Chapter 1

INTRODUCTION

The unplanned and indiscriminate exploitation of natural resources accompanied by unprecedented upsurge in industrial activities across the globe have resulted in the emergence of the problem of environmental pollution throughout the world — the harmful effects of which have drawn the focused attention of all segments of the society, more specifically during the past half century or so. A series of environmental disasters were witnessed in 1960s (Eccleston and March, 2011). Also, a number of books and reports including “The Population Bomb” (Ehrlich, 1968) and “Silent Spring” (Carson, 1962) appeared in 1960s which brought to the light “the then existing” and “probable” threats to our environment and humanity.

Water, being highly integrated component of most ecosystems, is highly prone to pollution — be it natural or anthropogenic. In fact, as the 21st century begins, the world is faced with water quality crisis which has resulted from (i) population explosion; (ii) urbanization; (iii) land use changes; (iv) rapid industrialization; (v) environment–unfriendly food production practices; and (vi) lack of appropriate worldwide management strategies (Anon, 2015). Many areas of the world, especially developing countries, are already experiencing the problem of water scarcity. Water crises in India, China and the United States have already become an impending problem (Harvey, 2006).

Industries — especially discharges from the industries—constitute one of the major sources of environmental pollution including water pollution. In fact, the effluents discharged into the environment from a variety of industries carry a range of inorganic as well as organic pollutants including phenols, formaldehyde, dyes and heavy metals. These pollutants pose a serious threat to the environment (Bhole et al., 2004; Arellano-Cardenas et al., 2005; Gopalakrishnan and Jeyadoss, 2011; Abdel-Ghani and El-Chaghaby, 2014; Mustapha and Halimoon, 2015; Joseph and Chinonye, 2016).
The contamination of environment by “heavy metals” from natural sources as well as a result of human activities is a matter of global concern (Dushenkov et al., 1995; Crusberg et al., 2004; Piccardo et al., 2009; Shamim and Rehman, 2012; Garcia et al., 2016). The term “heavy metals” has been used inconsistently by a number of workers, based on different parameters like: (i) density or specific gravity (Scott and Mercer, 1996; Akpor and Muchie, 2010); (ii) atomic mass (Rand et al., 1995); (iii) atomic number (Lyman, 1995); and (iv) toxicity (Crompton, 1997). However, strictly speaking, the term “heavy metals” refers to the metallic elements with atomic density higher than 6 gm/cm$^3$ (Akpor and Muchie, 2010) and which are able to form sulphides (Adrino, 1986). The metals with atomic number 23 onwards (with the exception of R, Sr, Y, Ba and Fr) are usually included in the category of heavy metals. As micronutrients, many heavy metals are essential for plants and animals (Wintz et al., 2002) but at elevated concentrations these become potentially toxic (Gadd and White, 1989; Garcia et al., 2016). Amongst the heavy metals Al, Cd, Co, Cu, Cr, Fe, Hg, Mn, Ni, Pb and Zn deserve immediate attention as these are known to be regularly discharged into the environment (Son et al., 2004; El-Shafey, 2010; Vinodhini et al., 2010; Abu Hasan et al., 2012; Guo et al., 2013). Once these reach the soil matrix, these enter the food web through ground water aquifers (Walton, 1995; Athar and Ahmad, 2002), ultimately getting biomagnified to toxic concentrations (Herawati et al., 2000). In the soil matrix itself, these affect soil microbial communities and consequently the biogeochemical cycle and plant growth. The present study focussed on only two metals i.e. lead and cadmium. The effluents from industrial units involved in the manufacturing of batteries, gasoline additives, paint pigments, alloys and sheets etc. often contain high concentrations of lead (Tunali et al., 2012). The wastes from industrial processes such as electroplating, smelting, alloy manufacturing, paints and pigments, cadmium-nickel batteries, fertilizers and pesticides serve as routes for contamination of environment with cadmium (Grayson and Othumer, 1978; Salim et al., 1992; Rao et al., 2010).
In addition to the heavy metals, the dyes also constitute a major cause of water pollution. The dyes find use in a variety of industries including textile, rubber, paper and pulp, plastic, cosmetic, tannery, pharmaceutical and food-processing units. More than 10,000 types of dye stuff are available throughout the world with annual production of about $7 \times 10^5$ metric tons (Rafatullah et al., 2010; Kuberan et al., 2011; Mahmoud, 2014). The dyestuff, especially those in current use, have been designed to resist fading as well as light-induced oxidation (Nigam et al., 2000; Casieri et al., 2008). Consequently, these acquire persistence, recalcitrance and become resistant to microbial degradation (Kabbout and Taha, 2014). The discharge of such dyes into the environment causes damage to the latter in a variety of manners.

About 11 percent of the pollution of industrial effluents results from the dyes (2% from dye-manufacturing unit and 9% from colouring processes). The textile sector occupies top position in this respect (Jain et al., 2003; Kiran et al., 2009) as textile industries release huge amounts of waste water from dyeing, printing, cloth-processing and mercerizing units. Even at very small concentrations (as low as 1.0 ppm), the dyes impart strong colour to the water; not only this, a number of toxic degradation products may also be formed (Dutta, 1994; Asamudo et al., 2005; Moorthi et al., 2007). Such polluted water has an adverse impact on the receptor water bodies, soil as well as other abiotic and biotic components of the environment including human life. Consequently, a variety of problems arise including (i) reduced sunlight penetration of water leading to diminished photosynthesis and consequent disturbance of the ecosystem of the water bodies (Kuo, 1992); (ii) inhibition of microbial growth and processes, flora and fauna (Meyer, 1993); and (iii) allergy, toxicity and carcinogenicity to fish and mammalian life (Kuo, 1992). Cerebral and skeletal abnormalities in human beings have been attributed to some dyes (Ahmad and Hameed, 2009; Wu et al., 2009). Possible connection between intestinal cancers and the azo dyes has also been suggested (Saeed et al., 2009). Damages to liver, kidney, central nervous system and reproductive systems in human beings have also been reported (Kiran et al., 2009; Ehrampoush et al., 2011).
In the interest of environmental health, it is the bounden duty of industries and the administration to ensure that the effluents containing metals and/or dyes emanating from industrial units are allowed to be discharged into the environment only after appropriate treatment. Already, a variety of strategies are available for the purpose. These include (a) physical processes such as membrane technologies; (b) chemical processes such as oxidation and ozonation; (c) physico-chemical methods like chemical precipitation, flocculation and photolysis; as well as (d) electrochemical methods (Mckay et al., 1987; Gupta et al., 1990; Lin and Peng, 1996; Grant and Buchanan, 2000; Selvam, and Shanmuga, 2012).

Such methods, however, suffer from a number of disadvantages including (i) high cost; (ii) frequent operational problems; (iii) high energy and reagent requirements; (iv) generation of concentrated sludge (Stephensen and Sheldon, 1996; Stoltz, 2001; Ahalya et al., 2003; Patel and Suresh, 2006; Farah et al., 2007; Singanan, 2011). As a result, many industrialists, especially those having tight budgets at their disposal, tend to avoid establishing and/or operating effluent-treatment systems. If alternative low-cost treatment systems are fabricated and become available, most of the industrial units might aspire to meet the requirements of environment regulatory bodies.

During the past two decades, “biosorption” has fast emerged as potentially economic and eco-friendly wastewater treatment technology (Volesky and Holan, 1995; Michalak et al., 2013). Gadd (2009) has defined, biosorption as “the removal of substances from solution by biological material” or as “any system where a sorbate (e.g. an atom, molecule, a molecular ion) interacts with a biosorbent (i.e. a solid interface of a biological matrix) resulting in an accumulation at the sorbate-biosorbent interface, and therefore a reduction in the solution sorbate concentration”. In fact, biosorption is a process of rapid and reversible binding of atoms/molecules/ions from aqueous solutions onto functional groups present on the surface of the biomass — the process being independent of cellular metabolism (Davis et al., 2003). The adsorption of solute (i.e. adsorbate) on to adsorbent occurs through: (i) exchange from sorbent to solution; or (ii) physical adsorption due to inner wall forces; or (iii) chemical adsorption i.e. chemisorption (Das et al., 2002).
The “biosorption technology” holds great promise for removal and/or recovery of pollutants because of its (i) simplicity; (ii) operation being analogous to conventional ion exchange technology; (iii) high efficiency; (iv) economic viability due to almost inexpensive biomass (Veglio and Beolchini, 1997; Volesky, 2007). The advantages of biosorption over conventional wastewater treatment methods include (i) low cost; (ii) high efficiency; (iii) minimum chemical and/or biological sludge; (iv) no additional requirements for nutrients; (v) regeneration of biosorbent; and (vi) possibilities of metal/dye recovery (Kratochvil and Volesky, 1998). The biosorption technique has been found to be highly useful for effluent treatment processes especially for dyes and heavy metals (Sa, 2001; Volesky, 2007; Wang et al., 2008; Abdel-Ghani and El-Chaghaby, 2014; Gupta et al., 2015; Ali and Alrafai, 2016).

Activated carbon has been in use as adsorbent conventionally (Yeh et al., 1993) but its generation cost is quite high. Therefore, all kinds of microbial, plant and animal biomass as well as their derivatives have been tried for the purpose. A lot of attention has been devoted to agricultural waste material as well as industrial waste biomaterial in this context (Volesky and Holan, 1995; Guo et al., 2003; Nakagwa et al., 2004; Gadd, 2009; Michalak et al., 2013).

The microbial biomass have been the materials of choice for a large number of workers interested in biosorption of dyes and metals. High biosorptive efficiency, low cost, and availability in large amounts must be amongst the reasons behind it. The biomass of bacteria (including cyanobacteria), algae, actinomycetes, yeast, fungi and even lichens have been tried for use as biosorbents. A large number of workers have so far advocated the use of microbes for accumulating dyes and heavy metals from the environment (Volesky and Holan, 1995; Gadd, 2009; Wang and Chen, 2009; Prigione et al., 2012; Michalak et al., 2013; Geetha and Belagali, 2013; Abdel-Ghani and El-Chaghaby, 2014; Fosso-Kanheu and Mulaba-Bafubiandi, 2014; Patel et al., 2015; Garcia et al., 2016).

The applicability of fungal biomass as biosorbent for the removal of dyes and heavy metals from the polluted environment is now a well-established aspect of biotechnology (Siegel et al., 1990; Ramalakshmi et al., 2011a,b,c). A sizeable
variety of fungi (including yeasts) have been tried across the world for the purpose and many of these have been found useful (Volesky, 1990; Volesky and Holan, 1995; Gadd, 2009; Naja and Volesky, 2011; Wu et al., 2012; Michalak et al., 2013; Gupta et al., 2015).

The chemical constitution and surface organization of fungal cell wall is highly suitable for biosorption. Fungal surface can be regarded as a “mosaic of different functional groups” where coordination complexes with metals can be found (Sa, 2001). The fungi have been found to be highly efficient for biosorptive removal of pollutants largely because of their filamentous morphology and high proportion of cell wall (Addour et al., 1999). The use of fungi as biosorptive agents appears to be economically feasible also because: (i) it is easy to cultivate these at large scale within short time (due to short multiplication cycle) using unsophisticated fermentation techniques on almost inexpensive growth media (Dhankar and Hooda, 2011); (ii) ease of separation of useful biomass from reactants mixture after biosorption (Kabbout and Taha, 2014); (iii) amenability to genetic manipulation (Goksunger et al., 2005; Marques et al., 2007). Not only this, thousands of tons of residual fungal biomass are produced as “waste” in many fermentation industries, which have to be incinerated (Bailey and Ollis, 1977; Fourest and Roux, 1992). This “waste?” biomass may find excellent use as biosorbent. The industries can sell these waste biomass produced (thereby earning money) and at the same time can save the cost involved in their disposal (Wang and Chen, 2006; Talos et al., 2012). The yeasts, though not as efficient as many other fungi, might form a significant proportion of mycomass for biosorption industry because of its large scale availability as “waste” by-product of fermentation industry, in addition to other properties which make these highly suitable for large scale use in biosorption industry (Talos et al., 2012).

A comparative evaluation of biomass of yeasts and filamentous fungi from the angle of biosorptive efficiency as well as from economic angle would be fruitful for selecting the appropriate biosorbents for metals/dyes under study.

The chemical make up and structural organization of fungal cell wall is such that substantial proportion of metals/dyes get deposited either on the surface
of the cell wall or within its structure. Obviously, cell wall composition of the fungal cell wall is an important factor governing biosorption of pollutants. However, the fungi exhibit remarkable variations in their cell wall composition and organization (Bartnicki-Garcia, 1968; Ruiz-Herrera, 2012), which influence their biosorptive capacity. For example, some workers have attributed better efficiency of *Rhizopus*, as compared to *Aspergillus*, for biosorption of metals to higher chitosan content in the former’s cell wall (Sa, 2001). The biomass of different groups of fungi (like mucorales, ascomycetous fungi, and anamorphic fungi) with substantial differences in their cell wall composition need to be compared for their efficiency to adsorb different pollutants. This might add to inputs for more logical selection of adsorbate to be used in biomass-based effluent treatment systems.

The use of microbial biomass in “living state” for bioremediation of dyes and metals has been under active consideration of scientists for a long time. A large number of microbes have been isolated during last two decades or so which are able to degrade dyes (Banat et al., 1996; Ramalho et al., 2002); and to remove metals from effluents through metabolism-dependent processes (Volesky and Holan, 1995; Huang et al., 2012). However, the use of “dead biomass” has gained wider acceptance during past few years to overcome a variety of complications involved in handling living biomass. The complications include (i) sensitivity to pollutant concentration (toxicity effects); (ii) requirement of constant nutrient supply; (iii) limitation of operation within a narrow range of physiological conditions like temperature, freezing, pH etc. which are not so easy to maintain. On the other hand, the dead biomass (i) can be easily stored; (ii) is not adversely affected by pollutant toxicity; (iii) does not require nutrient supply; and (iv) can be easily recharged (Selatnia et al., 2004; Baysal et al., 2009). Therefore, in the present study also, only dead biomass of selected yeasts and fungi have been tried.

The efficiency of the fungal biomass to adsorb metals or dyes may also be influenced by the “age” of the biomass (Gadd, 2009). It is quite possible because the cell walls of fungi may undergo changes in chemical composition, structure or both during various differentiation stages (Ruiz-Herrera, 2012); and biosorption largely depends upon the configuration of cell wall. This aspect of biosorptive potential of
fungi with reference to metals and dyes had drawn the attention of some workers quite long back (Young and Banks, 1998) but was followed by only a limited number of studies so far (Netpae, 2012; Osman et al., 2015).

The size of sorbent particles is another important parameter influencing the efficiency of a given biomass to adsorb metals and dyes (Gadd, 2009; Gong et al., 2005). It appears to be quite obvious on the grounds that adsorption is directly related with surface area of biomass (Gong et al., 2005). However, while many workers agree with this assumption (Safa and Bhatti, 2010; Kabbout and Taha, 2014), the results to the contrary have also been reported (Yang and Volesky, 1999). Resolution of this aspect of biosorption is also required.

It has been speculated for quite some time that if a given biosorbate is subjected to some severe treatment regimen, its capacity to adsorb metals and dyes might improve substantially (Huang et al., 1988; Dhankar and Hooda, 2011). The pretreatments may be physical or chemical. The physical pretreatments include heat treatment, autoclaving, boiling, or even freeze-drying. The chemical pretreatments commonly include the use of acids, alkali, ethanol and acetone. The pretreatments are believed to cause either modifications in the cell wall components, or exposure of active binding sites in the cell wall, or the removal of surface impurities (Huang and Huang, 1996). However, the response of a given biomass to a given pretreatment as also the change in the biosorptive efficiency would, to a great extent, depend upon the type of biomass and adsorbate under consideration (Brady et al., 1994; Zhao and Duncan, 1998; Xinjiao, 2006; Javaid et al., 2011; Kirova et al., 2015).

Most of the research on biosorption of heavy metals has mainly focussed on single metal (or dye)-biosorbent systems. Not much attention has been paid to biosorption in binary or multi-metal systems, though the significance of such studies was recognized much earlier (Chong and Volesky, 1996; Sag and Kutsal, 1996). A few studies covering this aspect do exist (Sag and Kutsal, 1998; Tsezos et al., 2009). Similarly, the presence of one or more metal species in the effluents carrying dyes might possibly affect the biosorptive removal of dyes (Pointing
et al., 2000; Ramachandran et al., 2013; Kumar, 2013). There do exist lacunae in our knowledge on this front also.

In view of the above considerations, the present study was conducted to work out the following aspects of metals and dyes biosorption:

1. Comparison of fungal strains of different cell wall compositions (Aspergillus flavus Link, Mucor rouxii Calmette, Penicillium spinulosum Thom, Rhizopus oryzae Went and Gerlings, and Saccharomyces cerevisiae Meyen ex-Hansen) to biosorb/accumulate dyes (malachite green and basic fuchsin)/metals (cadmium and lead).

2. To study the effect of adsorbent biomass of different particle sizes (0.50 mm – 0.25 mm (X), 0.25 mm – 0.125 mm (Y) and 0.125 mm or lesser (Z)) on the biosorption of dyes/metals.

3. To study the effect of age (48 hrs, 96 hrs, 144 hrs and 192 hrs) of biomass on the biosorption of dyes/metals.

4. To study the effect of different types of pretreatments (formaldehyde and glutaraldehyde) on the biosorption of dyes/metals by microbes (Aspergillus flavus, Rhizopus oryzae and Saccharomyces cerevisiae).

5. Effect of the presence of metals (Fe and Pb) on the potential of given biomass to adsorb given dyes.