Heavy Metal Removal from Industrial Wastewater Using Fungi: Uptake Mechanism and Biochemical Aspects

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Abstract: In this review, recent developments pertaining to the use of fungi as biosorbent for heavy metals removal from wastewater has been presented with critical analysis of the present status of the subject. Undoubtedly fungi have emerged as an interesting biosorbent family. They are superior to other microorganisms as they can be easily grown, produce large biomass, and genetic and morphological manipulation is easily possible with them. Various aspects of this field, such as classification, general characteristics, composition and role of the cell wall, and metal uptake mechanisms have been critically analyzed. The superiority of dead biomass of fungi and immobilization was paid enough attention. The use of fungal species as biosensors for metal detection in the environment was also presented. **DOI: 10.1061/(ASCE)EE.1943-7870.0000983.**

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Introduction

The technological importance of heavy metals has resulted in the intensification of their application (Kumar and Singh 2007; Kumar et al. 2008, 2009, 2012, 2013, 2014; Rao et al. 2010, 2012, 2013, 2014; Saleem et al. 2013, 2014) in industries leading to the release of some portion of them into the environment as waste resulting in metal pollution (Gautam et al. 2014). Industrial sources of metal pollution are well documented (Wang and Ren 2014). Industrial effluents containing cadmium, chromium, copper, lead, mercury, nickel, palladium, and zinc are of particular concern for treatment because of their toxic behavior in the environment (Barakat 2011).

During last couple of decades, environmental management greatly focused on the control of hazardous materials especially toxic heavy metals leading to a requirement of a cost-effective technique for treating metal-containing industrial wastewaters. Different conventional technologies such as precipitation, reduction, coagulation, membrane filtration, ion exchange, and adsorption have been developed to treat heavy metals in the industrial effluents (Barakat 2011; Fu and Wang 2011). In view of various disadvantages such as expensiveness and inappropriateness for low-strength wastewaters (Farooq et al. 2010) and the production of sludge (Say et al. 2001; Khezami and Capart 2005; Ngah and Hanafiah 2008; Srivastava and Majumder 2008) during use, many of these techniques are less preferred over adsorption (Eccles 1999). However, the search for an effective adsorbent, which is abundantly available and is low cost still continues, and recent developments in this context have been reviewed elsewhere (Barakat 2011; Fu and Wang 2011). Consequent to increased environmental awareness and the quest for development of clean remediation techniques for pollution abatement, biosorption has emerged as an attractive and promising technique for the removal of metal species from wastewater (Gautam et al. 2014). It involves the uptake of pollutants by the living or dead cells through the physico-chemical adsorption or ion exchange. Many researchers have focused on it during the past two decades (Izquierdo et al. 2010; Srinath et al. 2002; Spinti et al. 1995), and the existing definitions of biosorption, bioadsorption, and bioabsorption have been recently reviewed (Fomina and Gadd 2014). Several biological materials, such as bacteria, algae and fungi, have emerged as potential sorbents of heavy metals. The reason behind the increasing attention toward the use of biological materials as biosorbent of heavy metals may be attributed to their high performance, cost efficiency and low need for the technical support, ease in handling, no requirement of chemicals, and their renewable nature (Fourest et al. 1994; Volesky 1994; Sprocati et al. 2006; Lo et al. 2014). The applicability of fungal biomass for adsorption of heavy metals was discovered during the mid-1970s and has now become an important biosorbent. The number of research papers published dealing with heavy metals removal and recovery using this kind of biomass (Melgar et al. 2007; Zafar et al. 2007; Hanif et al. 2008; Khambhaty et al. 2009; Prigione et al. 2009; Velmurugan et al. 2010; Iskandar et al. 2011; Kocaoba and Arısoy 2011; Sepehr et al. 2012; Hu et al. 2013; Verma et al. 2013; Aftab et al. 2014; Kurniati et al. 2014) has increased to a great extent in the recent past. The removal of numerous metals from the aqueous solution has been studied, but this work reviews the recent researches related to the biosorption of toxic heavy metals employing fungal biosorbents.

Fungi

In nature, fungi act as decomposers of dead material, have a role in nutrient cycling, and establish a mutualistic or antagonist

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relationship with plants or animals (Simonesco and Ferdes 2012). Spoilage of synthetic and natural materials, i.e., plant biomass, paint, leather fabrics, and even plastic, mostly own to these morphologically diverse organisms, which possess complex fruiting bodies. They are producers of many substances with high economic importance like biofuel from bagasse decomposition, citric acid, polysaccharides, antibiotics, and essential amines (Azila 2008).

Characteristics and Classification of Fungi

The kingdom Mycetae can be divided into two groups: (1) macroscopic fungi, and (2) microscopic fungi. Microscopic fungi represent a large group that includes yeasts and molds. Yeasts are made up of budding cells (spherical in shape), whereas molds are comprised of hyphae (cylindrical shaped filaments), which give rise to mycelia. The molds, such as *Aspergillus* and *Penicillium*, are filamentous fungi. Fungi are a thalloid species. Their complexity and size are variable. For example, yeasts are unicellular microscopic structures whereas molds are multicellular. A unit of filaments (i.e., single filament) is known as hypha. Generally, growth of several hyphae organizes into a mycelium. Fungi have been divided in different classes (Table 1). All the fungal groups are closely related except Oomycetes, which have a phylogenetic distinction. Yeasts are included in ascomycetes. Molds or yeasts differ from each other in terms of the type of development of the thallus. Both asexual (via development of buds and transverse division) and sexual (through formation of spores) reproduction occur in yeasts. In case of a mold, thread-shaped, elongated, branching filaments form the hyphae. These hyphae are twisted untidily, and the tangled mass is a mycelium. Hyphae may have several partitions (known as septate) or may not be partitioned (known as coenocytic or nonseptate). Every mycelium is capable of forming reproductive structures (Prescott et al. 2002).

Most fungi are filamentous structures. The width of hyphae is typically between 5 and 10 μm. However, many variations between 0.0005 and 1.00 mm (depending on the type of species) have also been noticed (Lester and Birkettn 1999). The mycelium is a complex structure made up of several filaments or hyphae. The hyphal walls are made up of chitin or cellulose or both. The same type of cytoplasm is present in the hyphae. Therefore cellular consortium of fungi can be divided into three categories: (1) coenocytic or aseptate, where the hypha contains many nuclei in cytoplasm; (2) septate, where uninucleate protoplasts are present due to the division of hypha by partitions, and a single nucleus occupies every compartment; and (3) septate where each compartment is occupied with multinucleate protoplasts. In the case of septate species, a pore exists in the center of a septum to connect the cytoplasm of adjoining cells and permit the flow of both cytoplasm and nuclei (Lester and Birkettn 1999) across the partitions.

Table 1. Different Classes of Fungi (Adapted from Madigan et al. 2000, p. 729)

Group	Commonly known as	Status of partition in hyphae	Typical representative
Ascomycetes	Sac fungi	Septate	*Neurospora* *Saccharomyces* *Morchella*
Basidiomycetes	Club fungi, mushroom	Septate	*Amanita* *Agaricus*
Zygomycetes	Bread molds	Coenocytic	*Mucor* *Rhizopus*
Oomycetes	Water molds	Coenocytic	*Allomyces*
Deuteromycetes	Fungi imperfecti	Septate	*Penicillium* *Aspergillus* *Candida*

Generally, the size of yeast cells is bigger than that of bacterial cells and considerable variation in size depending upon the type of fungus has been noticed. The width of a representative yeast cell is between 0.0025 and 0.01 mm and length varies between 4.5 and 21 μm. The morphology of a yeast cell depends on the type of species of yeast, level of nutrition, and cultural condition. Commonly, they are spherical to oval-shaped. Growth of the majority of minuscule fungi takes place in colonies that exist as a loose association of these organisms. Only a single cell is involved in reproduction of most of the yeast; however, formation of tendril under certain conditions by some yeast is not exceptional. Some yeasts reproduce sexually by a peculiar process that is known as mating. Two different strains of *Saccharomyces cerevisiae* are among the most useful examples (Madigan et al. 2000) for commercial purposes as they are used in brewing and baking and hence, known as the baker's and brewer's yeasts. Many important aspects have been studied in eukaryotic biology using the yeast cells as models. *S. cerevisiae* was the first eukaryote, the genome of which was completely sequenced. It is a popular model eukaryote to carry out various scientific studies nowadays. In cell, as well as molecular biology, it has been considered as a eukaryotic model organism for highly intensive studies.

S. cerevisiae is a species of budding yeast and is probably the most applicable yeast because of its utility in baking and brewing since the long distant past. Day-to-day fermentation is carried out using only this organism. *Saccharomyces* (derived from Greek) means "sugar mold" and *cerevisiae*, which has come from Latin, means "of beer." It is believed that it used to be extracted originally from the outer covering layer of grapes. Visibility of the yeast as an ingredient of the white film on the outer covering layer of few fruits (which are dark in color, such as plums) can be easily noticed. The diameter of *S. cerevisiae* cells is 5–10 μm. These cells undergo a reproduction process (known as budding), which involves their division. Some results have been published (Chen and Wang 2006, 2007a, b, c, d, e, 2008a, b, c; Wang 2002; Wang and Chen 2006) on research related to *S. cerevisiae* in context of the peculiar features of interaction between metal and microorganism and metal biosorption.

Cell Wall and Its Composition

The fungal cell walls are rigid and provide structural support and shape. Polysaccharides comprise bulk (80–90%) of composition of the walls. The remaining content includes inorganic ions, polyphosphates, lipids, and proteins. Chitin, which is a pliable nitrogen-containing polysaccharide (made up of N-acetylglucosamine residues), is an important constituent of a typical fungal cell wall. Ultrastructural inspection of the fungal cell reveals the presence of bilayers in walls (Figs. 1 and 2): a thin outer layer and a thick inner layer. Mixed glycans (such as glucans, mannans, or galactans) are the main constituents of the outer layer, whereas the inner microfibrillar layer is made up of polysaccharide fibers. This polysaccharide may either be chitin or cellulose. Chemical structures of different ingredients of a cell wall are described in Fig. 3.

Plasma Membrane

The plasma membrane is a thin sheet consisting of two layers. It is made up of lipids (such as sterols and phospholipids) and protein molecules. The structure and behavior of sterols are different from those of phospholipids. The ratio of lipid content and protein

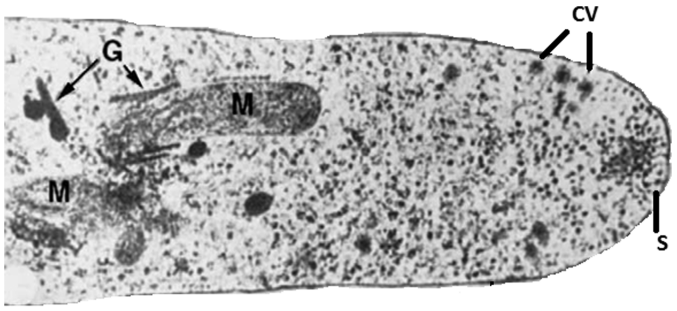

Fig. 1. Photomicrograph of glycocalyx structure of cell wall; the general structure of a fungal cell wall which was shown in cross section through the tip (S = growing tip; CV = coated vesicles; G = Golgi apparatus; M = mitochondrion)

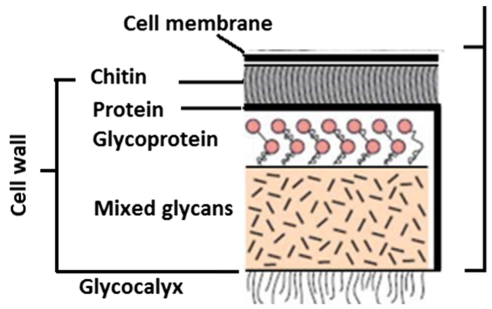

Fig. 2. Different layers of polysaccharides and proteins (reprinted from Wang and Chen 2009, with permission)

molecules in a membrane is approximately 40:60. According to fluid mosaic theory, which is a broadly accepted model for structural features of membranes, plasma membrane is a bilayer structure made by lipids. The orientation of polar heads of lipid is toward the outside, whereas that of nonpolar heads is toward the center of the membrane. Globular proteins of variable size are embedded in the membrane at several sites. Selective permeability during transport is the important feature of cytoplasmic membranes. Many individual organelles, which are membrane bound and account for 60% to 80% of total volume, are also present in the cells (Prescott et al. 2002; Talaro and Talaro 2002).

Cytoplasm

The cytoplasm contains various organelles such as ribosomes, mitochondria, and a substantial endoplasmic reticulum. The presence of vacuoles carrying different storage substances (such as volutin, glycogen, and lipids) is also noticeable. In unicellular fungi, including the *Saccharomyces* species, the protoplast is surrounded by the plasma membrane, which is semipermeable in nature and remains covered with a cell wall (inflexible). However, in the case of filamentous organisms, the protoplasm is found in the tips of the juvenile and emerging hyphae. Metabolic inactivity and the presence of large-sized vacuoles in the cytoplasm is a usual characteristic of older hyphae. The fungi are heterotrophic and do not contain chlorophyll at all. Normally, the germination (i.e., sprouting) of a spore or a reproductive cell leads to the development of a mycelium. Initially, a single long hypha is produced after germination, which subsequently undergoes the process of branching and ramifying to form the mycelium, i.e., a mass of hyphae.

The role of cytoplasm is important during the interaction of living cells with metal ions. After the entry of such ions into the cell, they are distributed into various subcellular organelles (e.g., mitochondria, vacuole). The strategies for accumulation of metal ions (especially strategies for internal compartmentalization) have been summarized by Vijver et al. (2004). In the case of nonessential and essential metal ions, metal accumulation strategies may differ from each other. For essential metals, the main strategies include limited metal uptake or active excretion, conversion of metal in chemically inactive form and storage of such forms, and excretion of stored metal. In the case of nonessential metals, excretions of the excess of metal and internal retention without expelling are the main strategies. An increase in the external concentration of metal leads to the elevation of metal concentration in the cells. Mainly, two types of the cellular sequestration mechanisms were proposed: the generation of definite encompassing bodies and coordination of metals to thermally stable proteins. The formation of three types of granules takes place in the case of the first type: Type A includes calcium phosphates for metals such as zinc; Type B is represented by mainly acid phosphatase for accumulation of cadmium, copper, silver, and mercury; and Type C involves the storage of an excess of iron in granules as insoluble haemosiderin. The other type of mechanism describes the role of metallothioneins (MT), which are a peculiar type of protein having the ability to bind with metals. These are of low molecular weight and cysteine-rich. Ions of heavy metals (such as cobalt, cadmium, zinc, copper, mercury) as well as many other substances can induce MT (Vijver et al. 2004).

The research outputs regarding the role of vacuole detoxification of metal ions have revealed that high susceptibility and diminished uptake of nickel, cobalt, zinc, and manganese are shown by vacuole-deficient strain (Ramsay and Gadd 1997). However, wild type and mutant of *S. cerevisiae* show insignificant variations in cadmium and copper uptake and susceptibility to ions of both metals. It has been reported (Gharieb and Gadd 1998) that vacuolar-lacking and vacuolar-defective mutants of *S. cerevisiae* show higher susceptibility to CrO_4^{2-} and TeO_3^{2-} with the diminishing cellular content of each metal, whereas the tolerance to SeO_3^{2-} with the enhancement on the cellular content of selenium. It has been confirmed by Avery and Tobin (1992) that the vacuole was the organelle of the living cell that accumulated bivalent cations of strontium in *S. cerevisiae*.

Functional Groups Involved in Biosorption

Pearson in 1963 and Nieboer with Richardson in 1980 classified the metals on the basis of their affinity for different types of ligands as illustrated in Table 2 (Remacle 1990). R is a symbol that indicates the presence of an alkyl component of a ligand, such as CH_2- and CH_3CH_2-. Oxygen of the ligands of Type I is preferred by Class A metal ions for binding. More affinity for ligands of Type III is shown by Class B metal ions, but strong coordination with ligands of Type II is also possible. Different preferences are shown by borderline metal ions while binding with these three kinds of donors/ligands.

According to the hard and soft acid base principle (HSAB), hard ions (such as Na^+, Ca^{2+}, and Mg^{2+}) that exhibit strong binding with a fluoride ion, can also be involved in forming a stable bonding association with O of oxygen-containing ligands, such as hydroxide (OH^-), HPO_4^{2-}, carbonate (CO_3^{2-}), R─COO^-, and carbonyl (>C═O). However, soft ions (heavy metal ions such as divalent ions of mercury and lead) coordinate a strong bond with nitrogen and sulfur atoms present in different groups such as NH_2^-, R─S^-, HS^-, CN^-, and imidazol. Divalent cations of zinc

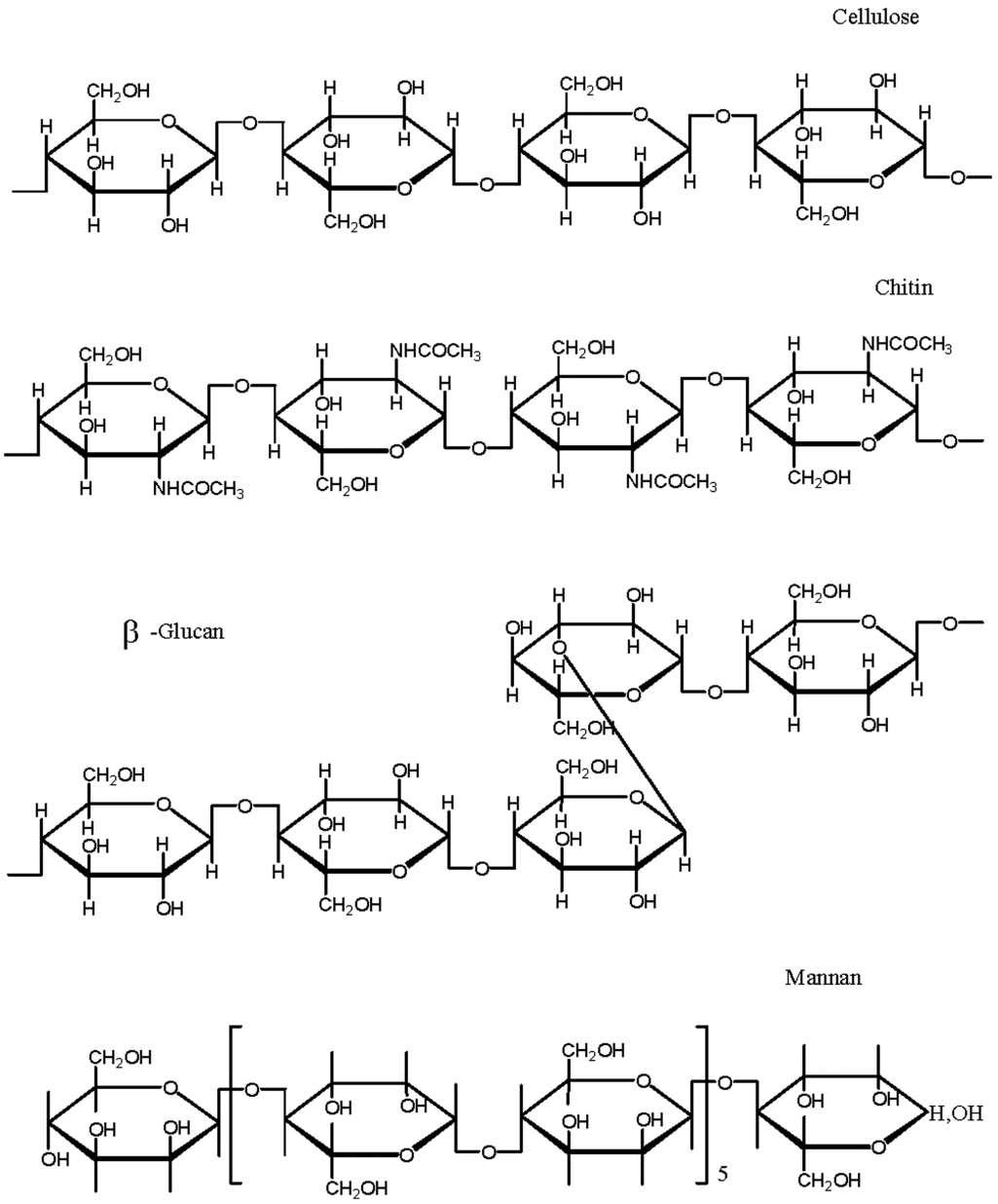

Fig. 3. Structures of different constituents of cell wall (reprinted from Wang and Chen 2009, with permission)

Table 2. Different Ligands Found in Fungi and Division of Metals into Three Classes (Data from Nieboer and Richardson 1980; Pearson 1963; Remacle 1990)

Ligand class	Ligands	Metal classes
I: Ligands preferred to Class A	F^-, O^{2-}, OH^-, H_2O, CO_3^{2-}, SO_4^-, $ROSO_3^-$, NO_3^-, HPO_4^{2-}, PO_4^{3-}, ROH, $RCOO^-$, C═O, ROR	Class A: Li, Be, Na, Mg, K, Ca, Sc, Rb, Sr, Y, Cs, Ba, La, Fr, Ra, Ac, Al, lanthanides, actinides
II: Other important ligands	Cl^-, Br^-, N_3^-, NO_2^-, SO_3^{2-}, NH_3, N_2, RNH_2, R_2NH, R_3N, ═N─, ─CO─N─R, O_2, O_2^-, O_2^{2-}	Borderline ions: Ti, V, Cr, Mn, Fe, Co,Ni, Cu, Zn, Ga, Cd, In, Sn, Sb, As
III: Ligands preferred to Class B	H^-, I^-, R^-, CN^-, CO, S^{2-}, RS^-, R_2S, R_3As	Class B: Rh, Pd, Ag, Lr, Pt, Au, Hg, Tl, Pb, Bi

and cobalt are less toxic, which are the examples of intermediate or borderline metal ions. Binding shown by hard ions is mainly ionic in nature, whereas the same shows a high degree of covalent nature in the case of soft ions (Remacle 1990).

Metal biosorption takes place through cell surface, and hence, it depends on the components on the cell and the spatial orientation of the cell wall. Different polysaccharides play a significant role in metal coordination found in cell walls of fungi. These include alginate, chitin, cellulose, and glycan. Involvement of various proteins in metal binding has also been proved. Certain functional groups were confirmed to be involved and have been noticed in binding with metal ions, especially the carboxyl ($RCOO^-$) group.

General Formula of ligands (R + Functional Group)	Name of functional group	Class of Compounds
R—CH_2-NH_2	Amino	Proteins, Nucleic acids
R—CH_2-SH	Sulfhydryl	Cysteine (amino acid), proteins
R—OH	Hydroxyl	Alcohols, carbohydrates
R—C(=O)—H	Carbonyl, terminal end	Aldehydes, polysaccharides
R—C(=O)—OH	Carboxyl	Fatty acids, proteins, organic acids
R—C(=O)—CH_3	Carbonyl, internal	Ketones, polysaccharides
R—C(=O)—OR	Ester	Lipids
R—O—P(=O)(OH)—OH	Phosphate	DNA, RNA, ATP

Fig. 4. Different functional groups for various classes of organic compounds in biomass

Direct participation of S—, N—, O—, or P-containing groups in forming bonds with certain metals has been confirmed by some evidence.

Several techniques, such as titration, infrared (IR) and Raman spectroscopy, energy dispersive X-ray analysis (EDX), X-ray photoelectron spectroscopy (XPS), electron microscopy (transmission and/or scanning), powder X-ray diffraction (XRD) analysis, nuclear magnetic resonance (NMR), and X-ray absorption spectroscopy (XAS), have been used to determine the involvement of some donor sites for metal uptake. Carbonyl (ketone, >C═O), carboxyl (O═C—O^-), sulfhydryl (thiol), sulfonate (SO_3^-), thioether (R—S—R′), amine (RNH_2), imine (>C═N—), secondary amine (R_2NH), imidazole, phosphonate, amide (—$CONH_2$), and phosphodiester are among such important sites (Volesky 2007).

Functional groups, which are different for every category of ligands present in biomass, are shown in Fig. 4. The symbol R signifies the residue that may be different for compounds or ligands of the same class (Talaro and Talaro 2002).

Fungi-Based Biosorption

Yeast and fungi have an advantage over the bacterial cell, i.e., large cell size, easy to grow, high biomass yield, and easy morphological and genetic manipulation. Wide-scale use of fungal species for commercial-scale biogenesis eventually leads to overproduction of fungal biomass as a by-product. This biomass is cheap and easily procured in a rather substantial amount for metal sorption. This advantage allows the preference for fungal biomass over conventional sorbents (Wang and Chen 2006; Azila 2008).

In general, fungal cell walls contain a very high percentage of polysaccharide content (80–90%). Some inorganic ions, proteins, lipids, and polyphosphates are also found. Chitin, a common ingredient of the fungal cell wall is strong, but flexible, nitrogen containing polysaccharide consisting of *N*-acetylglucosamine residues. Interestingly, many negatively charged functional groups act as donor sites during biosorption, which have the ability to bind with numerous metal cations. Such groups include carboxyl ($RCOO^-$), hydroxyl (HO^-), sulphate (SO_4^{2-}), phosphate, and amino groups (Volesky 2007; Verma et al. 2013).

Different physicochemical mechanisms or a combination of various processes (such as ion exchange, complexation, coordination, chelation, adsorption, and electrostatic interaction) may be involved (Volesky 2001; Wang and Chen 2009) during passive binding of fungal biomass with metal ions. For example, biosorption of hexavalent chromium by using *Rhizopusarrhizus* was found to be 23 mg/g biomass (Prakasham et al. 1999). Table 3 presents the maximum metal-uptake capacity of different fungi with optimum pH.

The concept of using fungi as a biosorbent was developed during research on the toxic effect of heavy metals in the commercial production of fungi. Since then, a lot of work has been carried out in this area. Fungal species, such as *Pythium* sp., *Dictyuchus* sterile, and *Scytalidium lignicola*, were found to accumulate zinc, lead, and cadmium by adsorption on their mycelium from the freshwater shrimp culture pond (Duddridge and Wainwright 1980). Subsequently, different studies have investigated the efficiency of different fungal species for heavy metals removal from wastewater, and at present, fungi represent one of the most studied classes of microorganisms in the field of biological remediation of pollutants. These heterotrophic microorganisms can biotransform metal pollutants through the chemical modification or metabolic process. They can form extended mycelial networks and use pollutants as growth substrates, which makes them a suitable candidate for bioremediation of pollution (Harms et al. 2011).

Fungi are superior to other microorganisms because they can be easily grown, they produce large biomass, genetic and morphological manipulation is possible, they are environmentally friendly as waste by-products of industries can be reused and it cuts the cost of the disposal of waste by-products (White et al. 1995; Wang and Ren 2014). They can be isolated from different sources as listed in Table 4. Moreover, simple fermentation techniques using a cheap growth medium can be used for them. For instance, *Aspergillus caespitosus* is a fungus that can be easily isolated from soils and sugarcane bagasse and can be grown to produce high yields of biomass, or they are also be available as industrial waste product as they are used for the production of invertases, alkaline phosphatase, and xylanases (Aftab et al. 2014). In addition, they have high metal tolerance capacity, intracellular metal-binding capacity and wall-binding capacity (Ahluwalia and Goyal 2010). They possess a large surface area for metal binding and allow for the easy separation of solid and liquid (Mishra and Malik 2014).

Table 3. Maximum Metal Uptake Capacity of Different Fungal Strains with Optimum pH

Name of fungi	Metal	Maximum sorption capacity mg g^{-1}	pH	References
A. caespitosus immobilized glutaraldehyde-crosslinked calcium alginate beads	Pb	670	4.5	Aftab et al. (2014)
Amanita rubescents dried biomass	Pb	38.4	5	Sarı and Tuzen (2009)
	Cd	27.3		
A. caespitosus (immobilized with calcium alginate beads)	Pb	651.6–3.3	4.5	Aftab et al. (2014)
A. flavus dried biomass	Pb	12.44	—	Dwivedi et al. (2012)
	Ni	0.53		
	Cr	0.05		
A. flavus dried biomass	Zn	287.8	5	Aftab et al. (2013)
A. niger	Cr	185	5–5.2	Sepehr et al. (2012)
A. oryzae	Cr	208	5–5.2	Sepehr et al. (2012)
A. terreus Immobilized in polyurethane foam	Fe	164.5	4.5	Dias et al. (2002)
	Cr	96.5		
	Ni	19.6		
Claviceps paspali dead biomass	Zn	1.0	—	Luef et al. (1991)
Lentinus edodes heat inactivated	Hg	336.3	6	Moore and Ramamoorthy (1984), Falih (1997)
	Cd	78.6		
	Zn	33.7		
M. rouxii dead biomass	Pb	25.22	5	Yan and Viraraghavan (2003)
	Ni	6.34		
	Cd	8.36		
	Zn	16.62		
M. rouxii treated with NaOH	Pb	35.69	5	Yan and Viraraghavan (2003)
	Ni	11.09		
	Cd	8.46		
	Zn	7.75		
P. chrysosporium	Zn	57	6	Marandi et al. (2010)
P. chrysosporium	Pb	90.0	5	Gopal et al. (2002)
	Cd	17.0		
	Cu	43.0		
P. chrysosporium dried biomass	Pb	45.25	6	Say et al. (2001)
	Cd	13.24		
	Cu	10.72		
P. chrysosporium immobilised on ironoxide nanoparticles	Pb	176.33	5	Xu et al. (2012)
P. chrysosporium inactivated and treated with alkali	Cd	15.2	4.5	Li et al. (2004)
P. chrysosporium inactivated and treated with alkali	Pb	12.34	4.5	Li et al. (2004)
P. chrysosporium live biomass	Pb	1.33	—	Dey et al. (1995)
P. chrysosporium resting cells	Ni	77.96	4	Çeribasi and Yetis (2004)
	Pb	73.56		
P. chrysosporium surface modified	Cr	279.9	3	Chen et al. (2011)
Penicillium citrinum immobilised in alginate	Cu	25	5	Verma et al. (2013)
Rhizopus arrhizus	Pb	200	7	Fourest et al. (1994)
Streptoverticillium cinnamoneum dead biomass	Pb	57.7	3.5–4.5	Moore and Ramamoorthy (1984)
	Zn	21.3	5–6	

Different fungal species have been used for the biosorption of heavy metals such as *Lentinus edodes* for the removal of cadmium, mercury, and zinc (Bayramoglu and Arıca 2008), *Phanerochaete chrysosporium* for the removal of nickel and lead (Çeribasi and Yetis 2004), and *Neurospora crassa* for lead and nickel (Kiran et al. 2005) among others. Fungi have been used in filamentous, pelletized, or powder forms for the biosorption processes. The separation of fungal biomass from the liquid phase is problematic when biomass are used as dispersed mycelia and/or used in powdered forms (Järup 2003). However, use of biomass in pelletized form helps to solve these problems as they are easy to separate from the broth (Gomez et al. 1988). Some of the most commonly studied fungal species for the biosorption of heavy metals are *Aspergillus* spp., *Mucor* spp., *Rhizopus* spp., and *Penicillium* spp. The metal-binding capacity of fungi varies from species to species. For instance, the efficiency of *A. niger* to adsorb cadmium was higher than that of *M. rouxii* under same study conditions. *A. niger* falls under the chitin-glucan group, and *M. rouxii* falls under the chitosanchitin group (Mullen et al. 1992). Studies show that fungi are also metal selective (Iskandar et al. 2011). It was found that three species of filamentous fungi, viz *A. niger, Penicillium simplicissimum*, and *Trichoderma asperellum*, had better removal efficiency for lead compared to copper (Iskandar et al. 2011). Morley and Gadd (1995) made a comparative study of the adsorption of three heavy metals (copper, cadmium, and zinc) between two fungi-*R. arrhizus* and *T. viride* on a surface-area basis. *R. arrhizus* had higher metal

Table 4. Different Sources for Isolation of Fungi

Name of fungi	Sourse for isolation	Metal adsorbed	References
A. flavu	Forest soil	Hg	Kurniati et al. (2014)
A. niger	Tanning factory	Cr	Sepehr et al. (2012)
A. niger strain ATCC 34467 and *Mucor rouxii* strain ATCC 24905	Soil	Cd, Cu	Mullen et al. (1992)
A. niger	River sediment	Cu and Pb	Iskandar et al. (2011)
A. oryzae	Tanning factory	Cr	Sepehr et al. (2012)
Paecilomyces lilacinus	Tannery sludge	Cr	Sharma and Adholeya (2011)
Paecilomyces lilacinu	Smelter	Cd	Zeng et al. (2013)
Penicillium citrinum	Soil samples from Stainless Steel industry	Cu	Verma et al. (2013)
P. simplicissimum	River sediment	Cu and Pb	Iskandar et al. (2011)
R. arrhizus, Mucor miehei and *P. chrysogenum*	Fermentation industries	Ni, Zn, Cd and Pb	Fourest et al. (1994)
Rhizopus oryzae, Aspergillus lentulus, A. terreus	Soil samples from dumping sites	Cr and Cu	Mishra and Malik (2014)
Saccharomyces cerevisiae	Pure culture	Cu	Gohari et al. (2013)
T. asperellum	River sediment	Cu and Pb	Iskandar et al. (2011)

uptake capacity compared to *T. viride*. It was suggested that the difference in metal-uptake capacity may be due to the different cell wall composition and metal-binding significance of chitin. However, detailed study on this is still missing. Detailed characterization of the cell surface is needed to better understand the mechanism of fungal bioadsorption and step forward to its commercialization. Despite the availability of technologies, such as scanning electron microscopy with the energy-dispersive X-ray analysis (SEM–EDX), transmission electron microscopy with energy-dispersive X-ray analysis (TEM–EDX), the transmission electron, as well as atomic force microscopy (AFM), study on fungal biosorption seldom used these technologies for the characterization of a cell.

Since wastewater contains diverse types of metals pollutants, consortia of fungi can provide an efficient system to treat such types of water as they provide a richer metabolic network to treat metal contaminants (Mahapatra et al. 2014; Mishra and Malik 2014). Mishra and Malik (2014) studied a system of metal removal using a consortium of different fungal species: *R. oryzae*, *A. lentulus*, and *A. terreus* from wastewater containing heavy metals and dye. This system of three fungal consortium was 100% efficient for the removal of chromium and 81.6% for copper. Removal of these metal ions up to this extent indicates that collective performance is significantly higher than that of the individual performance of each species in the same study. The results can be related to their higher metal-resistance capacity compared to the pure cultures (Sprocati et al. 2006; Mishra and Malik 2014). This study suggested the possibility of applying the three fungal consortiums for the treatment of multimetal pollutants; however, further study was recommended on the cost effectiveness of the application of such consortium.

Live and Dead Fungi

A remarkable ability to take up toxic and precious metals from aqueous solutions (Kapoor et al. 1999; Fu and Viraraghavan 2001; Wang and Chen 2009) was shown by both dead and living fungi. However, dead biomass was considered to be superior to live ones for various reasons. The main reasons included the high surface area associated with the dead cells as adsorption of metal on fungal biomass is the physical adhesion of metal ions (adsorbate) on to the two-dimensional solid cell wall (adsorbent) due to interaction between them. On the other hand, small size and low mechanical strength of living fungal cells made them less ideal as biosorbent. As a result, a substantial hydrostatic pressure was required for suitable and efficient flow rate and disintegration. Other reasons included the absence of toxic effects to the dead biomass, and also the system could be operated in different pH and temperature. Moreover it was cost-effective to use dead biomass as it cuts the expenses of media for the fungal growth. Along with this, dead biomass can be further reused after regeneration of biomass, which is quite complicated in the case of live ones. Heat treatment destroys or alters the metal-binding ligands (present in the mycelial surface and responsible for the adsorption process), leading to a decreased metal adsorption capacity (Duddridge and Wainwright 1980).

Different methods have been applied to kill the fungal biomass for the application in the adsorption process. Physical and chemical treatments used to kill the biomass also alter the surface biomass characteristics enhancing the porosity, exposure of more functional groups, and purge impurities (Gohari et al. 2013). Such treatments include high-pressure homogenization, sonication, enzymatic methods, and using bead mills. Studies showed that disrupting the cells of *S. cerevisiae* exposed more functional groups of the cell were more efficient in copper adsorption compared to the nondisrupted cells of *S. cerevisiae* (Gohari et al. 2013).

Immobilization of Fungal Biomass

The immobilization of biomass (White et al. 1995) is a technique that helps to avoid some disadvantages of applying free living and dead biomass of fungi (such as difficulty of separating biomass and effluents and low mechanical strength). It provides mechanical strength and stability to the biomass. In addition to this, it allows the proper handling and reusability of the biomass (Chen et al. 2014). Different materials, including inert ones such as agar, alginates, cellulose, glycol, cross-linked ethyl acrylate-ethylene dimethylacrylate, silica gel, and polyacrylamide, have been used and studied for the immobilization of fungi. Immobilized live biomass form biofilms and have been applied in different types of reactors to treat wastewater continuously. Verma et al. (2013) made a comparative study between the biosorption capacity of free and immobilized *Penicillium citrinum* in alginate. The result showed that immobilized biomass had higher efficiency (76.2%) for metal removal using 0.10 g/100 mL biomass compared to the free biomass (74.1%) using 0.15 g/100 mL biomass. Even thought cell immobilization techniques have been incorporated in fungal biosorption studies, the information on the technical and economic feasibility behind its application in real-world study is still scarce. Recently

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magnetic nanoparticles are also getting more attention for the immobilization of biomass (Xu et al. 2013).

Continuous Experiments

Different studies have been carried out that demonstrated the application of fungi for the treatment of heavy metals-contaminated wastewater in the continuous system. For instance, Fourest et al. (1994) demonstrated the successful application of fixed-bed biosorbent columns using *Mucor miehei*, *Rhizopus arrhizus*, and *Penicillium chrysogenum* to treat zinc and lead in a continuous-flow system. Similarly, Aftab et al. (2014) demonstrated the successful removal (93% removal efficiency) of lead by *A. caespitosus* immobilized glutaraldehyde-crosslinked calcium alginate beads in a column reactor using real wastewater from the paint industry.

Recovery of Metals and Regeneration of Biomass

The possibility of metal recovery through the desorption process is another factor that makes biosorption more acceptable and promising technology for the removal of heavy metals. It allows regeneration of the biomass, which can be used for a further biosorption process and increase the possibility of the application of the biomass at industrial scale. Regeneration capacity of biomass also indicates the effectiveness of the biosorbent for metal removal. The absorption and desorption process helps to concentrate the pollutants in smaller volume and recover them in cost-effective way. Research on desorption of metals and biomass regeneration is still a very important topic in the field of biosorption studies (Gautam et al. 2014). Despite a large number of studies in the field of fungal biosorption, limited studies have focused on the desorption section and reuse of biomass after metal recovery.

The desorbing agent used for desorption of metals should be environment friendly, and it should also not damage the nature of the biosorbent used. Different chemicals such as acids, alkaline, and complexing agents are used for desorption of heavy metals from the biomass after the adsorption process, which are shown in the Table 5 below.

It can be depicted from Table 5 that after desorption, the metal-binding efficiency of the biomass may be increased, unaltered, or decreased. For instance, Yan and Viraraghavan 2003 reported the increase in metal (cadmium, nickel, and zinc) removal efficiency of the desorbed biomass of *Mucor rouxii* when used for five adsorption–desorption cycles. On the other hand, some other studies reported the decrease of biosorption capacity of biomass after regeneration (Puranik and Paknikar 1997; Kaçar et al. 2002). Mineral acids of low concentration seem to be effective eluent for metal desorption from fungal biomass after adsorption. In contrast to this, low-cost adsorbent like tree bark of sal (*Shorea robusta*), mango (*Mangifera indica*), and jackfruit (*Artocarpus integrifloria*) needed higher concentration of HCl for desorption of metals after metal removal (Reddy et al. 1997).

Understanding of desorption kinetics is also equally important as the adsorption kinetics for the practical implication of the biosorbents. Detailed study on the surface structure of biomass after desorption of metal using different eluents also helps to know the effect of the desorbing agent on the cells as it should not significantly damage the biomass. The forms of the biomass also affect the desorption process. For instance, the use of freely suspending biomass is difficult to regenerate (Fomina and Gadd 2014).

Metal Uptake

Metal accumulation on a large scale is carried out possibly by various growth-dependent or growth-independent metabolic processes of living cells. Even dead cells or polysaccharides secretions may also be involved in metal sorption. Former researches have proven that molds and yeast are favorable for metal biosorption over conventional treatment technologies (Wang and Chen 2006; Yahaya and Don 2014).

Adsorption of metal on fungal biomass is the physical adhesion of metal ions (adsorbate) onto the two-dimensional solid cell wall (adsorbent) and takes place as a primary result of fungal metal interaction. Adsorption performance of metal is not independent of cell surface area and its polarity. Ultimately it depends on the biosorbent's ionic state to a significant extent. If the percentage of cell wall in biomass is higher, the surface area retaining various functional groups accountable for metal binding as well sequestration ability will also be higher (Dursun 2006; Akar et al. 2007).

The physical process of biosorption is based on some of the mechanisms like ion interchange, outward complexation, and precipitation (Tunali et al. 2006). Metal biosorption by dead fungal cells has an advantage over living cells' metal accumulation (bioaccumulation). Metal uptake by a living fungal cell wall leads to intracellular accumulation via cell metabolic cycle or cell membrane. Toxic metal interaction interrupts cellular activities, inhibits growth, and eventually induces cell death. In the case of dead cell biomass this interruption can be avoided to a remarkable extent (Özer et al. 2004; Javaid and Bajwa 2008).

When the heavy metal uptake involves its passage into the cell across the cell membrane through the cell metabolic cycle, the mode of metal uptake is referred to as active uptake, while metal uptake by both active and passive modes can be termed as bioaccumulation. The growing *Aspergillus versicolor* was shown to accumulate heavy metals and a dye, both singly and in combination with heavy metal and/or dye levels using molasses as a C and energy source in a batch process (Tasten et al. 2010).

Role of Fungal Cell Wall in Metal Interaction

Shape, integrity and interaction of fungus cell with its surrounding predicate on mechanical strength provided by cell wall which constitutes 30% of total cell dry biomass (Dursun 2006; Simonesco and Ferdes 2012). The cell wall inundates the plasma membrane with an elastic framework of fibrillar (chitin, β-glucan, cellulose) and matricial (glucoproteins, α-glucan, polyuranides, chitosans, lipids, inorganic salts, and pigments). Approximately 80% of the cell wall is composed of polysaccharides to which a major portion are grabbed by heavily glycosylated proteins, anchored in different modes, fatty acids, pigments, and inorganic salts are present as well in a small proportion (Mashitah et al. 2008). The schematic diagram of fungal biomass–metal interaction under the influence of various environmental conditions has been presented in Fig. 5.

The presence of chitin was revealed by former researchers to have role in cell reproduction and division. $\beta[1\rightarrow3]$-glucan is another material in bulk with the capability of forming a stable triple helix of H_2 bonding. Side chains of glucose monomers controlled this helix and its packaging (Özer et al. 2004; Igwe et al. 2005). Some of the proteins with the side chains of mannose (polysaccharides) shaped the outer most layer of cell wall. All these aforementioned components of a cell wall appear to be interconnected. Fungal cell wall structure and functioning vary from specie to specie. In molds, 25–30% of cell walls have chitin as a polymer of *n*-acetylglucosamine, whereas in yeast, a major portion is grabbed by mannan (31%) and glucan (29%); protein lipids and ash are

Table 5. Recovery of Heavy Metals Using Different Eluents

Metal desorbed	Biosorbent used	Eluent	Number of absorption desorption cycles	Recovery (%)	Remark	References
Cd	*Aspergillus niger*	0.05 M nitric acid	5	98.9	No remarkable change in removal efficiency	Kapoor et al. (1999)
Cd	*Phanerochaete chrysosporium*	0.01M hydrochloric acid	3	97	Less than 3% decrease in metal removal efficiency	Kaçar et al. (2002)
Cd	*Mucor rouxii*	0.05 M nitric acid and regenerated with 0.2 M NAOH	5	>90	Increase in biosorption capacity	Yan and Viraraghavan (2003)
Cd	*Funalia trogii*	0.01 M hydrochloric acid	5	97	No change in biosorption capacity	Yakup Arıca et al. (2004)
Cu	*Aspergillus niger*	0.05 M nitric acid	5	99.2	No remarkable change in removal efficiency	Kapoor et al. (1999)
Cu	*Pycnoporus sanguineus*	0.1M hydrochloric acid	4	95	No remarkable change in removal efficiency	Zulfadhly et al. (2001)
Cu	*Trametes versicolor*	0.01 M hydrochloric acid	5	97	No remarkable change in removal efficiency	Bayramoğlu et al. (2003)
Hg	*Funalia trogii*	0.01 M hydrochloric acid	5	97	No remarkable change in removal efficiency	Yakup Arıca et al. (2004)
NI	*Aspergillus niger*	0.05 M nitric acid	5	99.3	No remarkable change in removal efficiency	Kapoor et al. (1999)
Ni	*Mucor rouxii*	0.05 M nitric acid and regenerated with 0.2 M NAOH	5	>90	Increase in biosorption capacity	Yan and Viraraghavan (2003)
Pb	*Aspergillus niger*	0.05 M nitric acid	5	98.9	No remarkable change in removal efficiency	Kapoor et al. (1999)
Pb	*Pycnoporus sanguineus*	0.1M hydrochloric acid	4	100	No remarkable change in removal efficiency	Zulfadhly et al. (2001)
Pb	*Trametes versicolor*	0.01 M hydrochloric acid	5	97	No remarkable change in removal efficiency	Bayramoğlu et al. (2003)
Pb	*Mucor rouxii*	0.05 M nitric acid and regenerated with 0.2 M NAOH	5	>90	Increase in biosorption capacity	Yan and Viraraghavan (2003)
Pd	*Streptoverticillium cinnamoneum*	0.1 M nitric acid	3	90	Metal loading decreased in subsequent cycle by 26–44%	Puranik and Paknikar (1997)
Pd	*Streptoverticillium cinnamoneum*	0.1 M EDTA	3	90	Metal loading decreased in subsequent cycle by 26–44%	Puranik and Paknikar (1997)
Pd	*Streptoverticillium cinnamoneum*	0.05 M sulphuric acid	3	10	Metal loading decreased in subsequent cycle by 26–44%	Puranik and Paknikar (1997)
Zn	*Streptoverticillium cinnamoneum*	0.05 M sulphuric acid	3	90	Metal loading decreased in subsequent cycle by 26–44%	Puranik and Paknikar (1997)
Zn	*Trametes versicolor*	0.01 M hydrochloric acid	5	97	No remarkable change in removal efficiency	Bayramoğlu et al. (2003)
Zn	*Mucor rouxii*	0.05 M nitric acid and regenerated with 0.2 M NAOH	5	>90	Increase in biosorption capacity	Yan and Viraraghavan (2003)
Zn	*Funalia trogii*	0.01 M hydrochloric acid	5	97	No remarkable change in removal efficiency	Yakup Arıca et al. (2004)
Zn	*Streptoverticillium cinnamoneum*	0.1M hydrochloric acid	3	90	Metal loading decreased in subsequent cycle	Puranik and Paknikar (1997)
Zn	*Streptoverticillium cinnamoneum*	0.1M nitric acid	3	90	Metal loading decreased in subsequent cycle by 26–44%	Puranik and Paknikar (1997)

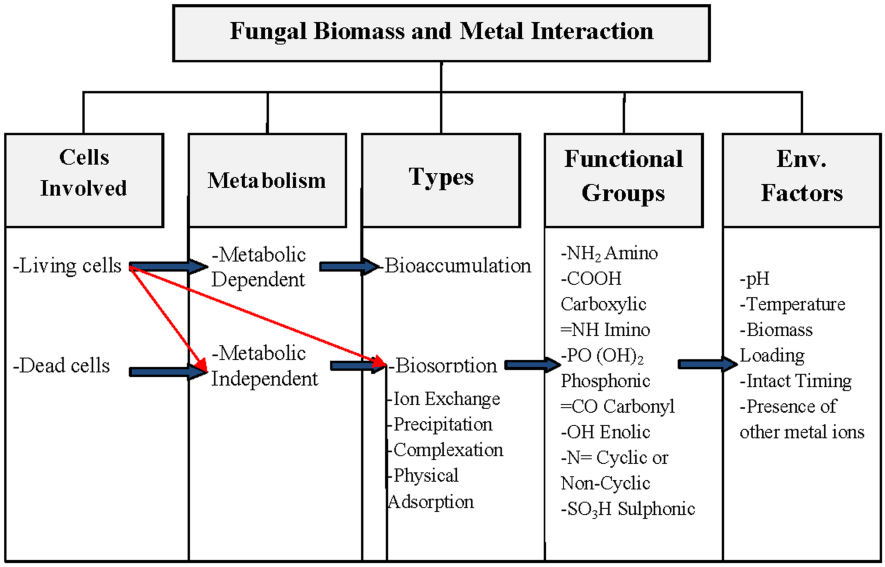

Fig. 5. Schematic representation of fungal biomass–metal interaction including functional groups of cell wall involved in metal binding and environmental factors affecting the interaction process

present in small amounts, i.e., 13%, 8.5%, and 3% respectively. Basically, polymers contain metal-binding groups (Table 6) like PO_4^{3-}, SO_4^{2-}, OH^-, R—NH_2, RSH, and $C_3H_4N_2$, but their availability is highly dependent on fungal specie (Wang and Chen 2006; Yahaya and Don 2014).

Factors Affecting Fungal Biomass–Metal Interaction

Factors influencing the fungal–metal interaction are pH, temperature, biomass loading, biomass contact time, type of fungal specie, and presence of other metal ions (Fig. 5). At acidic pH, lower metal uptake has been observed due to increased competition between H^+ and metal ions. Neutral pH maintenance is important as at alkaline pH, metal absorption becomes restricted due to precipitation. Dead cells biomass of *P. sanguineus* has been reported to absorb significant Pb, Cd, and Cu at optimal pH (0.5–0.7) (Dursun 2006; Simonesco and Ferdes 2012).

Temperature fluctuations have also been identified as an interrupter of metal interaction with cell biomass. Biosorption of copper (II) has been examined by Masitah et al. (2008) using dead biomass of *P. sanguineus* while keeping the temperature range of 30–40°C. Above 40°C, the biosorption process becomes exothermic in nature and inhibits efficient metal sorption. It can be attributed to temperature-dependent cell wall damage. However, at elevated temperature, an opposite trend by immobilized *P. sanguineus* was also noticed during the interaction with cadmium and copper. Similarly, chemical adsorption is thought to be directly dependent upon temperature changes (Dhankhar and Hooda 2011).

An increase in metal ion concentration favors an increased rate of absorption by cell biomass owing to the increment in metal's mass transfer driving force. High metal concentration, on the other hand, is known to limit the availability of metal-binding sites at the biomass cell surface, which eventually leads to lower metal adsorption (Javaid and Bajwa 2008). An increase in biomass concentration lowers the metal biosorption capacity as observed in dead *P. sanguineus* cell while interacting with copper. Some researchers claimed that lower biomass loading favors the metal adsorption by increasing the surface area, as cell-to-cell distance increases. A screen effect (reported at high biomass loading) results in decreased metal sorption by the thick outermost layer (Dursun 2006; Simonesco and Ferdes 2012).

Mechanism of Fungal Metal Uptake

In comparision to other microbes, filamentous fungi have been relatively less explored for characterization of metal uptake, particularly when the heavy metals are present at different and low concentrations (Tsekova et al. 2010). Gadd (1990) suggested that at high metal concentrations encountered in wastewaters, the metal uptake by the active mode does not contribute significantly to the total uptake by fungal microorganisms. Dursun et al. (2003) investigated copper and cadmium bioaccumulation properties of *A. niger* in an enrichment medium and observed no microbial growth or copper uptake over 100 mg L^{-1} initial copper concentration.

The cell wall is the first cell organelle exposed to heavy metal followed by plasma membrane and cytoplasm. As discussed earlier, the cell surfaces of fungi have the ability to bind metal cations (Chen and Hao 1998) due to the presence of various negatively charged (i.e., anionic) structures within cell wall. These anions allow the fungal cell wall to offer multiple metal-binding active sites. Electron microscopy and cell fractionation studies showed that 70–80% of the accumulated copper was associated with the cell wall.

A two-step metal-binding process (Fig. 6) has been reported. The first step involves the interaction of metal with the reactive metal-binding group on the cell wall, which is a stoichiometric interaction followed by an inanimate deposition of the interacted metal. This process (known as ion exchange) is complex in nature and comparable with commercial resin (Göksungur et al. 2005). Variation in cell wall composition and the presence of different functional groups are responsible for controlling the amount and type of metal ion to be absorbed. A number of metal-binding and sequestering mechanisms are attributed to the fungal cell wall's formulation. In a living fungal cell, metal sorption will be metabolic dependent or independent but in dead cell biomass it will be the latter one (Dursun 2006; Akar et al. 2007).

Three kinds of metal interaction and sorption can take place: (1) outer surface uptake, (2) intracellular accumulation, and

Table 6. Metal Interaction with Functional Groups of Various Fungal Biomasses

Metal	Fungal species	Status of Biomass	Metal binding group	Efficiency (%)	References
		Absorption			
Hg	*Mucor rouxii* IM-80	Living cells	Amino group	95.3	Martínez-Juárez et al. (2012)
	Mucor rouxii mutant	Living cells	Carboxyl group	88.7	Martínez-Juárez et al. (2012)
	Mucor rouxii Sp.1	Living cells	N_2 and O_2 of peptide bond	80.4	Martínez-Juárez et al. (2012)
Cd	*Saccharomyces cerevisiae*	Immobilized	Carboxyl, PO_4^{3-}, OH^-, amino, S^-	80–85	Tonk et al. (2011)
Pb, Cd & Cu	*Galerina vittiformis*	Fruiting body	Extra cellular polysaccharides	90	Damodaran et al. (2013)
Cd, Pb& Cu	*Galerina vittiformis*	Mycelia biomass	Extra cellular polysaccharides	75–80	Damodaran et al. (2013)
Ni, Cu, Zn, Cd, and Pb	*Cladosporium cladosporoides*	Living cell	Fungal melanin	85–90	Fogarty and Tobin (1996)
		Adsorption to cell surface			
Sb	*Agaricus campester*	Dead cells		95	Tomko et al. (2006)
Cu	*Trametes gibbosa*	Dead biomass	Carboxyl group	90	Tomko et al. (2006)
Pb	*Aspergillus niger*	Pretreated dead biomass	Amino group, OH^- group	75–80	Raja et al. (2013)
Ni	*Aspergillus niger*	Pretreated dead biomass	Amino group, OH^- group	97	Raja et al. (2013)
^{241}Am	*Aspergillu sniger*	Spore	—	80–85	Yang et al. (2004)
^{241}Am	*Aspergillus niger*	Hyphae	—	75–80	Yang et al. (2004)
Cr	*Aspergillus terreus*	Immobilized in polyurethanee foam	Carboxyl, PO_4^-, OH^-, amino, S^-	96.5	Dias et al. (2002)
Cd	*Penicillium canescens*	Dead biomass	Carboxyl, PO_4^-, OH^-, amino, S^-	96	Say et al. (2003)
Pb	*Penicillium canescens*	Dead biomass	Carboxyl, PO_4^-, OH^-, amino, S^-	95	Say et al. (2003)
Cr	*Trichoder magamsii*	Acid treated biomass	multiple-site type binding	97	Kavita and Keharia (2012)
		1on exchange			
Pb	*Mucor rouxii*	Metabolically inactive	K^+, Ca^+	90	Lo et al. (1999)
Cd, Cu, Zn, Ni & Co	*Aspergillus niger*	Alkali extracted mycelial biomass	Carboxyl group	70–80	Kapoor et al. (1997)
Co	*Penicillium cyclopium*	Raw dead biomass	OH^-, amide and carboxyl group	100	Tsekova et al. (2006)
Mo, Vd	*Rhizopus arrhizus*	Dead biomass	Amino group	90	Tobin et al. (1984)
		Metal complexation			
Cu	*Penicillium cyclopium*	Raw dead biomass	OH^-, amide and carboxyl group	100	Tsekova et al. (2006)
Cr, Mn, Cu, Zn and Cd	*Rhizopus arrhizus*	Dead raw biomass	Phosphate and carboxylate group	95–90	Tobin et al. (1984)
Hg, Pb2 and Ag	*Rhizopus arrhizus*	Dead biomass	Phosphate and carboxylate group	75–80	Tobin et al. (1984)

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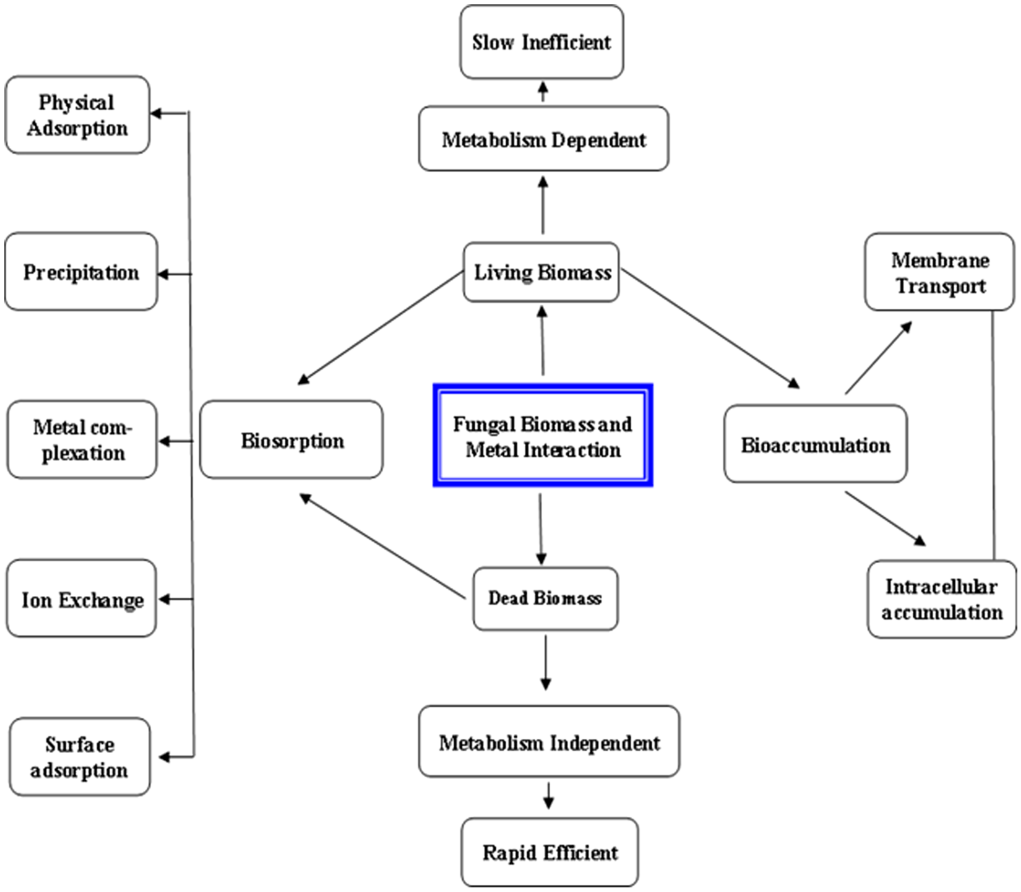

Fig. 6. Metal and fungal biomass interaction

(3) precipitation outside the cell. The premier cell surface sorption takes place by physicochemical interaction between active functional groups of the fungal cell surface and metal present in the solution (Cho and Kim 2003). These physicochemical interactions are ion exchange, surface adsorption, chemical sorption (complexation), metal co-ordination, and metal chelation (Fig. 5). All are rapid irreversible and do not depend upon cell metabolism. The cell metabolism-dependent metal uptake mechanism via cell membrane yields metal accumulation inside the cell. It takes place only in viable cells and is also considered as an active defense mechanism of fungi as a response to metal toxicity (Ahluwalia and Goyal 2007).

Precipitation of metals outside the cell occurs on the cell surface as well as in the solution. Precipitation is considered to be a metabolically dependent process when the fungal cell secretes such substances that favor precipitation; otherwise it is independent because no intracellular secretions are involved and metal–surface chemical interactions induce precipitation. It has been reported (Göksungur et al. 2005; Javaid and Bajwa 2008; Simonesco and Ferdes 2012) that various mechanisms work independently but with mutual relation to augment overall metal uptake.

Fungal strains have been isolated from tannery effluent, and process parameters are optimized in the presence of the toxic form of chromium with biotechnological methods for its removal from tannery effluent and soil (Srivastava and Thakur 2006). Live or dead fungal biomass can be used for the removal of toxic metal ions (Kapoor et al. 1999). The use of dead cells seems to be more advantageous than using live cells in metal ion removal. However, live biomass was able to remove nickel more effectively than pretreated biomass, indicating that *A. niger* biomass may have taken up some nickel intracellularly (Kapoor et al. 1999).

There are reports of live microbial systems for the purpose of remediation of contaminated soils and waters (Kratochvil et al. 1998). Growing microbes can be used (Malik 2004) as a feasible alternative to remove metal contamination from industrial effluents, and such a removal may be purely biosorptive in nature. Isolating fungi from polluted environments would provide the metal-resistant strains appropriate for the bioremediation purpose (Zucconi et al. 2003; Malik 2004). In a study of fungi isolated from the tanning effluent on EQ-Cr by Prigione et al. (2009), *F. solani* was selected for biosorption experiments because it was the only fungus able to grow in vitro into the tanning effluent, demonstrating a real adaptation to such a polluted environment. It was recommended that in the future, it would be very interesting to assess the chromium (III) active uptake capacity of this fungus. *A. versicolor* has been shown to be capable of accumulating heavy metals such as chromium (VI), copper (II), and nickel (II) (Tasten et al. 2010).

Similarly, several other fungal-based biomasses have been reported to be extremely useful for extensive biosorption of hexavalent cations of chromium (Table 7). In view of various advantages of the biosorption process, such as reusability of biomaterial, small period for operation, heavy metal removal from wastewater irrespective of toxicity, and production of no secondary compound, it has become more important than conventional methods (Modak and Natarajan 1995). However, any of these biosorbents has been sparsely applied to remove hexavalent chromium from wastewater (Wang and Chen 2009; Chojnacka 2010).

Table 7. Comparison of Different Fungal Biomasses for Biosorption of Hexavalent Cations of Cr

Fungal biosorbents	Maximum biosorption capacity (mg/g biomass)	Citation
Aspergillus sp.	8.40	Das and Guha (2009)
Neurospora crassa	12.70	Tunali et al. (2005)
Rhizopus arrhizus	30.5	Aksu and Balibek (2007)

Toxic Metal Tolerance and Stress Response in Fungi

Various types of analyses (such as deletome, genome, interactome, transcriptome, proteome, and metabolome) carried out in baker's yeast cultures are the main basis of available information related to metal toxicity in fungi. Presently available knowledge regarding the mode of action (i.e., mechanism) and the tolerance against toxic metals corresponds to yeast-based models. Numerous studies have revealed that it can be quite relevant to higher eukaryotic organisms including fungi (Tamás et al. 2005; Wysocki and Tamás 2010). On the other hand, yeast-based models cannot be fully applicable for mapping of pathways related to regulatory and metabolic activities in other fungi (even in the case of ascomycetes) due to the wide evolutionary distances between other major fungal taxons and hemiascomyctes. This limitation can be exemplified considering the Yap8p-dependent regulation of As (III) stress response, which is a pathway in baker's yeast. As it seems to be clearly specific for hemiascomycete, its exploitation in other fungi (Wysocki and Tamás 2010) cannot be made. Some additional models of fungi, which are evolutionarily closer to the mycorrhizal species (a heavy metal-exposed fungal taxon) have also been set up (González-Guerrero et al. 2009). In the recent past, three main lines of defense have been described in the context of fungi.

First Line of Defense: Extracellular Chelation and Binding to Cell Wall Constituents

Low molecular mass metabolites, peptides, and proteins play a crucial role via chelation with metals during almost all the processes of metal detoxification (Tamás et al. 2005; González-Guerrero et al. 2009; Wysocki and Tamás 2010) and, hence, overestimation of the significance of extracellular and cytosolic chelation reactions must be avoided. GSH-homeostasis in yeast involves the secretion of glutathione (GSH) under different conditions, and GSH-secretion by yeast cells gets intensified under exposure to trivalent cations of arsenic to relieve the intracellular pathways of detoxification (Wysocki and Tamás 2010).

Second Line of Defense: Transport, Intracellular Coordination with Bidentate and/or Multidentate Ligands and Compartmentalization

Channels and transporters facilitate the entrance of heavy metals into cells. These are also responsible for the normal uptake of essential micronutrients like iron, manganese, and zinc, anions including phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}) as well as sugars like glucose, and sugar derivatives (glycerol) (Tamás et al. 2005; Wysocki and Tamás 2010). Elimination of the channel (used for the transportation of a specific toxic metal ion) may be one of the simplest and most effective ways to keep off toxic metals outside the cell. Oxalate secretion has been reported in both white-rot and brown-rot fungi, and stimulation of this process seems to occur under stress related to divalent cations of copper and cadmium (Clausen and Green 2003; Jarosz-Wilkołazka et al. 2006). An efficient way for preventing the entrance of ions of toxic metals into cells of fungi (Jarosz-Wilkołazka and Gadd 2003) is the formation of crystals of metal-oxalate in bulk. These crystals are not soluble in water. Maintenance of the lignolytic system in case of white rot basidimycetes (Schlosser and Höfer 2002) is also a primary importance of oxalate.

Third Line of Defense: The Antioxidative Defense System

Commonly, exposure of fungi to toxic metal stress also causes oxidative cell injuries due to reactive oxygen species (Avery 2001). Fungal cells show many antioxidants to handle the different types of oxidative stress. For example, remarkable efficiency has been noticed in neutralization of reactive oxygen species with GSH-dependent and GSH-independent enzyme activities (Pócsi et al. 2004).

Fungal Biosensors for Environmental Metal Pollution

Conventional heavy metal detection techniques include urbane chromatography, use of atomic absorption spectrometer, emission spectrophotometery, and inbuilt coupled mass spectrometry. These techniques need costly, sophisticated, powerful, highly selective, and sensitive instrumentation and rely on sample pre-preparation, a skilled technician, and lengthy calibrating periods (Su et al. 2011; Bereza-Malcolm et al. 2014).

Consequently, biosensors become an easy, rapid, renewable, and cost-effective method for metal detection (Dragone et al. 2014; Datta et al. 2013). Effective methods of detection are thus greatly needed as preventive measures. Biosensors are autonomous unified instruments that moderately detect and quantify metal using a biochemical receptor that acts as a fundamental recognition element. Biosensors are composed of two main installments: a biological receptor (nucleic acid, enzyme, antigen, antibodies, and lectins) and a physicochemical (electrical, electrochemical, Piezoelectric, optical, or thermal) transducer (Su et al. 2011). This unit attached with amplification element and a pollutant transport control eclectic membrane. The biological material coupled with transducer may comprise the whole cell organism or its parts like soluble proteins, exopolysacharides, receptors, and organelles. In a microbial biosensor, any bacterial or fungal species may be integrated with the physical transducer to sense a quantifiable signal proportional to the absorption of analytes. Fungi-based whole-cell biosensors have overcome prokaryotic bacterial biosensors in many ways (Bereza-Malcolm et al. 2014; Dragone et al. 2014; Gutierrez et al. 2015). Cell suspension, membrane entrapment, and cell immobilization are all used in the construction of fungal biosensors. There are two types of fungi-based biosensors known so far. These are electrochemical and optical microbial biosensors. Some of the classes of electrochemical microbial biosensors are amperometric, potentiometric, conductometric, and microbial fuel cell-based sensors, whereas optical microbial sensors contained bioluminescence, fluorescent, and colorimetric biosensors. Bioluminescent fungal biosensors are primarily and now widely used to monitor and scrutinize bioavailable metal as nutrients (Datta 2013; Bereza-Malcolm et al. 2014) as well as other toxic organic pollutants (Gutierrez et al. 2015). In contrast, biochemical oxygen demand (BOD) can also be efficiently measured using whole-cell fungal biosensors (Su et al. 2011).

For metal testing, fungi-based bioluminescence biosensors proved to be the most suitable tool. Both natural and genetically

modified bioluminescent fungi (filamentous and yeast) are used for metal detection; in addition, they have replaced the existing bioluminescent bacterial species. A total of nine genera's 42 natural fungi have been discovered, dominated by basidiomycetes bioluminescent. The most common species reported of luminescent basidiomycetes are *Omphalotus olearius*, *Armillaria melle*, and *Panellus stipticus*. Luminescence of fungal species has been reported in mycelia like in *Mycena* spp.; in contrast *P. stipticus and O. olearius* have luminescence in their fruiting bodies as well. Choe et al. (2012) reported *Aspergillus niger* as highly arsenic resistant and its use for arsenic detection (both arsenite and arsenate) using florescent transducer. *S. cerevisiae* cells can detect copper ions As^{3+}, Cd^{2+}, and Hg^{2} even at very low concentration (Su et al. 2011; Adeniran et al. 2014). Pradhan et al. (2014) suggested fungi as an efficient renewable alternative for Cu detection in polluted soil. Kushwah et al. (2014) extract and purify laccase enzyme from *Agaricus bisporous* and developed a low-cost metal biosensor. Although these biosensors have remarkable sensitivity and accuracy, the stability and storability of these bioanalytical tools are yet to be improved.

Conclusion

Classification and general characteristics of fungi including the composition of the cell wall and different kinds of organic ligands present in the cell wall have been discussed. Various aspects of heavy metal removal using this interesting class of biosorbents have been critically analyzed. The influence of factors like pH, temperature, biomass loading, biomass intact time, type of fungal specie, and presence of other metal ions on the fungal metal interaction has been addressed. Regeneration of fungal biomass, which can be used for further biosorption process and to increase the possibility of the application of the biomass at industrial scale, has been a part of the focus. Many fungal species are useful biosensors of environmental metal pollution.

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