Chapter 13
Metal Tolerance and Biosorption Potential of Soil Fungi: Applications for a Green and Clean Water Treatment Technology

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Abstract Heavy metals pose a significant ecological and public health hazard because of their toxic effects and their ability to accumulate in terrestrial and aquatic food chains. This chapter addresses the interactions of heavy metals with organisms for application in wastewater or soil treatment systems, with special emphasis on yeasts and fungi. Conventional techniques to remove metals from wastewaters have several disadvantages; however, biosorption has demonstrated significant metal removal performance from large volumes of effluents. One key step of treatment processes for cleanup of heavy metal-enriched water or soil involves growing resistant cells that accumulate metals to optimize removal through a combination of biosorption and continuous metabolic uptake. Fungal biosorption can be used for the removal of metals from contaminated water and soil; fungal biosorbents are less expensive and more effective alternatives for the removal of metallic elements, especially heavy metals, from aqueous solution. In this chapter, the biosorption abilities of fungal biomass toward metal ions are emphasized. The chapter also highlights the mechanisms involved in fungal biosorption and the factors affecting the biosorption process. The current status and achievements of fungal biosorption technologies are reviewed.

13.1 Introduction

Rapid industrialization and urbanization have resulted in elevated emissions of toxic heavy metals and radionuclides to the biosphere. Inorganic toxicants may occur as cations of metals such as mercury (Hg), cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni), and uranium (U). Toxic inorganics may also include alkylated or aromatized forms of metal ions, such as methylmercury and phenylmercury.

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The increasing quantities of toxic metals emitted into the biosphere pose potential hazards to ecosystems and influence the metabolism of living organisms (Gazso et al. 2001; Ansari and Malik 2007).

Heavy metals pose a significant threat to the environment and public health because of their toxicity and their accumulation in soil and food chains (Ceribasi and Yetis 2001; Chen et al. 2009; Gurel et al. 2010). Metal pollution of the biosphere by toxic metals has accelerated dramatically since the industrial revolution (Mclveen and Negusanti 1994). Agricultural application of wastewater and sludge and improper disposal of industrial effluents in developing countries, including India, have resulted in the accumulation of toxic heavy metals in soil. Most heavy metals (except Cd, Hg, and Pb) are required by living organisms in trace quantities; however, at elevated concentrations these become environmental toxins. Once the soil is contaminated with a metal, it is difficult and costly to remove from soil. In addition, microbial diversity and their activity in soil will be adversely affected, which may result in adverse effects on soil productivity (Ansari and Malik 2010).

Industrialized countries are increasingly concerned regarding the occurrence of toxic metals in the environment. The most effective policy to minimize their release from industrial or agricultural sources is the adoption of low waste-generating technologies coupled with effective effluent purification processes (Fourest et al. 1994; Sag et al. 2000).

In recent years, public awareness has increased regarding the long-term effects of wastewater containing toxic elements. Numerous industrial processes generate aqueous effluents contaminated with heavy metals. Metal concentrations must be reduced to meet ever increasing legislative standards and recovered where feasible. According to the World Health Organization, the metals of most immediate concern are Hg, Cr, Cd, Pb, Ni, aluminum (Al), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), and zinc (Zn) (Allen and Brown 1995).

Removal of heavy metal ions from wastewater is necessary due to their toxic properties. Chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, evaporative recovery, and solvent extraction are the most commonly used procedures for removing heavy metal ions from aqueous environments (Ucun et al. 2003; Babu et al. 2007; Acheampong et al. 2010). However, these technologies have several disadvantages such as unpredictable rates of metal ion removal, high reagent or energy requirements, and/or generation of toxic sludge, which is often difficult to dewater and requires extreme caution in its disposal. The search for new and innovative treatment technologies has focused attention on the effects of heavy metal toxicity on, and uptake by, microorganisms (Aksu et al. 1997). Using microorganisms as biosorbents for heavy metals offers a potential alternative to existing methods (Igwe and Abia 2006; Malik 2004). With the growing scarcity and increasing economic value of certain metals, this intrinsic property of microorganisms has also given importance to the study of microbial metal recovery.

The use of biomass for heavy metal removal or recovery has gained importance in recent years due to its promising performance and low cost. Among the various sources, both live and inactivated biomass of microorganisms (fungi, algae, bacteria, etc.)

exhibit promising metal-binding capacities. Their complex cell walls contain high concentrations of functional groups including amino, amide, hydroxyl, carboxyl, sulfhydryl, and phosphate, which have been associated with metal binding (Akhtar and Mohan 1995; Gardea-Torresdey et al. 2004). Specific constituents of fungal cell walls, e.g. chitin, have been documented as possessing significant metal binding abilities (Gadd et al. 2001).

Fungi possess many properties that influence metal mobility and toxicity, including the production of metal-binding proteins, organic and inorganic precipitation, active transport, and intracellular compartmentalization.

The uptake of heavy metals by fungi is of industrial relevance (Gadd 1986a). Fungi are well suited for removal of metal ions from wastewater, since they exhibit marked tolerance toward metals and other adverse conditions, e.g., low pH. Fungi have higher capacities of metal binding to cell walls than other microorganisms.

The scientific literature indicates that the use of fungi and other microorganisms as biosorbents for heavy metals offers a potential alternative to existing chemical and physical methods, which possess several disadvantages. It is expected that filamentous fungi of heavy metal-contaminated habitats exhibiting significant tolerance to toxic metals and demonstrating metal-complexing metabolites or activity will serve as efficient biosorbents for heavy metals.

Considering the problem of heavy metal pollution and the importance of fungi as a potential biosorbent for heavy metals, the present chapter discusses the diversity of soil fungi, interactions with metals, and the development and exploitation of metal tolerance. The potential of various fungi in the biosorption of heavy metals from aqueous environments and their future prospects are discussed.

13.2 Soil Fungi and Their Diversity

Fungi are eukaryotic organisms and are ubiquitous members of soil microbial communities. They comprise a varying proportion of the overall biomass in different systems. Fungi tend to dominate in soils containing high proportions of organic matter and of low pH and generally constitute a smaller proportion in intensively managed mineral soils. The fungi are an immensely diverse group of organisms, encompassing a wide range of forms from microscopic single-celled yeasts to large macrofungi, as exemplified by the well-known mushrooms and toadstools and the largest of fruiting bodies, the giant puffball (Bridge and Spooner 2001).

The majority of fungal species occur in the soil environment at some stage in their life cycle. Current knowledge of fungal diversity in soil is based largely on observations of fruiting bodies or cultures, which are obtained from soil isolation studies. Both approaches have limitations for the detection of the true diversity in any chosen environment. Approximately 17% of the known fungal species can be successfully grown in culture. Detecting exactly which fungi are present in a soil sample is not an easy task, one of the major problems being the fastidious nature of the great majority of species (Hawksworth et al. 1995). If the above figure was applied to the

1,200 culturable fungi species occurring in the biosphere as suggested by (Watanabe 1994), then an estimated 7,000 species of fungi may exist in soil. In addition, although some soil fungi can be grown in culture, in many cases it is not possible to germinate resting structures such as spores, so that only vegetative mycelium is available for detailed analysis. Surveys of soil fungal diversity, which were popular during the 1960s and 1970s, have reappeared in the literature with the advent of DNA-based, culture-independent methods of analysis.

Culture-based estimates of soil fungal diversity require considerable effort and taxonomic expertise (Cosgrove et al. 2007). Culture-dependent approaches to characterize microbial communities additionally have built-in biases in the isolation of microorganisms. Recent attempts have been made to develop new culture media to maximize the recovery of diverse microbial groups (Davis et al. 2005; Vieira and Nahas 2005).

Culture-independent methods have recently been used in preference to traditional isolation techniques for microbial community analysis, including denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), or temporal temperature gel electrophoresis (TTGE) methods (Muyzer et al. 1993; Torsvik et al. 1998; Muyzer 1999; Kirk et al. 2004; Kostanjsek et al. 2005). These techniques have proved highly successful in detecting soil microbial composition and diversity, thus providing insight into the response of soil ecosystems to environmental changes or anthropogenic disturbance. The combination of broad spectrum polymerase chain reaction (PCR) detection, coupled with single-strand conformation polymorphisms (SSCP) or DGGE, can provide more accurate answers to fundamental questions regarding ecosystem diversity. This technique does not, however, distinguish between active and resting stages (Bridge and Spooner 2001).

The most commonly isolated culturable soil fungi having significant ecological roles and functions belong to Glomeromycota, Zygomycota, Ascomycota, and Basidiomycota. Mycorrhizae and sugar fungi (zygomycetes) have been widely studied. The Ascomycota are the largest group in terms of number of species (approximately 33,000 plus another 16,000 known only as asexual forms), which span a range of nutritional modes from parasitic and pathogenic to plants, animals, and other fungi, through mutualists and saprotrophs (Kirk et al. 2001). A separate artificial phylum, Deutromycota or Fungi Imperfecti, has been devised for those fungi that lack known sexual reproduction, but the majority comprise asexual relatives of Ascomycota (Seifert and Gams 2001). The most familiar and economically important molds, including *Aspergillus* and *Penicillium,* are asexual forms of Ascomycota. Key literature in the identification of these groups of fungi appear in Gilman (2001), Ellis (1976), Domsch et al. (1993), Klich (2002), Mueller et al. (2004).

Some 20 functions of fungi were described by Christensen (1989), one of the main functions of fungi in soil being primary degraders. Many soil fungi have other roles and interactions, one of the most widely studied being mycorrhizal processes. Mycorrhizal relationships vary widely and may involve direct cross-feeding with plants, aiding in plant seed germination or the prevention of invasion by pathogens through niche exclusion (Brundrett et al. 1996).

Fungi are chemoheterotrophic organisms and are ubiquitous in subaerial and subsoil environments and are considered important decomposers and mutualistic symbionts of animal and plants. They are also pathogens and contribute to spoilage of natural and manufactured materials (Gadd 1993, 1999, 2006; Burford et al. 2003). Fungi also play an important role in the maintenance of soil structure due to their filamentous branching growth habit and exopolymer production. A fungal role in biogeochemical cycling of elements (e.g. carbon, nitrogen, phosphorus, sulfur, and metals) is well known and interlinked with the ability to adopt a variety of growth, metabolic and morphological strategies, their adaptive capabilities to environmental extremes, and their mutualistic associations with animals, plants, algae, and cyanobacteria (Burford et al. 2003; Gadd 2004; Braissant et al. 2004; Fomina et al. 2005a). Free-living fungi also have major roles in the decomposition of plant and other organic materials including cellulose, lignin, and chitin as well as the degradation of xenobiotics and the solubilization of minerals (Gadd 2004, 2005, 2006).

13.3 Heavy Metal Pollution in Water and Soil

Many researchers have reported heavy metal pollution in soil, especially in agricultural lands in different parts of the world (Sun et al. 2009; Fabiani et al. 2009; Yang et al. 2009; Nas et al. 2009). Fossil fuel combustion, mineral mining and processing, and the generation of industrial effluents and sludges, biocides and preservatives release a variety of toxic metal species into aquatic and terrestrial ecosystems, and this can have significant effects on biota (Gadd and Griffiths 1978; Gadd 1992a, 2000c, 2005, 2007b; Wainwright and Gadd 1997; Pokrovsky et al. 2008; Fabiani et al. 2009). Metal-rich habitats also occur due to natural localized ores and mineral deposits, and the weathering of rocks, minerals, soil, and sediments are a vast reservoir of metals. Restoration of metal-polluted habitats requires a functional microbial community for plant community establishment, soil development, and biogeochemical cycling.

Heavy metals are elements having a density over 5 g/cm^3. They are nondegradable and exist in number of inorganic and organic forms. Some heavy metals such as Fe, Cu, and Zn are essential trace elements but others, such as Cd and Pb, have no beneficial biological function and are toxic even in very small amounts. Cadmium, Pb, and Hg are regarded as the most toxic of the heavy metals. Another elemental toxicant, arsenic (As), is sometimes regarded as a heavy metal, although strictly speaking, it is a metalloid.

Contamination of soil and water by heavy metals has significant relevance, because metals cannot be degraded like most organic pollutants and they accumulate in terrestrial, aquatic and marine food chains (Smejkalova et al. 2003; Ortega-Larrocea et al. 2007). Metals such as Cd, Cr, Pb, Hg, As, copper (Cu), zinc (Zn), and nickel (Ni) are continuously being added to soils through agricultural activities such as long-term application of urban sewage sludge, and industrial activities such as waste disposal, waste incineration, and through vehicle exhausts. These sources

cause the accumulation of metals and metalloids in soils and pose threats to food safety and public health due to soil-to-plant transfer of metals.

Metals cause detrimental effects on both aquatic and terrestrial ecosystems and human health due to their mobilities and solubilities which determine their speciation (Kabata-Pendias 1992; Del Val et al. 1999). In some cases, soil may be contaminated to such an extent that it may be classified as a hazardous waste (Berti and Jacob 1996). Soil contamination with heavy metal mixtures is receiving increasing attention from the public as well as governmental agencies, particularly in developing countries (Yanez et al. 2002; Khan 2005).

Cadmium (Cd) is ubiquitous in the environment and has been recognized as one of the most hazardous of the heavy metal pollutants (Robards and Worsfold 1991; Christine 1997). Cadmium readily transfers from soil to food plants through root absorption and accumulates in tissues (Oliver 1997; Ortega-Larrocea et al. 2007), thereby potentially affecting human health (Adriano 1986; Smith 1996; Jose et al. 2002; Yao et al. 2003).

Cadmium concentrations ranging from 0 to 2.6 μg/L in drinking and natural waters have been reported from different parts of the world (Rajaratnam et al. 2002; Ho et al. 2003; Rosborg et al. 2003; Barton 2005; Virkutyte and Sillanpää, 2006; Asante et al. 2007). Natural Cd concentrations in water bodies rarely exceed the WHO guideline value of 3 μg/L (WHO 2006). High Cd concentrations in aquatic ecosystems are often reported in the surroundings of abandoned and active mines and metal smelters (Appleton et al. 2001; Miller et al. 2004; Lee et al. 2005a, b), especially where nonferrous metals are extracted (Florea et al. 2005). Phosphate fertilizers used in agriculture may also be enriched with Cd. Acidification of soil and water may release Cd bound to soil and sediments. Elevated Cd concentrations up to 57 μg/L (Seifert et al. 2000; Rajaratnam et al. 2002; Barton 2005) originating from soldered joints and zinc galvanized plumbing have been reported in tap water when first draw waters were studied. However, most of the households studied usually had acceptable Cd levels; for example, in Germany, Cd concentrations have been reported to exceed the WHO guideline value of 3 μg/L in only 0.7% of samples examined (Seifert et al. 2000).

In natural waters, Cd is distributed into three different fractions: dissolved, bound to suspended particulate matter (SPM), and precipitated/sedimented forms. Cadmium has a strong affinity for particulate matter, and sediments may contain over 100 mg/kg (Appleton et al. 2001; Woo and Choi 2001). As a result, soluble Cd concentrations in water are generally low, although the dissolved Cd fraction may be increased by high concentrations of dissolved organic matter (mainly fulvic and humic acids) (Linnik 2003) and low pH.

Excessive chromium (Cr) is present in the natural environment due to chrome plating and polishing operations, inorganic chemical production, cooling tower and steel mill effluents, and activities at wood-preserving facilities and petroleum refineries (USEPA 1990; Allen et al. 1998).

Chromium wastes pose a serious threat to public health and the environment. The chemical form of chromium (e.g., trivalent versus hexavalent) determines its toxicity, its mobility in the environment, and its availability to microorganisms.

Chromium(VI) has been shown to have carcinogenic, mutagenic and allergenic effects in humans and animals. In contrast, Cr(III) is considered a trace element essential for living systems (Costa 1997; Nies 1999). The toxicity of hexavalent chromium is from 100 to 1,000 times greater than that for the trivalent species (Onta and Hattori 1983; Wyszkowska et al. 2001). Chromium(VI) is toxic to biological systems due to its strong oxidizing potential that can damage cells (Kotas and Stasicka 2000). Within living cells, Cr(VI) complexes with organic compounds, interfering with metalloenzyme systems at high concentrations (Kotas and Stasicka 2000).

Lead contamination from anthropogenic sources is relatively common and high Pb concentrations have been detected in proximity to metal mines and smelters (Miller et al. 2004; Florea et al. 2005; Lee et al. 2005b). Point contamination on a smaller scale also occurs as a result of industrial emissions, agricultural practices, and improper disposal of sewage sludge. Before the introduction of unleaded fuels, the use of leaded gasoline was one of the major sources of Pb pollution to soil. Lead is also released into tap water from pipes, solders, and fittings of old plumbing systems (which may contain up to 50% Pb), and Pb concentrations up to 5,580 μg/L have been detected in tap water (Murphy 1993; Gulson et al. 1994; Gulson et al. 1997; Seifert et al. 2000; Barton et al. 2002; Rajaratnam et al. 2002; Fertmann et al. 2004).

The World Health Organization (WHO 2006) has established a limit of 10 μg/L for Pb in drinking water. Natural Pb concentrations of water bodies are generally low; background concentrations of <0.45–14 μg/L in groundwater have been reported (Smedley et al. 2002). Like Cd, Pb in water is distributed into three different fractions: dissolved, bound to SPM, and precipitated/sedimented. Lead has a strong affinity for particulate matter and, therefore, is mainly present in SPM and sediment fractions, but the dissolved amount is low (Balls 1988; Zarazua et al. 2006). However, Pb bound to SPM and sediments is at least partly reversible and may, therefore, be released to the surrounding water under suitable conditions (Chrastný et al. 2006).

13.4 Metal–Fungi Interactions and Development of Metal Resistance/Tolerance

Metals influence soil fungi by various means; for example, they can diminish total populations, impoverish fungal diversity, alter fungal morphology and physiological activity, and affect growth rate, reproduction processes, and enzyme production (Gadd 1992b, 1993; Martino et al. 2000). The response of *Penicillium* to heavy metals varies over a wide range of concentrations. Both sensitive and extremely resistant fungi of this genus have been reported in the literature. *Penicillium ochrochloron* is reported to grow in a saturated solution of copper sulfate (Stokes and Lindsay 1979). Metal effects vary not only among species and strains of fungi but also among different vegetative and reproductive forms of the same organism (Sabie and Gadd 1990). Fungal survival in the presence of toxic metals depends primarily on intrinsic

biochemical and structural properties, physiological and/or genetic adaptation including morphological changes, and environmental modification of metal speciation, and metal availability and toxicity. The relative importance of each of these is often difficult to determine (Gadd and Griffiths 1978; Gadd 1990, 1992b).

Heavy metal resistance in fungi has been investigated in detail in mutants isolated in the laboratory (Mohan and Sastry 1983) by gradual adaptation on toxic metal ion-containing media or by mutagenesis. A number of metal-resistant fungi isolated from polluted environments have also been reported (Ashida 1965; Gadd 1993; Zafar et al. 2007; Ahmad et al. 2006; Imran 2010); however, the mechanism of resistance in most cases was not studied. Resistance to heavy metals in fungi may be due to either of two mechanisms (1) transport blocks that restrict the entry of toxic metals into the cell and (2) intracellular sequestration into vacuoles or binding to specific proteins, viz. metallothioneins as described by Rao et al. (1997).

Numerous methods have been employed to determine metal tolerance in fungi. In vitro assays include sensitivity to spore germination, mycelia growth extension, and biomass production in the presence of various concentrations of metal salts in liquid and/or solid nutrient medium. These assays have demonstrated a range of levels of tolerance to different metals.

Metal-contaminated soil and wastewater harbor relatively more resistant fungal flora compared with noncontaminated media (Zafar et al. 2007; Ahmad et al. 2006; Ansari and Malik 2010); however, no strict criteria exist for the designation of a particular fungus as metal resistant/tolerant or sensitive. The minimum inhibitory concentration (MIC) of a metal sufficiently higher than MIC50 may be considered as tolerant or resistant.

13.5 Mechanisms of Metal Resistance and Tolerance

Metals and their compounds interact with fungi in various ways depending on metal species, organism, and environment. In addition, fungal metabolic activity can influence metal speciation and mobility.

Metal toxicity is greatly affected by the chemical behavior of the particular metal species, which is often influenced by the physical and chemical properties of the local environment. Metals exert toxic effects in many ways; for example, they can block the functional groups of important biological molecules such as enzymes, displace or substitute for essential metal ions, cause disruption of cellular and organellar membranes, and interact with systems which normally protect against harmful effects of free radicals generated during normal metabolism (Gadd 1992b, 1993; Avery et al. 1996; Howlett and Avery 1997). Fungi possess numerous qualities that influence metal toxicity including the production of metal-binding proteins (e.g., constituents of fungal cell walls [chitin, melanin] have significant metal binding abilities (Gadd and Griffiths 1978; Gadd 1993), organic and inorganic precipitation of metals, and active transport and intracellular compartmentalization. All these mechanisms are highly dependent on the metabolic and nutritional status

of the organism, as these will affect the expression of energy-dependent resistance mechanisms as well as synthesis of wall structural components, pigments, and metabolites, which affect metal availability and organism response (Gadd 1992b, 1993; Ramsay et al. 1999).

Fungi restrict entry of toxic metal species into cells by (1) reduced metal uptake and/or increased metal efflux; (2) metal immobilization, e.g., cell wall adsorption, extracellular precipitation of secondary neo-formed minerals (e.g. oxalates); and (3) extracellular metal sequestration by exopolysaccharides and other extracellular metabolites (Gadd 1993, 2001a, b, c; Macreadie et al. 1994; Blaudez et al. 2000; Perotto and Martino 2001; Baldrian 2003).

Metal-tolerant fungi survive in metal-enriched environments in part due to their abilities of intracellular chelation by the generation of metallothioneins and phytochelatins, and metal localization/sequestration within vacuoles. Fungal vacuoles play an important role in the regulation of cytosolic metal ion concentrations and the detoxification of potentially toxic metals (White and Gadd 1986; Gadd 1993; Gharieb and Gadd 1998; Liu and Culotta 1999). Metals preferentially sequestered by the vacuole include Mn^{2+} (Okorokov et al. 1985; Gadd and Laurence 1996), Fe^{2+} (Bode et al. 1995), Zn^{2+} (White and Gadd 1987), Co^{2+} (White and Gadd 1986), Ca^{2+} and Sr^{2+} (Okorokov et al. 1985; Borst-Pauwels 1989; Gadd 1993; Okorokov 1994), Ni^{2+} (Joho et al. 1995), and the monovalents K^+, Li^+, and Cs^+ (Okorokov et al. 1980; Perkins and Gadd 1993a, b). Recently, other researchers have discussed the mechanisms of metal resistance in soil fungi (Meharg 2003; Bučková et al. 2007; Gonçalves et al. 2007; Richie et al. 2007; Xiao et al. 2008).

13.5.1 Metal Solubilization

Solubilization of metal compounds is an important but unappreciated aspect of fungal physiology for the release of anions such as phosphate and essential metal cations into forms available for intracellular uptake, and transport through biogeochemical cycles. Fungal solubilization of metal compounds, including certain oxides, phosphates, sulfides and mineral ores, occurs by several mechanisms including (1) protonation of the anion of the metal compound, thereby decreasing its availability to the cation with the proton-translocating ATPase of the plasma membrane (production of organic acids being the source of protons) and (2) siderophore production (Gadd 1993, 1999; Sayer et al. 1995).

Organic acid anions are frequently capable of soluble complex formation with metal cations, thereby increasing mobility of the latter (White et al. 1997). Such complexation is dependent on relative concentrations of the anions and metals in solution, pH, and the stability constants of the various complexes (Denevre et al. 1996). A further mechanism of metal solubilization is the production of low-molecular-weight iron-chelating siderophores, which solubilize Fe^{3+}. Siderophores are the most common means of acquisition of Fe by bacteria and fungi and are effective over a wide range of soils, including calcareous soil. The most common fungal siderophore is ferrichrome (Crichton 1991).

Acidification of soil can lead to metal release via a number of obvious routes, e.g., competition between protons and metal in a metal–anion complex or in a sorbed form, resulting in the release of free-metal cations. Heterotrophic metabolism can also lead to leaching as a result of the efflux of organic acids and siderophores. Organic acids supply both protons and metal-complexing anions (Burgstaller and Schinner 1993; Gadd 1999; Gadd and Sayer 2000). Citrate and oxalate anions can form stable complexes with a large number of metals. Many metal citrates are highly mobile and not readily degraded (Francis et al. 1992). Oxalic acid can also act as a leaching agent for those metals that form soluble oxalate complexes, including Al and Fe (Strasser et al. 1994).

In many fungi, an important leaching mechanism occurs through the production of organic acids (e.g., oxalic acid and citric acid) (Adams et al. 1992; Francis et al. 1992; Denevre et al. 1996; Sayer et al. 1997; Gadd 1999, 2000a; Sayer and Gadd 2001; Jarosz-Wilkolazka and Gadd 2003; Fomina et al. 2005a; Ansari 2004; Imran 2010)

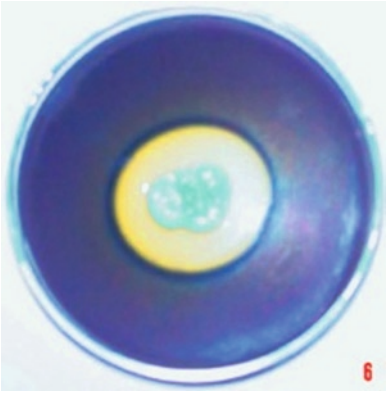

Fig. 13.1 Organic acid production by *Penicillium spp.* in plate (Ansari 2004)

Fig. 13.2 Organic acid production by *Aspergillus spp.* in plate (Ansari 2004)

(Figs. 13.1 and 13.2). Organic acid excretion by fungi is both inter- and intra-specific and can be strongly influenced by the presence of toxic metals (Sayer et al. 1995; Sayer and Gadd 2001; Fomina et al. 2004, 2005c).

13.5.2 Metal Immobilization

Toxic metal species including radionuclides can be bound, accumulated, and precipitated by fungi. Fungal biomass can act as a metal sink by (1) metal biosorption to biomass cell walls, pigments, and extracellular polysaccharides; (2) intracellular accumulation and sequestration (including uptake with complexation to ligands such as sulfur-containing peptides [e.g. metallothioneins] [Gadd 1993; Sarret et al. 1998, 2002; Fomina et al. 2005b]); or (3) precipitation of metal compounds onto and/or around hyphae. Some fungi can precipitate metals in amorphous and crystalline forms, such as oxalates and other secondary mycogenic minerals (Gadd 1999; Burford et al. 2003, 2006).

In addition to immobilizing metals, the above processes reduce the external free metal concentration and drive the equilibria to release more metal ions into soil solution (Gadd 1993, 2000a, b; Sterflinger 2000). Fungi can be highly efficient accumulators of soluble and particulate forms of metals (e.g., Ni, Zn, Ag, Cu, Cd, and Pb), especially from dilute external concentrations (Gadd 1993, 2000a, b, 2001b, c; Baldrian 2003).

13.5.3 Metal Transformations

Metal transformation embraces the mechanisms by which fungi (and other microorganisms) effect changes in metal speciation and mobility. Transformations are essential components of biogeochemical cycles for metals as well as all other elements including carbon, nitrogen, sulfur, and phosphorus. Fungi and other microorganisms transform metals via oxidation, reduction, methylation, and dealkylation (Gadd 1992a). Some enzymatic metal transformations may be involved in survival since certain transformed metal species are less toxic and/or more volatile than the original species. Reductions carried out by fungi include Ag^{+} to metallic Ag^{0} which is deposited in and around cells (Kierans et al. 1991) and Cu^{2+} to Cu^{+} by cell wall-associated compounds in *Debaryomyces hansenii* (Wakatsuki et al. 1988, 1991; Breuer and Harms 2006).

13.6 Biosorption

Biosorption is the process by which metals are sorbed or complexed to either living or dead biomass (Volesky and Holan 1995). Binding of metal ions onto cell walls and other external surfaces in fungal biomass (Gadd 1990, 1993;

Sterflinger 2000) can be an important passive process in concentrating metals in soils and contaminated aquifers (McLean et al. 1996; Berthelin et al. 1995). It has been suggested that stimulating the growth of indigenous microorganisms with metal biosorptive capacities may be a useful strategy for immobilizing metals in soils and preventing contamination of underlying groundwater supplies (Valentine et al. 1996). Furthermore, it is possible to envision a barrier of microorganisms with biosorptive abilities established in subsurface environments to remove metals from groundwater flowing through. Although small-scale bioremediation of mine drainage with biosorption has been documented (Ledin and Pedersen 1996), biosorption has been evaluated primarily as a strategy for removing metals from waste streams. Biosorption may be economically competitive with ion exchange or chemical precipitation for treating some waste streams (Eccles 1995). One strategy to enhance the applicability of biosorption over alternative techniques for metal removal is to survey for novel microorganisms with unique biosorption capacities (Hu et al. 1996; Vesper et al. 1996).

13.6.1 Biosorbents

Adsorptive removal of heavy metals from aqueous effluents, which has received much attention in recent years, is usually achieved using activated carbon or activated alumina (Faust and Aly 1987; Ouki et al. 1997; Hsisheng and Chien-To 1998; Ali et al. 1998; Ralph et al. 1999; Shim et al. 2001; Monser and Adhoun 2002; Igwe and Abia 2005).

Certain biosorbents bind and collect a wide range of heavy metals with no specificity, whereas others are specific for certain types of metals (Hosea et al. 1986; Volesky and Kuyucak 1988). When choosing biomass for metal biosorption experiments, its origin must be taken into account. Biomass can originate from

1. Industrial wastes, which should be obtained free of charge,
2. Organisms readily available in large quantities in nature, and
3. Organisms experiencing rapid growth and cultivated or propagated for biosorption purposes.

Biosorbents prepared from naturally abundant biomass are primarily of algae, fungi, moss, or bacteria that have been killed by washing with acids or bases or both, before drying and granulation (Brierley 1990; Kratochvil et al. 1997). Living or dead fungal biomass and fungal metabolites have been used to remove metal or metalloid species, metal compounds and particulates, radionuclides and organometal compounds from solution by biosorption (Gadd and White 1989, 1990, 1992, 1993; Wang and Chen 2006). These processes are ideally suited for use in bioreactors (Gadd 2000a).

13.6.2 Metal Binding to Cell Walls

The wall is the first cellular site of interaction with external metal species. Metal removal from solution may be rapid, although rates depend on numerous factors such as type of metal ion and biomass, metal concentration and environmental factors such as pH, oxidation–reduction status, presence of competing ions, etc. Metabolism-independent modes of metal binding to fungal walls include ion exchange, adsorption, complexation, precipitation, and crystallization (Mullen et al. 1992).

The fungal cell wall is composed primarily of polysaccharides, some of which may have associated proteins with other components including lipids and melanins. The specific components of the fungal cell wall include the following.

13.6.2.1 Skeletal Elements

Chitin: β-1-4-linked homopolymer of *N*-acetyl-D-glucosamine
β-Glucans: β-1-3-glucan homopolymer composed of D-glucose units with β-1-3- and β-1,6-glucosidic bonds (R-glucan)
Cellulose: β-1,4-linked homopolymer of glucose

13.6.2.2 Matrix Components

α-Glucan: α-1,3-homopolymer of glucose (s-glucan)
α-1,3- and α-1,4-Linked glucan (nigeran)
Glycoproteins
Mannoproteins

13.6.2.3 Miscellaneous Components

Chitosan: β-1,4-polymer of D-glucosamine
D-galactosamine polymers
Polyuronides
Melanins
Lipids

The fungal cell wall thus has important protective properties and so may act as a barrier controlling uptake of solutes, including potentially toxic metal species, into the cell (Gadd and Griffiths 1978; Gadd 1986a, b; Ono et al. 1988) and also indirectly affects the intracellular ionic composition by restricting cellular water.

13.6.3 Transport of Toxic Metal Cations

Most research on metal ion transport in fungi has concerned K^+ and Ca^{2+}, largely because of their importance in fungal growth, metabolism, and differentiation. The transport of toxic metal species is still poorly understood.

Transport systems in cell membranes are usually classified as either carrier or channel systems. In the carrier system, conformational changes in the transport protein are believed to result in alternate exposure of the transport binding site on each side of the membrane. Carriers include all metabolically coupled and H^+-gradient-driven transport systems. Fluxes through such systems saturate with respect to ligand concentration and, if a current is carried, with respect to membrane potential (Sanders 1990).

Ion channels are a class of protein that function as gated pores in the plasma membrane allowing the flow of ions down electrical and/or chemical gradients (Gustin et al. 1986). Channels have higher turnover rates than carriers, 10^{7-8} s^{-1} compared with 10^{2-5} s^{-1}, respectively (Sanders 1990).

13.6.4 Metal Uptake by Living Cells

Penicillium is known to remove a variety of heavy metals from aqueous solution. Spores of *Penicillium italicum* were shown to accumulate Cu (Somers 1963; Kapoor and Viraraghavan 1995). Metal accumulation by growing cells varied with age of the cell. Maximum metal uptake occurred during the lag period, or the early stages of growth and declined as cultures reached a stationery phase. *A. niger*, *P. spinolosum*, and *Trichoderma viride* showed a similar uptake pattern (Townsley and Ross 1985, 1986; Kapoor and Viraraghavan 1995). Other, researchers have also reported metal uptake by living cells (Bayramoglu et al. 2006; Zafar et al. 2007; Melgar et al. 2007; Akhtar et al. 2007; Pakshirajan and Swaminathan 2009).

The uptake of metals by living cells depends on contact time, solution pH, culture conditions, initial metal ion concentration, and the concentration of cells in aqueous solution (Kurek et al. 1982; Galun et al. 1987; Siegel et al. 1987). Huang et al. (1988) observed that Cd biosorption on various fungal strains was pH-sensitive. *Aspergillus oryzae*, *Fusarium solani*, and *Candida utilis* were found to take up higher concentrations of metal in the acidic range. *Mortierella ramannianc, Rhizopus sexualis*, *R. stolonifer*, *Zygorhynchus hetergamus*, *Z. moelleri*, *A. niger*, *Mucor recemosus*, *Penicillium chrysogenum*, and *T. viride* removed Cd from aqueous solutions (Azab et al. 1990; Kurek et al. 1982; Ross and Townsley 1986; Kapoor and Viraraghavan 1995).

13.6.5 Intracellular Fate of Toxic Metals

Both in laboratory and in field studies, it has been shown that the toxicity of a given metal depends on species and chemical properties as well as environmental factors

(e.g., adsorption to solid surfaces, complexation, or precipitation) (Hughes and Poole 1989; Gadd 1992a, b).

Toxic effects include ion displacement and/or substitution of essential ions from cellular sites and blocking of functional groups of important molecules, e.g., enzymes, polynucleotides, and essential transport systems. This results in denaturation and inactivation of enzymes and disruption of cell organelle membrane integrity.

Metal-binding proteins are important in the modulation of intracellular concentrations of both potentially toxic and essential metal ions. The superfamily of proteins called metallothioneins may achieve these by binding the metal ion to cysteine thiolate groups (Hamer 1986).

Polypeptides are designated as "metallothioneins," if they possess specific properties including low molecular mass, high metal content, high cysteine (cys) content, abundant cys-*x*-cys sequences (where *x* is an amino acid other than cys), and metal-thiolate clusters, and lack the aromatic amino acids and histidine.

The following subdivision of metallothioneins into three classes has been recommended (Rauser 1990).

Class I: Polypeptides with locations of cysteine closely related to those in equine renal metallothionein.

Class II: Polypeptides with locations of cysteine only distantly related to those in equine renal metallothionein.

Class III: Typical, nontranslationally synthesized metal thiolate polypeptides.

13.6.6 Metal Transformations Within Fungi

Microbes play key geoactive roles in the biosphere, particularly in the areas of element biotransformations and biogeochemical cycling, metal and mineral transformations, decomposition, bioweathering, and soil and sediment formation. Numerous categorie of microbes, including prokaryotes and eukaryotes, and their symbiotic associations with each other and "higher organisms," can contribute actively to geological phenomena, and central to many such geomicrobial processes are transformations of metals and minerals. Fungi possess a variety of properties that can effect changes in metal speciation, toxicity, and mobility, as well as mineral formation or mineral dissolution or deterioration. Such mechanisms are important components of natural biogeochemical cycles for metals as well as associated elements in biomass, soil, rocks, and minerals, e.g., sulfur and phosphorus, and metalloids, actinides, and metal radionuclides (Gadd 2010).

Fungi are intimately involved in biogeochemical transformations at local and global scales, and although such transformations occur in both aquatic and terrestrial habitats, it is the latter environment where fungi probably have the greatest influence. Within terrestrial aerobic ecosystems, fungi may exert an especially profound influence on biogeochemical processes, particularly when considering soil, rock and mineral surfaces, and the plant root–soil interface. The geochemical transformations that take place can influence plant productivity and the mobility of toxic elements and

substances and are therefore of considerable socioeconomic relevance, including human health. Of special significance are the mutualistic symbioses, lichens, and mycorrhizas. Some fungal transformations have beneficial applications in environmental biotechnology, e.g., in metal leaching, recovery and detoxification, and xenobiotic and organic pollutant degradation. They may also cause adverse effects when these processes are associated with the degradation of foodstuffs, natural products, and building materials, including wood, stone, and concrete (Gadd 2007a).

13.6.7 Metal Sorption by Dead Cells

The biosorption capacity of dead cells may be greater to, equivalent to, or less than that of living cells. Recently, several researchers reported metal removal by dead microbial cells (Naeem et al. 2006; Chen and Wang 2007; Akhtar et al. 2007; Bishnoi et al. 2007; Tsekova et al. 2007).

Use of dead biomass in industrial applications offers certain advantages over that of living cells. Systems using living cells are likely to be more sensitive to metal-ion concentration (i.e., toxicity effects) and adverse operating conditions (pH and temperature). Furthermore, a constant, regulated nutrient supply is required for systems using living cells (with increased operating cost for the management of waste streams), and recovery of metal and regeneration of biosorbent are more complicated for living cells.

In such a biosorption system cells can be killed by physical treatment methods using heat (Siegel et al. 1987), autoclaving, and vacuum drying (Tobin et al. 1984; Huang et al. 1988), or chemicals such as acids, alkalis, and detergents (Tsezos and Volesky 1981; Ross and Townsley 1986; Huang et al. 1988; Rao et al. 1993; Kapoor and Viraraghavan 1995).

13.6.8 Mechanism of Biosorption

Veglio and Beolchini (1997) described biosorption mechanisms on the basis of cellular metabolism (i.e., metabolism-dependent and metabolism-independent) and according to location where the removed metal is located.

The process of biosorption may be classified as follows:

1. Extracellular accumulation/precipitation
2. Cell surface sorption/precipitation
3. Intracellular accumulation

13.6.8.1 Extracellular Accumulation/Precipitation

Some prokaryotic (bacteria and archaea) and eukaryotic (algae and fungi) microorganisms produce or excrete extracellular polymeric substances (EPS) such as polysaccharides,

glycoprotein, lipopolysaccharides, soluble peptides, etc. These substances possess a substantial quantity of anionic functional groups which can adsorb metal ions. Research published on metal biosorption with EPS focuses mainly on the bacteria, such as *Bacillus megaterium*, *Acinetobacter, Pseudomonas aeruginosa,* sulfate-reducing bacteria (SRB), cyanobateria, or activated sludge (Liu et al. 2001), whereas EPS studies for fungi and algae are limited (Wang and Yang 1996); Flemming and Wingender (2001) discovered that the initial rate of Pb^{2+} uptake by live cells of *Saccharomyces cerevisiae* is lower than that of dead cells, while in the case of *A. pullulans*, both the capacity and the initial rate of Pb^{2+} accumulation in live cells are greater than those in dead cells. This result was due to the presence of EPS in live *A. pullulans*.

13.6.8.2 Cell Surface Sorption/Precipitation

Numerous chemical groups have been suggested to contribute to metal biosorption either by whole organisms such as algae (Crist et al. 1981; Greene et al. 1987) and bacteria (Brierley 1990; Mann 1990) or by molecules such as biopolymers (Hunt 1986; Macaskie and Dean 1990). These groups comprise hydroxyl, carbonyl, carboxyl, sulfhydryl, thioether, sulfonate, amine, imine, amide, imidazole, phosphonate, and phosphodiester groups. The importance of any given group for biosorption of a particular metal by particular biomass depends on factors including:

1. The number of sites occurring within the biosorbent material
2. The accessibility of the sites
3. The chemical state of the site (i.e. availability)
4. Affinity between site and metal (i.e. binding strength)

The cell wall consists of a variety of polysaccharides and proteins and hence offers a number of active sites capable of binding metal ions (Kuyucak and Volesky, 1989). Thus, it is regarded as a complex ion exchanger, similar to a commercial resin. Differences in cell wall composition among different groups of microorganisms, viz. algae, bacteria, cyanobacteria and fungi, and intra group differences can thus result in significant differences in type and amount of metal ion binding (Horikoshi et al. 1981; Friis and Myers-Keith 1986; Muraleedharan et al. 1991). The various groups involved in metal binding have been discerned by modification/blocking of the groups (Tobin et al. 1990).

The cell wall tends to be the first cellular structure to come into contact with metal ions, excluding a possible extracellular layer mainly related to bacterial cells. Two basic mechanisms of metal uptake by the cell wall are (1) stoichiometric interaction between functional groups of the cell wall including phosphate, carboxyl, and amine as well as phosphodiester and (2) physicochemical inorganic deposition via adsorption or inorganic precipitation.

Other mechanisms such as complexation, ion exchange, adsorption (by electrostatic interaction or van der Waals force), inorganic microprecipitation, oxidation, and/or reduction have been proposed to explain metal sorption by organisms (Volesky 1990a, b; Liu et al. 2002).

Kapoor and Viraraghavan (1997) showed that in dried fungal biomass of *A. niger*, amine and carboxyl groups were important functional groups involved in Pb, Cd, and Cu biosorption, and they reported that phosphate groups and the lipid fraction of the biomass did not play a significant role in biosorption of the metal ions studied.

Brady and Tobin (1995) found that the total metal ions displaced accounted for only a small portion of the metal ions taken up in the biosorption of metal ions by freeze-dried *R. arrhizus***.** This indicates that ion exchange is neither the sole nor the main mechanism for metal biosorption by fungi. However, Davis et al. (2003) believed ion exchange was the main mechanism for metal ion uptake by brown algae.

Precipitation and redox reactions of heavy metals on the cell surface are also reported by many researchers. A research group at Xiamen University in China found that precious metal ions such as Pd^{2+} (Liu et al. 2003; Xie et al. 2003a), Pt^{4+} (Xie et al. 2003b), Au^{3+} (Lin et al. 2005), Ag^{+} (Lin et al. 2001), and Rh^{3+} (Lin et al. 2001) were unexceptionally bound to the cell wall of yeast and then reduced in situ to the corresponding solids.

Biosorption of heavy metals often involves many mechanisms. Kratochvil et al. (1998) proved that the maximal uptake of Cr^{6+} by protonated *Sargassum* biomass at pH 2 was due to simultaneous anion exchange and the reduction of Cr^{6+} to Cr^{3+}.

13.6.8.3 Intracellular Accumulation/Precipitation

Metal transport across the cell membrane results in intracellular accumulation, which is dependent on cellular metabolism. This implies that this mode of biosorption may take place only within viable cells (Veglio and Beolchini 1997).

After entering the cell, metal ions are compartmentalized into different subcellular organelles (e.g. mitochondria and vacuoles). Vijver et al. (2004) summarized metal ion accumulation strategies, in particular the internal compartmentalization strategies. The mechanism primarily relates to the presence of low-molecular-weight metal-binding proteins, the metallothioneins (MT), which are cysteine-rich and occur in the animal kingdom, plants, eukaryotic microorganisms, and some prokaryotes. MT can be induced by many substances, including heavy metal ions such as Cd, Cu, Hg, Co, and Zn (Vijver et al. 2004).

In addition to MT, other cellular thiols influencing the sensitivity to toxic metals include glutathione (GSH), phytochelatins (β cadystins (α-Glu-Cys) nGly), and labile sulfide (Perego and Howell 1997; Gharieb and Gadd 2004). Tripeptide glutathione (GSH) is a typical low-molecular-weight cellular thiol and functions as a storage form of endogenous sulfur and nitrogen as well as for detoxification of metal ions. GSH in *S. cerevisiae* may account for 1% of cell dry weight (Gharieb and Gadd 2004). The role of the vacuole in the detoxification of metal ions was investigated by Ramsay and Gadd (1997), who showed that a vacuole-deficient strain displayed much higher sensitivity and a lower biosorption capacity for Zn, Mn, Co, and Ni.

Many genes involved in the tolerance to uptake or detoxification of metal ions have been identified (Rosen 2002). For example, the *S. cerevisiae* Arr4p plays an important role in the tolerance to As^{3+}, As^{5+}, Co^{2+}, Cr^{3+}, Cu^{2+}, and VO_4^{3-} (Shen et al. 2003). Genetic technologies, including cell surface display technologies have been applied to improve the performance of biomass in metal removal from solution (Bae et al. 2003; Kuroda et al. 2002; Wang 2005). Kuroda et al. (2002) constructed a cell surface-modified yeast *S. cerevisiae*, which produces histidine hexapeptide. This engineered yeast can chelate Cu ion and possesses the property of self-aggregation, which indicates the potential application for bioremediation of heavy metal pollution.

13.6.9 Factors Affecting Heavy Metal Biosorption

Bioremoval of a heavy metal using microorganisms is affected by several factors, including the specific surface properties of the microorganism and the physicochemical parameters of the solution such as temperature, pH, metal ion concentration, metal solubility, metal valence, concentration of complexing agents, and particle size (Brown and Lester 1979). Butter et al. (1998) showed temperature variations from 15 to 35°C did not affect Cd sorption by dead *Streptomyces* biomass. Also, Kasan (1993) found that the complexation/removal of Cr, Pb, and Zn by living activated sludge was independent of temperature.

Several studies are reported in the literature which have investigated the effect of pH on biosorption of metals. Most investigators have reported negligible metal sorption at pH values <4.0 (Tien and Huang 1987; Delgado et al. 1998; Wang et al. 1999). These results could be explained by the competition between hydrogen ions and metal ions for the sorption sites of cells. At very low pH values, metal cations and protons compete for binding sites on cell walls, which results in lower metal uptake. Biosorbent concentration has also been shown to be an important factor in the biosorption process. Metal uptake increased when biomass concentration decreased (Esposito et al. 2001); as an increase in biomass concentration leads to interference between binding sites (Veglio et al. 1997; Esposito et al. 2001).

Another factor affecting biosorption is initial metal concentration. It has been reported that adsorption rate increases with increasing initial metal concentration. For example, adsorption of Fe^{2+}, Pb^{2+}, and Cd^{2+} by *S. leibleini* increased with increasing initial metal ion concentrations up to 150 mg/L. At high concentrations, the adsorption rates did not change (Ozer et al. 1999).

13.6.9.1 Biomass Pretreatment Effect on Biosorption

Living cells have been pretreated using physical and chemical methods to increase metal biosorption capacity. Physical pretreatment methods have included heat treatment, autoclaving, freeze-drying, and boiling. Chemical pretreatment methods such

as reacting cells, especially fungal cells, with acids, alkaline and organic chemicals have been reported (Wase and Forster 1997; Kapoor and Viraraghavan 1998; Zhao and Duncan 1998).

Yan and Viraraghavan (2000) studied the effect of pretreatment of *Mucor rouxii* biomass on bioadsorption of Pb^{2+}, Cd^{2+}, Ni^{2+}, and Zn^{2+}. Pretreatment with detergent and alkalis such as NaOH, Na_2CO_3, and $NaHCO_3$ improved or maintained the bioadsorption capacity in comparison with live *M. rouxii* biomass. Acid pretreatment using HCl, H_2SO_4, and $C_2H_4O_2$ resulted in a significant reduction in bioadsorption capacity. To improve the bioadsorption capacity for metal ions by dead biomass, alkali pretreatment was an effective method (Yan and Viraraghavan 2000).

Bai and Abraham (2002) reported that the treatment of the biosorbent with mild alkalies (0.01 N NaOH and ammonia solution) and formaldehyde (10% w/v) deteriorated biosorption efficiency. However, extraction of biomass powder in acids (0.1 N HCl and H_2SO_4), alcohols (50% v/v, CH_3OH and C_2H_5OH), and acetone (50% v/v) improved Cr uptake capacity. Reaction of cell wall amino groups with acetic anhydride reduced biosorption potential drastically. Blocking of COOH groups by treatment with water-soluble carbodiimide resulted in an initial lag in Cr binding. Biomass modification experiments conducted using cetyl trimethyl ammonium bromide (CTAB), polyethylenimine (PEI), and amino propyl trimethoxy silane (APTS) improved biosorption efficiency to exceptionally high levels.

13.7 Biosorption Potential of Fungal Biomass

Heavy metal biosorption potential of different treatment fungi are known and vary greatly. This variation is probably due to the different methods used and types of biomass and their pretreatment (Table 13.1). Heavy metal biosorption preference by various fungi in single/multimetal solutions is also variable as reported by various workers (Table 13.2).

Sorption of Pb by nonliving *P. chrysogenum* biomass was strongly affected by pH (Niu et al. 1993). Within a pH range of 4–5, the saturated sorption uptake of Pb^{2+} was 116 mg/g dry biomass, higher than that of activated charcoal and other microorganisms. At pH 4.5, *P. chrysogenum* biomass exhibited sorption preference for metals in the following order: Pb > Cd > Cu > Zn > As. Sorption of Pb^{2+} remained unchanged in the presence of Cu^{2+}, and As^{3+} decreased in the presence of Zn^{2+} and increased in the presence of Cd^{2+}. Volesky and May-Phillips (1995) found that living and nonliving biomass of *S. cerevisiae* differs in uptake of uranium (U), Zn, and Cu at the optimum pH of 4–5. Dead cells of *S. cerevisiae* removed approximately 40% more U or Zn than corresponding live cultures. The maximum Pb biosorption capacity at pH 6 of *M. rouxii* was estimated at 769 mg/g dry biomass, significantly higher than that of most microorganisms. Biomass of *M. rouxii* showed specific selectivity for Pb^{2+} over Zn^{2+}, Ni^{2+}, and Cu^{2+} (Lo et al. 1999). Bai and Abraham (2001) reported that the optimum pH for biosorption of Cr^{6+} was 2.0. Adsorption capacity of biomass increased with increasing concentration

Table 13.1 Heavy metal biosorption potential of different fungi

Biosorption capacity (mg/g)	Biosorbent	Treatment	Metals	References
0–10	*Mucor rouxii*	Immobilized	Pb, Cd, Ni, Zn	Yan and Viraraghavan (2001)
	Aspergillus foetidus		Cr	Prasanjit and Sumathi (2005)
	Aspergillus spp.		Cr, Cd	Zafar et al. (2007)
	Mucor rouxii	Dead	Ni, Zn	Yan and Viraraghavan (2003)
	Rhizopus spp	–	Cr, Cd	Zafar et al. (2007)
	S. cerevisiae	Immobilized	Pb	Zhang et al. (2009)
11–20	*Aspergillus flavus*	Dead	Pb, Cu	Akar and Tunali (2006)
	Mucor rouxii	Dead	Cd	Yan and Viraraghavan (2003)
	Penicillium chrysogenum		Ni	Tan and Cheng (2003)
	Penicillium spp	Dead	Cr, Ni, Cd	Ahmad et al. (2006)
	Aspergillus niger	Dead	Cr, Cd	Ahmad et al. (2006)
	Trichoderma viride	Immobilized	Cr	Bishnoi et al. (2007)
	Rhizopus oryzae	Living	Cu	Bhainsa and D'souza (2008)
	Aspergillus niger	Free	Cu	Tsekova et al. (2010)
21–30	*Mucor rouxii*	Dead	Pb	Yan and Viraraghavan (2003)
	Penicillium canescens		As	Say et al. (2003a)
	Penicillium chrysogenum		Cr, Zn	Tan and Cheng (2003)
	Aspergillus niger	Dead	Ni	Ahmad et al. (2006)
	Aspergillus niger		Cu	Mukhopadhyay et al. (2007)
	Rhizopus arrhizus	Dead/living	Cu	Subudhi and Kar (2008)
31–50	*Funalia trogii*	Immobilized (live)	Zn	Yakup et al. (2004)
	Penicillium canescens		Hg	Say et al. (2003b)
	Penicillium cyclopium		Cu	Ianis et al. (2006)
	Penicillium purpurogenum		Cr	Say et al. (2003b)
	Aspergillus niger	Immobilized	Cu	Tsekova et al. (2010)

(continued)

Table 13.1 (continued)

Biosorption capacity (mg/g)	Biosorbent	Treatment	Metals	References
51–100	*Funalia trogii*	Immobilized (heat inactivated)	Zn	Yakup et al. (2004)
	Phanerochaete chrysosporium	Resting cells	Ni, Pb	Ceribasi and Yetis (2001)
	Penicillium simplicissimum		Cd, Zn, Pb	Fan et al. (2008)
	Aspergillus niger	Free/immobilized	Cd	Tsekova et al. (2010)
100–200	*Funalia trogii*	Immobilized (live/heat inactivated)	Cd	Yakup et al. (2004)
	Penicillium canescens		Cd	Say et al. (2003b)
More than 200	*Funalia trogii*	Immobilized (live/heat inactivated)	Hg	Yakup et al. (2004)
	Penicillium canescens		Pd	Say et al. (2003b)

Table 13.2 Heavy metal biosorption preference by various fungi in single/multimetal solutions

pH	Biosorbent	Biosorption preference/order	References
0–2	*Aspergillus niger*	Cr^{6+}	Chhikara and Dhankhar (2008)
2–4	*Trichoderma viride* (immobilized)	Cr^{6+}	Bishnoi et al. (2007)
4–6	*Penicillium chrysogenum*	Pb > Cd > Cu > Zn > As	Niu et al. (1993)
	Sacchromyces cerevisiae	U > Zn > Cd > Cu	Volesky and May-Phillips (1995)
	Baker's yeast (nonliving)	Zn > Cd > U > Cu	Volesky and May-Phillips (1995)
	Baker's yeast (living)	Zn > Cu = Cd > U	Volesky and May-Phillips (1995)
	Mucor rouxii	Pb > Zn > Cd > Ni	Yan and Viraraghavan (2003)
	Aspergillus flavus (heat inactivated)	Pb > Cu	Akar and Tunali (2006)
	Phanerochaete chrsosporium	Cd > Cu	Pakshirajan and Swaminathan (2009)
6–8	*Mucor rouxii*	Pb > Zn > Cd > Ni	Yan and Viraraghavan (2003)
	Aspergillus niger	Ni	Amini et al. (2009)
	Funalia trogii (immobilized live)	Hg^{2+} > Cd > Zn	Yakup et al. (2004)
	Sacchromyces cerevisiae	Ni > Cd	Fereidouni et al. (2009)

of ions, temperature, and agitation speed, and optimum sorption capacity was determined at 45°C and 120 rpm.

Biosorption in the order Cu > Zn > Cd was observed in *Microcystis sp*. in single-, bi-, and trimetallic combinations. The order of inhibition of Cu, Zn, and Cd biosorption in bi- and trimetallic combinations suggested possible screening or competition of the binding sites on cell surfaces (Pradhan and Rai 2001). Yan and Viraraghavan (2001) studied the biosorption capacity of *M. rouxii* biomass and immobilized it in a polysulfone matrix. For single-component metal solutions, the metal removal capacities of the beads for Pb, Cd, Ni, and Zn were 4.06, 3.76, 0.36, and 1.36 mg/g, respectively. For a multicomponent metal solution, the capacities were 0.36, 0.31, and 0.40 mg/g for Cd, Ni, and Zn, respectively. Say et al. (2001) studied the biosorption of Cd^{2+}, Pb^{2+}, and Cu^{2+} from artificial wastewaters onto dry biomass of *Phanerochaete chryosporium* in the concentration range of 5–500 mg/L. Maximum absorption of metal ions on fungal biomass was obtained at pH 6.0. The experimental biosorption data for Cd^{2+}, Pb^{2+}, and Cu^{2+} ions were in good agreement with those calculated by the Langmuir model.

Immobilized mycelia of *Rhizopus delemar* showed an occasional increase in uptake compared with that of free cells. Metal ion accumulation from a mixed solution decreased slightly for Co and Fe and decreased considerably for Cu ions. Metal uptake was examined in immobilized column experiments; >92% heavy metal removal was achieved from a mixed solution during five cycles (Tsekova and Petrov 2002). Similarly, Yan and Viraraghavan (2003) reported that biosorption by dead biomass of *M. rouxii* was reduced in the presence of other metal ions but total biosorption capacity increased, indicating the capability of *M. rouxii* biomass in adsorbing multimetal ions. *A. niger* removed >98% Ni from a liquid medium after 100 h growth but did not remove other metals (Magyarosy et al. 2002).

Tan and Cheng (2003) used alkaline pretreatment of *P. chrysogenum* to remove proteins and nucleic acids from cells, thus increasing adsorption capacities from 18.6 to 27.2 mg/g for Cr^{3+}, from 13.2 to 19.2 mg/g for Ni^{2+}, and from 6.8 to 24.5 mg/g for Zn^{2+}. Yakup et al. (2004) measured maximum adsorption of metals on calcium alginate and both live and inactivated immobilized fungal preparations of *Funalia trogii* at pH 6.0. Metal biosorption capacities of heat inactivated-immobilized *F. trogii* for Hg^{2+}, Cd^{2+}, and Zn^{2+} were 403.2, 191.6, and 54.0 mg/g, respectively, while biosorption capacities of the immobilized live cells was 333.0, 164.8, and 42.1 mg/g, respectively. The same order of affinity on a molar basis was observed for single- or multi-metal ions.

Heat inactivated biomass of *Aspergillus flavus* showed maximum biosorption values of 13.5 mg/g for Pb^{2+} and 10.8 mg/g for Cu^{2+} at pH 5.0 with an equilibrium time of 2 h. The results indicated that *A. flavus* is a suitable biosorbent for removal of Pb^{2+} and Cu^{2+} ions from aqueous solution (Akar and Tunali 2006). Ahmad et al. (2006) reported that alkali-treated, dried, and powdered mycelia of metal-tolerant fungal isolates of *Aspergillus* and *Penicillium* have high biosorption capacities for Cr, Ni, and Cd. Biosorption of all metals was found to be higher at 4 mM initial metal concentration when compared with that at 2 and 6 mM. At 4 mM initial metal concentration, Cr biosorption was 18.1 and 19.3 mg/g of *Aspergillus* and *Penicillium* biomass, respectively. Similarly, biosorption of Cd and Ni ions was maximal at 4 mM initial metal concentration by *Aspergillus* (19.4 mg/g for Cd and 25.1 mg/g for Ni) and *Penicillium* (18.6 mg/g for Cd and 17.9 mg/g for Ni). Dried mycelial biomass of Co-resistant fungi belonging to *Mortierella* isolated from serpentine soil of Andaman (India) removed almost 50% of 4.0 mM Co from aqueous solution (Pal et al. 2006). The metal biosorption capacity of the isolate accelerated with increasing Co concentration, while the reverse occurred with increased initial biomass. The optimum pH and temperature for Co^{2+} removal were 7.0 and 30°C, respectively. Co^{2+} uptake was inhibited in the presence of other metals (Pb, Cd, Cu, Ni, Cr, and Zn), however (Pal et al. 2006). Untreated, heat- and alkali-treated Lentinus sajor-caju (white rot fungus) mycelia were used for the recovery of U from aqueous solution by Bayramoğu et al. (2006). He reported that the alkali-treated form had a high biosorption capacity (378 mg/g) compared with 268 mg/g for untreated and 342 mg/g for heat-treated fungal mycelia. Optimum biosorption was observed at pH 4.5 for all the tested fungal preparations and was independent of temperature (5–35°C). Naeem et al. (2006) studied H^+, Cd, Pb, Sr, and Zn adsorption onto *S. cerevisiae*. They modeled the acid/base properties of the fungal cell wall by invoking a nonelectrostatic surface complexation model with four discrete surface organic acid functional group types, with average *p*Ka values of 3.4 ± 0.4, 5.0 ± 0.2, 6.8 ± 0.4, and 8.9 ± 0.6. The affinity of the fungal cells for the metal ions followed the trend: Pb > Zn > Cd > Sr. The authors used the metal adsorption data to determine site-specific stability constants for the important metal fungal surface complexes. Their results showed that *S. cerevisiae* may represent a novel biosorbent for the removal of heavy metal cations from aqueous waste streams. Pokhrel and Viraraghavan (2006) reported potential removal of As from an aqueous solution by nonviable fungal biomass of *A. niger* coated with Fe. *A. niger* biomass coated with

iron oxide showed maximum removal (approximately 95% of As^{5+} and 75% of As^{3+}) at pH 6. No strong relationship was observed between the surface charge of the biomass and As removal.

Biosorption of Zn, Cu, Hg, Cd, or Pb by living or nonliving biomass of *A. macrosporus* from an acid solution, acid solution supplemented with P and K, and an alkaline solution showed maximum uptake of all metals (Cu 96% and Pb 89%) at alkaline pH. With living biomass, metal biosorption was greater and more rapid in P/K-supplemented acid media than in nonsupplemented acid media (Melgar et al. 2007). Zafar et al. (2007) observed in vitro Cr and Cd biosorption capacity among fungi isolated from wastewater-treated soil, which belonged to genera *Aspergillus*, *Penicillium*, *Alternaria*, *Geotrichum*, *Fusarium*, *Rhizopus*, *Monilia*, and *Trichoderma*. Maximum biosorption of Cr and Cd ions was detected at 6 mM initial metal concentration. *Aspergillus* sp.1 accumulated 1.2 mg of Cr and 2.7 mg Cd/g of biomass. Accumulation of these metals by metal-tolerant *Aspergillus* sp.2 isolate was at par with relatively less tolerant *Aspergillus* sp.1 isolate. *Rhizopus* sp. accumulated 4.3 mg of Cr and 2.7 mg Cd/g of biomass. These findings indicate promising capabilities for biosorption of Cd and Cr by *Rhizopus* and *Aspergillus* spp. from aqueous solution. There is little, if any, correlation between metal tolerance and biosorption properties of the test fungi.

Bishnoi et al. (2007) reported that the biosorption efficiency of powdered *T. viride* biomass entrapped in a polymeric matrix of calcium alginate compared with that of cell-free calcium alginate beads. Biosorption of Cr^{6+} was pH-dependent and maximum adsorption (16.1 mg/g) was observed at pH 2.0. The maximum adsorption capacity was observed at a dose of 0.2 mg in 100 ml of Cr^{6+} solution. The experimental results were fitted satisfactorily to both Langmuir and Freundlich isotherm models. The hydroxyl (–OH) and amino (–NH) functional groups were responsible for the biosorption of Cr^{6+} with fungal biomass.

Fungal strain *T. harzianum* was found to be a comparatively better candidate for uranium biosorption than algae. The process was highly pH-dependent. At optimized experimental parameters, the maximum uranium biosorption capacity of *T. harzianum* was 612 mg U g^{-1}, whereas maximum values of uranium biosorption capacity exhibited by algal strains (RD256 and RD257) were 354 and 408 mg U g^{-1}, respectively, and much higher in comparison with commercially available resins (Dowex-SBR-P and IRA-400). Uranium biosorption by algae followed the Langmuir model while fungus exhibited a more complex multilayer phenomenon of biosorption and followed pseudo-second-order kinetics. Mass balance studies revealed that uranium recovery was 99.9% for *T. harzianum*, and 97.1 and 95.3% for RD256 and RD257, respectively, by 0.1 M hydrochloric acid, which regenerated the uranium-free cell biomass facilitating the sorption–desorption cycles for better economic feasibility (Akhtar et al. 2007)

Das and Guha (2007) found biomass of *Termitomyces clypeatus* (TCB) to be the most effective for biosorption of all fungal species tested. Sorption of Cr^{6+} by live TCB depends on pH of the solution, with the optimum pH value being 3.0. The biomass amino, carboxyl, hydroxyl, and phosphate groups chemically interacted with the chromate ion forming a cage-like structure as depicted by scanning electron microscopic

(SEM) and Fourier-transformed infrared spectroscopic (FTIR) results. Desorption and FTIR studies also showed that Cr^{6+} was reduced to trivalent chromium on binding to the cell surface. The maximum Pb^{2+} biosorption capacity of *Aspergillus parasiticus* was found to be 4.02×10^{-4} mol/g at pH 5.0 and 20°C in a batch system. The biosorption equilibrium was reached in 70 min (Akar et al. 2007).

Chen and Wang (2007) used waste biomass of *Saccharomyces* as a biosorbent to react with ten metal ions, and maximum biosorption capacity (q (max)) was determined by the Langmuir isotherm model. They reported that values of q (max) decreased in the following order (in mM/g): Pb^{2+} (0.413) > Ag^{+} (0.385) > Cr^{3+} (0.247) > Cu^{2+} (0.161) > Zn^{2+} (0.148) > Cd^{2+} (0.137) > Co^{2+} (0.128) > Sr^{2+} (0.114) > Ni^{2+} (0.108) > Cs^{+} (0.092). It is suggested that the greater the covalent index value of the metal ion, the greater the potential to form covalent bonds with biological ligands such as sulfhydryl, amino, carboxyl, hydroxyl, etc. on the biomass surface and the higher the metal ion biosorption capacity. Fan et al. (2009) studied the isotherms, kinetics, and thermodynamics of Cd^{2+}, Zn^{+2}, and Pb^{2+} biosorption by *Penicillium simplicissimum* in a batch system. The effects of pH, initial metal ion concentration, biomass dose, contact time, temperature and presence of co-ions on biosorption were studied. The results of the kinetic studies at different temperatures showed that adsorption rate followed pseudo-second-order kinetics. The thermodynamic constants ΔG^{o}, ΔH^{o}, and ΔS^{o} of the adsorption process showed that biosorption of Cd^{2+}, Zn^{+2}, and Pb^{2+} ions on *P. simplicissimum* were endothermic and spontaneous.

The quantity of metals retained through bioaccumulation by fungal strains *Penicillium sp.* A1 and *Fusarium* sp. A19 and of a consortium of the two types of strains (A1+A19) was significantly higher than that through biosorption by these fungi. The highest quantities of Cd, Cu, and Zn accumulated by fungal biomass was obtained in the presence of $Cd^{2+} + Cu^{2+} + Zn^{2+}$ in potato dextrose agar compared with the individual A1 or A19 used in PDB. A1+A19 accumulated greater quantities of Cu and Pb in the presence of $Cd^{2+} + Cu^{2+} + Pb^{2+}$ and greater quantities of Pb in the presence of $Cd^{2+} + Cu^{2+} + Zn^{2+} + Pb^{2+}$. There was no simple relationship between metal biosorption by fungal biomass and fungal metal tolerance. The biomass of A1 + A19 cultivated in PDB absorbed greater quantities of metals than A1 or A19 in the presence of single metals and their combinations (Pan et al. 2009). The results suggest that the applicability of growing fungi tolerant to heavy metals provides a potential biotechnology system for the treatment of wastewaters contaminated with heavy metals (Pan et al. 2009).

Effect of biosorbent dosage, initial solution pH and initial Ni^{2+} concentration on uptake of Ni^{2+} by NaOH-pretreated biomass of *A. niger* from aqueous solution was investigated by Amini et al. (2009). Optimum Ni^{2+} uptake (4.8 mg Ni^{2+}/g biomass, 70.3%) was achieved at pH 6.25, biomass dosage 2.98 g/L, and initial Ni^{2+}concentration 30.0 mg/L Ni^{2+}. Langmuir and Freundlich isotherms described the biosorption fairly well; however, the prediction of Ni^{2+} biosorption using Langmuir and Freundlich isotherms was relatively poor in comparison with response surface methodology (RSM) approaches. Pakshirajan and Swaminathan (2009) studied biosorption of Cu^{2+} and Cd^{2+} by live *Phanerochaete chrysosporium*

immobilized by growing onto polyurethane foam in individual packed bed columns over two successive cycles of sorption–desorption. Initial pH and metal concentrations in their respective solutions were set to optimal levels (4.6 and 35 mg/L in the case of Cu and 5.3 and 11 mg/L for Cd). The breakthrough curves obtained for the two metals during sorption in both cycles exhibited a constant pattern at various bed depths in the columns. The maximum yield of the columns in removing these metals was found to be 57 and 43% for Cu and Cd, respectively. Recovery values of the sorbed Cu and Cd from the respective loaded columns using 0.1 N HCl as eluant exceeded 65 and 75%, respectively, at the end of desorption in both the cycles. In recent years, Tsekova et al. (2010) reported biosorption of Cu^{2+} and Cd^{2+} from aqueous solution by free and immobilized biomass of *A. niger*. Tsekova et al. (2010) study investigated the ability of *A. niger* resting cells entrapped in a polyvinyl alcohol (PVA) network to remove Cu^{2+} and Cd^{2+} from single-ion solutions. The performance of free and immobilized biosorbent was evaluated by equilibrium and kinetic studies. The PVA-immobilized fungal biosorbent removed Cu^{2+} and Cd^{2+} rapidly and efficiently with maximum metal removal capacities of 34.1 and 60.2 mg/g, respectively. These values of metal uptake at equilibrium were higher than the quantity of Cu^{2+} and Cd^{2+} removed by free biomass (17.6 and 69.4 mg/g, respectively). Biosorption equilibrium data were best described by Langmuir isotherm models. The biosorption kinetics followed the pseudo-second-order model and intraparticle diffusion equation. The results obtained suggest that the immobilized biosorbent holds great potential for wastewater treatment applications.

Based on the above literature search, it is concluded that there is sufficient scientific data on the potential exploitation of fungal biomass for heavy metal removal from aqueous solutions. Therefore, further efforts should be focused on the development of specific technologies for metal removal and recovery from fungal biomass systems. More data are needed to assess the factors influencing metal removal in wastewater treatment systems and to build upon these issues.

13.8 Conclusions

Many modes of nonactive metal removal by microbial biomass are documented. Any one or a combination can be functional in immobilizing metallic species on biosorbents. Soil fungi seem to be well adapted to metals and could effectively be used as a metal biosorbent, either in living, dead and/or immobilized states. Metal tolerance appears to be an added advantage when using live cells for metal removal. A number of anionic ligands participate in metal removal: phosphoryl, carbonyl, sulfhydryl, and hydroxyl groups can all be active to various degrees in binding the metal. Due to the accumulated knowledge and due to the significant economic margin for application in metal removal/detoxification processes, new biosorbent materials are currently well poised for commercial exploitation. However, there are no limits to expanding the science of biosorption required to provide a deeper understanding of the phenomenon and to support effective application attempts.

Human populations need technologies to treat water supplies and diminish the environmental dangers posed by certain industrial and agricultural practices. Biosorption can be a solution to providing abundant clean water and treating soils contaminated by heavy metals. Research in the past two decades has provided a better understanding of metal sorption by certain potential biosorbents. Application aspects are being aimed at biosorption process optimization and development of strategies for further processing of biosorbent as a greener and cleaner technology.

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