiment were sterilized in hot-air oven at 160°C for 3h before use. Streptomycin was added to the medium aseptically just before pouring the medium in the plates.

### 3.3 RECORDS OF THE FUNGI ISOLATED

The inoculated Petri dishes under incubation were observed for the fungal growth from the third day itself when the fast-growing fungi started appearing in the Petri dishes. The slow-growing fungi were transferred onto other Petri plates just after their appearance so as to prevent them from being overrun by the fast-
growing fungi. A complete record of the fungal species and their numbers (CFUs: Colony Forming Units) in the Petri dishes was maintained. The identification of the fungal species was done on the basis of their morphology and cultural characteristics with the help of keys and manuals published by Gilman (1957), Ellis (1971, 1976), Subramanian (1971), Barnett and Hunter (1972), Domsch and Gams (1972), Domsch et al. (1980) and Nagamani et al. (2006).

The total number of colonies and the number of colonies of each fungal species growing in every Petri dish were recorded to determine their frequencies. The frequency classes were expressed as mentioned by Saksena (1955).

The axenic cultures of the fungal species, obtained by routine hyphal cut method, were maintained on the slants of PDA medium and were then stored in a refrigerator.

3.4 PREPARATION OF FUNGAL BIOMASS

3.4.1 Selection of fungal species for biomass preparation

Nineteen species of fungi were isolated from different metal-polluted soil samples while twenty four species of fungi were isolated from dye-polluted soil samples. Out of these, four fungal species i.e. (i) Aspergillus flavus Link, (ii) Mucor rouxii Calmette, (iii) Penicillium spinulosum Thom and (iv) Rhizopus oryzae Went and Gerlings, were selected for further studies. The biomass of these four selected species were prepared in vitro following the procedure explained in the section 3.5.2.

The spent biomass of yeast (Saccharomyces cerevisiae Meyen ex-Hansen) was obtained from Messrs Sab Miller (a brewery industry, located at Meerut Cantt.), Meerut (U.P.).

The selection of the species was done on the basis of their dominance as also on the basis of divergence in the cell wall composition (Bartnicki-Garcia, 1968).

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Cell Wall Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A. flavus, P. spinulosum</td>
<td>Chitin + Glucan</td>
</tr>
<tr>
<td>2</td>
<td>R. oryzae, M. rouxii</td>
<td>Chitosan + Chitin</td>
</tr>
<tr>
<td>3</td>
<td>S. cerevisiae</td>
<td>Mannan + Glucan</td>
</tr>
</tbody>
</table>
3.4.2 Preparation of biomass of molds

Axenic cultures of the four experimental molds were prepared on potato dextrose agar plates. 100 ml MGYP broth (Atlas, 1946) were taken in each of a set of 20 flasks of 250 ml capacity. The composition of the broth is as under:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malt extract</td>
<td>3.0 gms</td>
</tr>
<tr>
<td>Glucose</td>
<td>10 gms</td>
</tr>
<tr>
<td>Peptone</td>
<td>3.0 gms</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.0 gms</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
<tr>
<td>pH</td>
<td>6.8</td>
</tr>
</tbody>
</table>

After sterilization, these flasks (containing MGYP media) were inoculated with the given fungal strain from the prepared plates. The flasks were incubated at 27±1°C in a BOD-incubator shaker for one week. After one week of incubation, all flasks were autoclaved. The fungal biomass of each flask was harvested by filtration, washed with generous amounts of distilled water; and resuspended in water followed by filtration to obtain wet mycelial biomass. The biomass so obtained was dried in hot air oven at 60±2°C for 24 hrs. This dried biomass was crushed with the help of mortar and pestle to obtain it in powder form. The dried powder contained particles of different size ranges i.e. (i) X (0.5 mm to 0.25 mm), (ii) Y (0.25 mm to 0.125 mm) and (iii) Z (0.125 mm or lesser) the proportion of X: Y: Z being approximately 2: 1: 1.

3.5 PREPARATION OF SOLUTIONS OF METALS AND DYSES

Two different metals i.e. lead (as lead sulphate) and cadmium (as cadmium sulphate) as well as two different dyes i.e. malachite green (MG) and basic fuchsin (BF) were used to assess the ability of the fungal biomass to adsorb metals and dyes. Stock solutions of metals were prepared in a manner so as to obtain different concentrations (250 ppm, 500 ppm and 750 ppm) of metals. The 500 ppm stock solution of each dye was prepared. The aforementioned concentrations of metals and dyes solutions were tried because the concentrations of metals and dyes in the effluents quite often reached these levels (Tiwari, 2010).
3.6 BIOSORPTION BY MYCOMASS

3.6.1 BIOSORPTION OF LEAD AND CADMIUM BY THE BIOMASS OF YEAST AND SELECTED MOLDS

In each of a set of 18 flasks of 250 ml capacity (set IA), 100 ml of 250 aqueous solution of lead sulphate were taken. Similarly, two sets of 18 flasks of 250 ml capacity were used — one set (set IB) for 500 ppm solution of lead sulphate and another set (set IC) for 750 ppm solution of lead sulphate. Thus, 54 flasks were used for lead sulphate solution. To 45 of these flasks, 10 mg dry powdered biomass of yeast (*Saccharomyces cerevisiae*) or the given fungal (*Aspergillus flavus* / *Mucor rouxii* / *Penicillium spinulosum* / *Rhizopus oryzae*) were added as under:

(IA₁) 10 mg *A. flavus* + 250 ppm lead sulphate solution: 3 flasks
(IA₂) 10 mg *P. spinulosum* + 250 ppm lead sulphate solution: 3 flasks
(IA₃) 10 mg *R. oryzae* + 250 ppm lead sulphate solution: 3 flasks
(IA₄) 10 mg *M. rouxii* + 250 ppm lead sulphate solution: 3 flasks
(IA₅) 10 mg *S. cerevisiae* + 250 ppm lead sulphate solution: 3 flasks

One set of 3 flasks (IA₆) without biomass served as control

(IB₁) 10 mg *A. flavus* + 500 ppm lead sulphate solution: 3 flasks
(IB₂) 10 mg *P. spinulosum* + 500 ppm lead sulphate solution: 3 flasks
(IB₃) 10 mg *R. oryzae* + 500 ppm lead sulphate solution: 3 flasks
(IB₄) 10 mg *M. rouxii* + 500 ppm lead sulphate solution: 3 flasks
(IB₅) 10 mg *S. cerevisiae* + 500 ppm lead sulphate solution: 3 flasks

One set of 3 flasks (IB₆) without biomass served as control

(IC₁) 10 mg *A. flavus* + 750 ppm lead sulphate solution: 3 flasks
(IC₂) 10 mg *P. spinulosum* + 750 ppm lead sulphate solution: 3 flasks
(IC₃) 10 mg *R. oryzae* + 750 ppm lead sulphate solution: 3 flasks
(IC₄) 10 mg *M. rouxii* + 750 ppm lead sulphate solution: 3 flasks
(IC₅) 10 mg *S. cerevisiae* + 750 ppm lead sulphate solution: 3 flasks

One set of 3 flasks (IC₆) without biomass served as control

All these flasks were then placed on a BOD-incubator shaker for 10 minutes at 27±1°C. After a contact period of 10 minutes, the fungal biomass from each flask was harvested separately by filtering the reaction mixture through 120 BSS (0.125 mm) “Standard” test sieves. The filtrate was further processed for assessing the concentration of lead remaining in the solution.
A few drops of aqua-regia were added to the filtrate from each flask for acidification. Thereafter, the contents of 3 flasks of each sub-set (eg. IA1/IA2/IB1/IB2 etc.) were pooled together to get a composite solution for atomic absorption spectrophotometry to quantify the amount of metal (lead) remaining in the filtrate. Before the measurement, the filtrates were diluted with appropriate amount of double-distilled water so as to bring the metal concentration in range measureable by the Atomic Absorption Spectrophotometer (AAS). The absorbance of these diluted solutions were recorded on AA–7000 model Atomic Absorption Spectrophotometer. The dilutions were taken into account while calculating the metal concentrations.

Similar procedure was repeated for the solutions of cadmium (as cadmium sulphate) i.e. sets IIA to IIC using biomass of same fungal strains as for sets IA to IC.

### 3.6.2 BIOSORPTION OF MALACHITE GREEN AND BASIC FUCHSIN DYES BY BIOMASS OF YEAST AND SELECTED MOLDS

100 ml of 500 ppm solution of malachite green (MG) were taken in each of a set of 18 flasks (set III). To 15 of these flasks, dead fungal biomass were added as under:

| (IIIA)  | 10 mg *A. flavus*  | + 500 ppm MG solution: 3 flasks |
| (IIIB)  | 10 mg *P. spinulosum*  | + 500 ppm MG solution: 3 flasks |
| (IIIC)  | 10 mg *R. oryzae*  | + 500 ppm MG solution: 3 flasks |
| (IID)   | 10 mg *M. rouxii*  | + 500 ppm MG solution: 3 flasks |
| (IIIE)  | 10 mg *S. cerevisiae*  | + 500 ppm MG solution: 3 flasks |

A set of 3 flasks (500 ppm solution of malachite green: set IIIF) without added fungal biomass served as control.

Similarly, a set of 18 flasks (set IV) was used for basic fuchsin dye (BF) solution.

| (IVA)   | 10 mg *A. flavus*  | + 500 ppm BF solution: 3 flasks |
| (IVB)   | 10 mg *P. spinulosum*  | + 500 ppm BF solution: 3 flasks |
| (IVC)   | 10 mg *R. oryzae*  | + 500 ppm BF solution: 3 flasks |
| (IVD)   | 10 mg *M. rouxii*  | + 500 ppm BF solution: 3 flasks |
| (IVE)   | 10 mg *S. cerevisiae*  | + 500 ppm BF solution: 3 flasks |
| (IVF)   | Control  | + 500 ppm BF solution: 3 flasks |
All these flasks were then placed on a BOD-incubator shaker for 10 minutes at 27±2°C. After a contact period of 10 minutes, the fungal biomass from each flask was separated by filtering the reaction mixture through 120 BSS (0.125 mm) “standard” test sieves. The unadsorbed dye (that remaining in the supernatant) was estimated using a *uv−vis* spectrophotometer (ELICO India, Model SL−159) at 618 nm (malachite green) and 550 nm (basic fuchsin) wave lengths.

### 3.6.3 CALCULATION OF SPECIFIC UPTAKE OF METALS/DYES BY BIOMASS

The specific uptake (Q−value) of metals (Pb and Cd) and dye (malachite green and basic fuchsin) were calculated as follows:

\[
Q = \frac{V (C_i - C_f)}{m}
\]

Where,  
- \(Q\) = metal/dye uptake (mg metal/dye/g biosorbent)  
- \(V\) = the liquid sample volume (ml)  
- \(C_i\) = the initial concentration of the metal/dye in solution (mg/l)  
- \(C_f\) = the final concentration of the metal/dye in solution (mg/l)  
- \(m\) = the amount of added biosorbent on the dry weight basis (g)

The biosorptive efficiency of particular biomass was interpreted as under:

- 0−10 : Very poor  
- 10−20 : Poor  
- 20−40 : Moderate  
- 40−60 : Good  
- 60−80 : Very good  
- 80−100 : Excellent

### 3.7 FT−IR ANALYSIS OF METAL/DYE−LOADED AND UNLOADED FUNGAL BIOMASS

FT−IR analyses of the samples of the biomass of the five fungal species (loaded with metal/dye and unloaded) were carried out to assess the relationship, if any, between the functional groups on the biomass and their biosorptive capability. Two mg of given fungal biomass powder were mixed with 98 mg of dry powdered
potassium bromide (KBr) and finely grounded. The material was used for preparing pellets by applying pressure of 10,000–15,000 psi. IR spectra of these were recorded on JASCO– 4100 model FT–IR spectrophotometer at high resolution (≤ 0.001 cm$^{-1}$).

Two empirical models were employed to describe the biosorption equilibria of the test fungi — one proposed by Freundlich (1906) and another by Langmuir (1916) for single solute system.

Freundlich equation: \( q_e = K_F C_e^{1/n} \)

Langmuir equation: \( q_e = \frac{q_{\text{max}} K_L C_e}{1 + K_L C_e} \)

where,
- \( q_e \) = metallic ions adsorbed per unit weight of adsorbent at equilibrium (mg/g);
- \( q_{\text{max}} \) = maximum possible amount of metallic ions adsorbed per unit of weight of adsorbents (mg/g);
- \( K_L \) = constant related to the affinity of binding sites for metal ions (L/mg);
- \( C_e \) = equilibrium (residual) concentration (mg/L);
- \( K_F \) = Freundlich characteristic constant of the system, incorporating parameters affecting the adsorption process, such as adsorption capacity;
- \( n \) = Freundlich characteristic constant of the system, incorporating parameters such as effect of concentration on the adsorption capacity and represents the adsorption intensity (dimensionless)

### 3.8 EFFECT OF AGE OF THE BIOMASS ON BIOSORPTIVE POTENTIAL

The biomass of two fungal species *i.e. Aspergillus flavus* and *Rhizopus oryzae* were selected for these studies because of their better performance in the biosorption of dyes and metals (chapter IV). The biomass of different ages (48 hrs, 96 hrs, 144 hrs and 192 hrs) were obtained for each organism to evaluate the effect of age of biomass on adsorption. Since the lesser the age the lower is the amount of biomass, it became necessary to use greater number of flasks (containing MGYP media) to obtain substantial amount of biomass with lesser age. Therefore, 30 flasks containing MGYP media inoculated with a given species were harvested after 48 hrs of incubation, 25 flasks after 96 hrs, 20 flasks after 144 hrs and only 15
3.8.1 Biosorption of lead and cadmium by mycomass of different ages

In each of a set (set V) of 27 flasks of 250 ml capacity, 100 ml of 250 ppm aqueous solution of lead sulphate were taken. Similarly, two sets of 27 flasks of 250 ml capacity were used — one set (set VI) for 500 ppm solution of lead sulphate and another set (set VII) for 750 ppm solution of lead sulphate. Thus, 81 flasks were used for lead sulphate solution. To 72 of these flasks (VA to VIIH), 10 mg of fungal biomass of different ages were added as under:

(VA) 10 mg *A. flavus* (48 hrs) + 250 ppm lead sulphate solution: 3 flasks
(VB) 10 mg *A. flavus* (96 hrs) + 250 ppm lead sulphate solution: 3 flasks
(VC) 10 mg *A. flavus* (144 hrs) + 250 ppm lead sulphate solution: 3 flasks
(VD) 10 mg *A. flavus* (192 hrs) + 250 ppm lead sulphate solution: 3 flasks
(VE) 10 mg *R. oryzae* (48 hrs) + 250 ppm lead sulphate solution: 3 flasks
(VF) 10 mg *R. oryzae* (96 hrs) + 250 ppm lead sulphate solution: 3 flasks
(VG) 10 mg *R. oryzae* (144 hrs) + 250 ppm lead sulphate solution: 3 flasks
(VH) 10 mg *R. oryzae* (192 hrs) + 250 ppm lead sulphate solution: 3 flasks

(VIA) 10 mg *A. flavus* (48 hrs) + 500 ppm lead sulphate solution: 3 flasks
(VIB) 10 mg *A. flavus* (96 hrs) + 500 ppm lead sulphate solution: 3 flasks
(VIC) 10 mg *A. flavus* (144 hrs) + 500 ppm lead sulphate solution: 3 flasks
(VID) 10 mg *A. flavus* (192 hrs) + 500 ppm lead sulphate solution: 3 flasks
(VIE) 10 mg *R. oryzae* (48 hrs) + 500 ppm lead sulphate solution: 3 flasks
(VIF) 10 mg *R. oryzae* (96 hrs) + 500 ppm lead sulphate solution: 3 flasks
(VIG) 10 mg *R. oryzae* (144 hrs) + 500 ppm lead sulphate solution: 3 flasks
(VIH) 10 mg *R. oryzae* (192 hrs) + 500 ppm lead sulphate solution: 3 flasks

(VIIB) 10 mg *A. flavus* (96 hrs) + 750 ppm lead sulphate solution: 3 flasks
(VIIC) 10 mg *A. flavus* (144 hrs) + 750 ppm lead sulphate solution: 3 flasks
(VIID) 10 mg *A. flavus* (192 hrs) + 750 ppm lead sulphate solution: 3 flasks
(VIIE) 10 mg *R. oryzae* (48 hrs) + 750 ppm lead sulphate solution: 3 flasks
(VIIF) 10 mg *R. oryzae* (96 hrs) + 750 ppm lead sulphate solution: 3 flasks
(VIIG) 10 mg *R. oryzae* (144 hrs) + 750 ppm lead sulphate solution: 3 flasks
(VIIF) 10 mg *R. oryzae* (192 hrs) + 750 ppm lead sulphate solution: 3 flasks

A sub-set of 3 flasks of set V with 250 ppm solution of lead sulphate with no fungal biomass served as control. Similarly, two sub-sets of 3 flasks (sets VI & VII) each with 500 ppm and 750 ppm solution of lead sulphate with no fungal biomass served as control.
Similar procedure was repeated for the assessing the effect of age on the capacity of fungal biomass to adsorb cadmium from aqueous solutions. Using sets VIII, IX and X as under:

(VIIIA) 10 mg *A. flavus* (48 hrs) + 250 ppm CdSO$_4$ solution: 3 flasks
(VIIIB) 10 mg *A. flavus* (96 hrs) + 250 ppm CdSO$_4$ solution: 3 flasks
(VIIIC) 10 mg *A. flavus* (144 hrs) + 250 ppm CdSO$_4$ solution: 3 flasks
(VIIID) 10 mg *A. flavus* (192 hrs) + 250 ppm CdSO$_4$ solution: 3 flasks
(VIIIE) 10 mg *R. oryzae* (48 hrs) +250 ppm CdSO$_4$ solution: 3 flasks
(VIIF) 10 mg *R. oryzae* (96 hrs) +250 ppm CdSO$_4$ solution: 3 flasks
(VIIG) 10 mg *R. oryzae* (144 hrs) +250 ppm CdSO$_4$ solution: 3 flasks
(VIIF) 10 mg *R. oryzae* (192 hrs) +250 ppm CdSO$_4$ solution: 3 flasks

(VIJA) 10 mg *A. flavus* (48 hrs) + 500 ppm CdSO$_4$ solution: 3 flasks
(VIJB) 10 mg *A. flavus* (96 hrs) + 500 ppm CdSO$_4$ solution: 3 flasks
(VIJC) 10 mg *A. flavus* (144 hrs) + 500 ppm CdSO$_4$ solution: 3 flasks
(VIJD) 10 mg *A. flavus* (192 hrs) + 500 ppm CdSO$_4$ solution: 3 flasks
(VIJE) 10 mg *R. oryzae* (48 hrs) + 500 ppm CdSO$_4$ solution: 3 flasks
(VIJE) 10 mg *R. oryzae* (96 hrs) + 500 ppm CdSO$_4$ solution: 3 flasks
(VIJK) 10 mg *R. oryzae* (144 hrs) + 500 ppm CdSO$_4$ solution: 3 flasks
(VIJK) 10 mg *R. oryzae* (192 hrs) + 500 ppm CdSO$_4$ solution: 3 flasks

(VIKA) 10 mg *A. flavus* (48 hrs) + 750 ppm CdSO$_4$ solution: 3 flasks
(VIKB) 10 mg *A. flavus* (96 hrs) + 750 ppm CdSO$_4$ solution: 3 flasks
(VIKC) 10 mg *A. flavus* (144 hrs) + 750 ppm CdSO$_4$ solution: 3 flasks
(VIKD) 10 mg *A. flavus* (192 hrs) + 750 ppm CdSO$_4$ solution: 3 flasks
(VIKE) 10 mg *R. oryzae* (48 hrs) + 750 ppm CdSO$_4$ solution: 3 flasks
(VIKE) 10 mg *R. oryzae* (96 hrs) + 750 ppm CdSO$_4$ solution: 3 flasks
(VIKF) 10 mg *R. oryzae* (144 hrs) + 750 ppm CdSO$_4$ solution: 3 flasks
(VIKH) 10 mg *R. oryzae* (192 hrs) + 750 ppm CdSO$_4$ solution: 3 flasks

A sub-set of 3 flasks each of set VIII, set IX and set X with 250 ppm, 500 ppm and 750 ppm solution of cadmium sulphate without biomass served as controls.

Further procedure for biosorption-reaction and estimation of Pb and Cd remaining unadsorbed in the filtrate was as outlined in section 3.6.1.
3.8.2 Biosorption of Malachite Green and Basic Fuchsin by mycomass of Different Ages

100 ml of 500 ppm solution of malachite green (MG) were taken in each of a set (set XI) of 27 flasks. To each of these flasks, dead fungal biomass of different ages were added as under:

(XIA) 10 mg *A. flavus* (48 hrs) + 500 ppm MG solution: 3 flasks
(XIB) 10 mg *A. flavus* (96 hrs) + 500 ppm MG solution: 3 flasks
(XIC) 10 mg *A. flavus* (144 hrs) + 500 ppm MG solution: 3 flasks
(XID) 10 mg *A. flavus* (192 hrs) + 500 ppm MG solution: 3 flasks
(XIE) 10 mg *R. oryzae* (48 hrs) + 500 ppm MG solution: 3 flasks
(XIF) 10 mg *R. oryzae* (96 hrs) + 500 ppm MG solution: 3 flasks
(XIG) 10 mg *R. oryzae* (144 hrs) + 500 ppm MG solution: 3 flasks
(XIH) 10 mg *R. oryzae* (192 hrs) + 500 ppm MG solution: 3 flasks

3 flasks of the set XI without added fungal biomass served as control. Similarly, a set of 27 (24 + 3) flasks (set XII) was used for evaluating uptake of basic fuchsin from its aqueous solution by mycomass.

3.9 EFFECT OF PARTICLE SIZE ON BIOSORPTIVE POTENTIAL OF MYCOMASS

Since there does exist difference of opinion regarding the effect of biomass particle size on biosorption of pollutants (chapter 1), it was decided to evaluate the influence of particle size of *A. flavus* and *R. oryzae* mycomass on their capacity for biosorption of metals and dyes under study. The mycomass of ages 96 hrs (for *R. oryzae*) and 192 hrs (for *A. flavus*) were selected for the study because of the better performance of the mycomass of these ages. The biomass of two test fungi (*A. flavus* and *R. oryzae*) of 192 hrs and 96 hrs ages respectively were prepared through the procedure already outlined in section 3.8.

The biomass so obtained was dried in hot air oven at 60±2°C for 24 hrs. The dried biomass was crushed with the help of mortar and pestle to obtain it in powder form. The powder of biomass was screened through “Standard” test sieves of different pore size *i.e.*, 0.50 mm (30 BSS), 0.25 mm (60 BSS) and 0.125 mm (120 BSS) to obtain biomass of three different size ranges: (i) 0.50 mm to 0.25 mm,
denoted as “X”; (ii) 0.25 mm to 0.125 mm, denoted as “Y”; and (iii) 0.125 mm or lesser denoted as “Z”.

3.9.1 Biosorption of Lead and Cadmium by Mycomass of Different Particle Sizes

In each of a set of 30 flasks of 250 ml capacity (set XIII), 100 ml of 500 ppm aqueous solution of lead sulphate were taken. To these flasks, 10 mg of fungal biomass of different ages and particle sizes (i) X (0.50 mm to 0.25), (ii) Y (0.25 mm to 0.125 mm), (iii) and Z (0.125 mm or lesser) were added as under:

(XIII A1) 10 mg \(A. \text{flavus} \) X (192 hrs) + 500 ppm PbSO\(_4\) solution: 3 flasks
(XIII A2) 10 mg \(A. \text{flavus} \) Y (192 hrs) + 500 ppm PbSO\(_4\) solution: 3 flasks
(XIII A3) 10 mg \(A. \text{flavus} \) Z (192 hrs) + 500 ppm PbSO\(_4\) solution: 3 flasks
(XIII B1) 10 mg \(R. \text{oryzae} \) X (96 hrs) + 500 ppm PbSO\(_4\) solution: 3 flasks
(XIII B2) 10 mg \(R. \text{oryzae} \) Y (96 hrs) + 500 ppm PbSO\(_4\) solution: 3 flasks
(XIII B3) 10 mg \(R. \text{oryzae} \) Z (96 hrs) + 500 ppm PbSO\(_4\) solution: 3 flasks
(XIII C1) 10 mg \(S. \text{cerevisiae} \) X + 500 ppm PbSO\(_4\) solution: 3 flasks
(XIII C2) 10 mg \(S. \text{cerevisiae} \) Y + 500 ppm PbSO\(_4\) solution: 3 flasks
(XIII C3) 10 mg \(S. \text{cerevisiae} \) Z + 500 ppm PbSO\(_4\) solution: 3 flasks

Three flasks of the set XIII, without fungal biomass, served as control.

Similar procedure was repeated for the assessing the effect of age and particle size on the capacity of fungal biomass to adsorb cadmium from aqueous solutions using sets XIV, as under:

(XIVA1) 10 mg \(A. \text{flavus} \) X (192 hrs) + 500 ppm CdSO\(_4\) solution: 3 flasks
(XIVA2) 10 mg \(A. \text{flavus} \) Y (192 hrs) + 500 ppm CdSO\(_4\) solution: 3 flasks
(XIVA3) 10 mg \(A. \text{flavus} \) Z (192 hrs) + 500 ppm CdSO\(_4\) solution: 3 flasks
(XIVB1) 10 mg \(R. \text{oryzae} \) X (96 hrs) + 500 ppm CdSO\(_4\) solution: 3 flasks
(XIVB2) 10 mg \(R. \text{oryzae} \) Y (96 hrs) + 500 ppm CdSO\(_4\) solution: 3 flasks
(XIVB3) 10 mg \(R. \text{oryzae} \) Z (96 hrs) + 500 ppm CdSO\(_4\) solution: 3 flasks
(XIVC1) 10 mg \(S. \text{cerevisiae} \) X + 500 ppm CdSO\(_4\) solution: 3 flasks
(XIVC2) 10 mg \(S. \text{cerevisiae} \) Y + 500 ppm CdSO\(_4\) solution: 3 flasks
(XIVC3) 10 mg \(S. \text{cerevisiae} \) Z + 500 ppm CdSO\(_4\) solution: 3 flasks

Three flasks of the set XIV without fungal biomass, served as control.

Further procedure for biosorption-reaction and estimation of Pb and Cd remaining in the filtrate followed was as outlined in section 3.6.1.
3.9.2 Biosorption of Malachite Green and Basic Fuchsin by Mycomass of Different Particle Sizes

100 ml of 500 ppm solution of malachite green (MG) were taken in each of a set of 30 flasks (set XV). To these flasks, dead fungal biomass of different particle sizes and ages were added as under:

(XVA) 10 mg *A. flavus* X (192 hrs) + 500 ppm MG solution: 3 flasks
(XVB) 10 mg *A. flavus* Y (192 hrs) + 500 ppm MG solution: 3 flasks
(XVC) 10 mg *A. flavus* Z (192 hrs) + 500 ppm MG solution: 3 flasks
(XVD) 10 mg *R. oryzae* X (96 hrs) + 500 ppm MG solution: 3 flasks
(XVE) 10 mg *R. oryzae* Y (96 hrs) + 500 ppm MG solution: 3 flasks
(XVF) 10 mg *R. oryzae* Z (96 hrs) + 500 ppm MG solution: 3 flasks
(XVG) 10 mg *S. cerevisiae* X + 500 ppm MG solution: 3 flasks
(XVH) 10 mg *S. cerevisiae* Y + 500 ppm MG solution: 3 flasks
(XVI) 10 mg *S. cerevisiae* Z + 500 ppm MG solution: 3 flasks

A set of 3 flasks (with 500 ppm solution of malachite green) without fungal biomass served as control.

Similarly, a set of 30 flasks (set XVI) was used for mycomass of different ages and particle sizes with basic fuchsin dye solution.

3.10 EFFECT OF PRETREATMENT OF FUNGAL BIOMASS ON BIOSORPTIVE POTENTIAL

In order to assess the effect of pretreatments with gluteraldehyde (2% and 5%) as well as formaldehyde (10% and 15%) on biosorptive capacity of dead biomass of *Aspergillus flavus*, *Rhizopus oryzae* and *Saccharomyces cerevisiae*, the dead biomass of each of these organisms were treated as under:

(a) 30g of dead biomass were boiled for 15 minutes in 500 ml of 2% and 5% gluteraldehyde solution separately designated as G2% and G5%;
(b) 30g of dead biomass were boiled for 15 minutes in 500 ml of 10% and 15% formaldehyde solution separately designated as F10% and F15%; and
(c) 30g of dead biomass were not given any treatment (control).
The biomass so obtained were washed with generous amounts of distilled water separately, followed by drying at 60±2°C for 24 hrs in hot air oven.

3.10.1 Biosorption of Metals by the Pretreated Biomass

Since the biomass of particle size 'X' (0.50 mm to 0.25 mm) did not prove to be optimum particle size, the biomass of this size (X) was not considered for further experiments. The particle size 'Z' (0.125 mm or lesser) was optimum in case of *Rhizopus oryzae* and *Saccharomyces cerevisiae*, while the particle size 'Y' gave optimum results in case of *Aspergillus flavus*. Therefore, for further experiments particle size 'Y' only was tried in case of *Aspergillus flavus* and 'Z' in case of *Rhizopus oryzae* and *Saccharomyces cerevisiae*.

Similarly, the biomass of age 96 hrs and age 192 hrs yielded optimum results, in case of *Rhizopus oryzae* and *Aspergillus flavus* respectively. Therefore, the mycomass of 96 hrs old *Rhizopus oryzae* and 192 hrs old *Aspergillus flavus* were taken for study of these fungi respectively.

500 ppm lead sulphate solution were dispensed into each of 48 flasks (set XVII); while a set of another 48 flasks was used for 500 ppm cadmium sulphate solution (set XVIII). Thus, a total of 96 flasks used for this experiment. To these flasks (sets XVII and XVIII), 10 mg dead fungal biomass pretreated with glutaraldehyde (G2% and G5%) and formaldehyde (F10% and F15%) were added.

(a) To each of a set of 45 flasks (set XVII) containing 100 ml of 500 ppm lead sulphate aqueous solution, 10 mg of G2% or G5% or F10% or F15% biomass of *Aspergillus flavus/ Rhizopus oryzae/ Saccharomyces cerevisiae* were added as under:

(XVIIA₁) 10 mg *A. flavus* (G2%) + 500 ppm PbSO₄ solution: 3 flasks
(XVIIA₂) 10 mg *A. flavus* (G5%) + 500 ppm PbSO₄ solution: 3 flasks
(XVIIA₃) 10 mg *A. flavus* (F10%) + 500 ppm PbSO₄ solution: 3 flasks
(XVIIA₄) 10 mg *A. flavus* (F15%) + 500 ppm PbSO₄ solution: 3 flasks
(XVIIB₁) 10 mg *R. oryzae* (G2%) + 500 ppm PbSO₄ solution: 3 flasks
(XVIIB₂) 10 mg *R. oryzae* (G5%) + 500 ppm PbSO₄ solution: 3 flasks
(XVIIB₃) 10 mg *R. oryzae* (F10%) + 500 ppm PbSO₄ solution: 3 flasks
(XVIIB₄) 10 mg *R. oryzae* (F15%) + 500 ppm PbSO₄ solution: 3 flasks
(XVIIIC₁) 10 mg *S. cerevisiae* (G2%) + 500 ppm PbSO₄ solution: 3 flasks
A set of three flasks to which untreated *Aspergillus flavus* biomass were added, served as control 1AF; another set with untreated *Rhizopus oryzae* as control 1RO; and still another set with *Saccharomyces cerevisiae* served as control 1SC; one set without any biomass served as control C2.

(b) another set of 45 flasks (set XVIII) with 500 ppm cadmium sulphate solution was subjected to the same treatment as was given to set XVII:

A set of three flasks to which untreated *Aspergillus flavus* biomass were added, served as control 1AF; another set with untreated *Rhizopus oryzae* as control 1RO; and still another set with *Saccharomyces cerevisiae* served as control 1SC; one set without any biomass served as control C2.

### 3.10.2 Biosorption of Dyes by the Pretreated Mycomass

100 ml of 500 ppm solution of malachite green (MG) were taken in each of a set of 48 flasks (set XIX). To each these flasks, 10 mg of dead biomass of *Rhizopus oryzae* (Z, 96 hrs)/*Aspergillus flavus* (Y, 192 hrs) pretreated with glutaraldehyde (2%/5%) and formaldehyde (10%/15%) were added as under:

(XIXA₁) 10 mg *A. flavus* (G2%) + 500 ppm MG solution: 3 flasks
(XIXA₂) 10 mg *A. flavus* (G5%) + 500 ppm MG solution: 3 flasks
(XIXA₃) 10 mg *A. flavus* (F10%) + 500 ppm MG solution: 3 flasks
(XIXB₁) 10 mg *R. oryzae* (G2%) + 500 ppm CdSO₄ solution: 3 flasks
(XIXB₂) 10 mg *R. oryzae* (G5%) + 500 ppm CdSO₄ solution: 3 flasks
(XIXB₃) 10 mg *R. oryzae* (F10%) + 500 ppm CdSO₄ solution: 3 flasks
(XIXC₁) 10 mg *S. cerevisiae* (G2%) + 500 ppm CdSO₄ solution: 3 flasks
(XIXC₂) 10 mg *S. cerevisiae* (G5%) + 500 ppm CdSO₄ solution: 3 flasks
(XIXC₃) 10 mg *S. cerevisiae* (F10%) + 500 ppm CdSO₄ solution: 3 flasks
(XIXC₄) 10 mg *S. cerevisiae* (F15%) + 500 ppm CdSO₄ solution: 3 flasks
A set of three flasks with untreated biomass of *Aspergillus flavus* served as control “C1AF”; another with *Rhizopus oryzae* as “C1RO” and still another with *Saccharomyces cerevisiae* as “C1SC”.

A set of 3 flasks (3 with 500ppm solution of malachite green) without added fungal biomass served as control 2.

Similarly, a set of 48 flasks (set XX) was used for evaluating the effect of pretreatment on potential of yeast, *Aspergillus flavus* and *Rhizopus oryzae* biomass to adsorb basic fuchsin.

### 3.11 Effect of the Presence of Metals on Biosorption of Dyes by Mycomass

In order to assess the effect of presence of metals Fe (as FeSO₄) and Pb (as PbSO₄) on biosorptive capacity of the yeast and selected fungal biomass (chapter 1), the metal (Fe/Pb) solutions of different concentrations (50 ppm, 100 ppm, 250 ppm and 500 ppm) were prepared in 500 ppm solution of malachite green (MG) and basic fuchsin (BF) separately. In the effluents from industries, the level of iron (Fe) and lead (Pb) solution reached upto 500 ppm (Tiwari, 2010). For this experiment, a set (set XXI) of 180 flasks were used as under:

(XIXA₁) 10 mg *A. flavus* (F15%) + 500 ppm MG solution: 3 flasks
(XIXA₂) 10 mg *A. flavus* (G5%) + MG in 50 ppm FeSO₄ solution: 3 flasks
(XIXB₁) 10 mg *R. oryzae* (G2%) + MG in 50 ppm FeSO₄ solution: 3 flasks
(XIXB₂) 10 mg *R. oryzae* (G5%) + MG in 50 ppm FeSO₄ solution: 3 flasks
(XIXB₃) 10 mg *R. oryzae* (F10%) + MG in 50 ppm FeSO₄ solution: 3 flasks
(XIXB₄) 10 mg *R. oryzae* (F15%) + MG in 50 ppm FeSO₄ solution: 3 flasks
(XIXC₁) 10 mg *S. cerevisiae* (G2%) + 500 ppm MG solution: 3 flasks
(XIXC₂) 10 mg *S. cerevisiae* (G5%) + MG in 50 ppm FeSO₄ solution: 3 flasks
(XIXC₃) 10 mg *S. cerevisiae* (F10%) + MG in 50 ppm FeSO₄ solution: 3 flasks
(XIXC₄) 10 mg *S. cerevisiae* (F15%) + 500 ppm MG solution: 3 flasks

Similarly, a set of 48 flasks (set XXI) was used for evaluating the effect of pretreatment on potential of yeast, *Aspergillus flavus* and *Rhizopus oryzae* biomass to adsorb basic fuchsin.

(XIXD₁) 10 mg *A. flavus* (G5%) + MG in 50 ppm PbSO₄ solution: 3 flasks
(XIXD₂) 10 mg *A. flavus* (F15%) + MG in 50 ppm PbSO₄ solution: 3 flasks
(XIXD₃) 10 mg *A. flavus* (F15%) + MG in 50 ppm PbSO₄ solution: 3 flasks
(XIXE₁) 10 mg *R. oryzae* (G2%) + MG in 50 ppm PbSO₄ solution: 3 flasks
(XIXE₂) 10 mg *R. oryzae* (G5%) + MG in 50 ppm PbSO₄ solution: 3 flasks
(XIXE₃) 10 mg *R. oryzae* (F15%) + MG in 50 ppm PbSO₄ solution: 3 flasks
(XIXE₄) 10 mg *R. oryzae* (F15%) + MG in 50 ppm PbSO₄ solution: 3 flasks
A subset of 12 flasks (3 with 50 ppm, 3 with 100 ppm, 3 with 250 ppm and 3 with 500 ppm) of FeSO₄ and 12 flasks for PbSO₄ solution each without added fungal
biomass served as control (C1).

A subset each of 3 flasks with only 500 ppm MG solution (without metal) was used for *Aspergillus flavus/ Rhizopus oryzae/ Saccharomyces cerevisiae* as control 2.

Another subset of 3 flasks with MG solutions only served as C3.

Similarly, a set (set XXII) of 180 flasks were used for basic fuchsin dye. The biosorptive efficiencies of the biomass of the test fungi were assessed as already outlined in section 3.6.2.