Review of Literature
2.1 A Short Introduction to Soil Fungi

Fungi are eukaryotic organisms and form a separate kingdom from plants and animals. Phyla ascomycota and basidiomycota comprise about 80% of the all described fungal species and are either single celled organisms growing as yeast, or multi-cellular organisms growing as filamentous hyphae (Berbee and Taylor, 1999). The hyphal growth mode of filamentous fungi is well adapted to explore and exploit nutrient sources in the highly heterogeneous soil environment (Robson, 1999).

In many basidiomycetes, hyphal aggregates, called rhizomorphs, are formed behind the mycelial front. In these, elonged vessels may develop, surrounded by closely packed hydrophobic hyphae. Rhizomorphs are units of spread, survival and long-distance translocation of nutrients and water in the mycelia (Jennings and Lysek, 1996). By applying concurrent explorative and exploitative growth strategies the mycelia of rhizomorph-forming basidiomycetes grow and differentiate in response to the spatial distribution of nutrient and moisture resources within the growth substrate (Ritz and Crawford, 1990). A fungalmycelium acts as a single, interconnected functional unit, translocating resources within the network of hyphae and rhizomorphs. The ability of mycelia to connect different mineral and carbon sources enables translocation of heterogeneously distributed nutrients and moisture through the mycelia. This makes resource utilisation more effective and increases stress tolerance in filamentous fungi compared to single celled organisms (Hirsch et al., 1995).

Filamentous fungi have the ability to adopt both explorative and exploitative growth strategies, and the formation of linear organs of aggregated hyphae for protected fungal translocation (Fomina et al., 2005a and 2005b). Some fungi are polymorphic, occurring as both filamentous mycelium and unicellular yeasts or yeast-like cells, as in black meristematic or microcolonial fungi colonizing rocks (Sterflinger, 2000; Gorbushina et al., 2002a, 2002b and 2003). Fungi can also grow inside their own parental hyphae, utilizing dead parts of the colony under the protection of parental cell walls (Gorbushina et al., 2003). The ability of fungi to translocate nutrients through the mycelial network is important feature for exploring heterogeneous environments (Lindahl and Olsson, 2004; Jacobs et al., 2002a, 2002b and 2004; Boswell et al., 2002, 2003 and 2006).
2.2 Significance of Soil Fungi

Fungi are chemoheterotrophic organisms and have ubiquitous nature in subaerial and subsoil environments, and considered as important decomposers, mutualistic symbionts of animal and plants. They are also pathogens, and spoilage organisms of natural and manufactured materials (Gadd 1993a, 1999 and 2006; Burford et al., 2003a). Fungi also have an important role in the maintenance of soil structure, due to their filamentous branching growth habit and frequent exopolymer production. A fungal role in biogeochemical cycling of the elements (e.g. carbon, nitrogen, phosphorus, sulphur, metals) is obvious 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., 2003a; Gadd, 2004; Braissant et al., 2004; Fomina et al., 2005a). In aerobic environments they are of great importance, especially when considering rock surfaces, soil and the plant root–soil interface (Gadd, 2005 and 2006; Fomina et al., 2005a and 2005b; Gadd et al., 2005a, 2005b and 2006). For example, mycorrhizal fungi are associated with 80% of plant species, and are involved in major mineral transformations and redistributions of inorganic nutrients, e.g. essential metals and phosphate, as well as carbon flow (Paris et al., 1995; Hoffland et al., 2002; Fomina et al., 2004 and 2005b). Free-living fungi have also major roles in the decomposition of plant and other organic materials like cellulose, lignin, chitin etc, including xenobiotics, as well as mineral solubilization (Gadd, 2004).

Fungi are also major biodeterioration agents of stone, wood, plaster, cement and other building materials, and they are also important components of rock-inhabiting microbial communities with significant roles in mineral dissolution and secondary mineral formation (Hughes and Lawley, 2003; Burford et al., 2003a, 2003b and 2006; Fomina et al., 2005a and 2005b). There is even some evidence that several fungi can dissolve minerals and mobilize metals at higher pH values, and over a wider redox range, faster and more efficiently than bacteria (Gu et al., 1998; Castro et al., 2000; Burford et al., 2003a). The majority of fungi inhabit soil environments, which are seemingly much more hospitable than bare rock surfaces. Fungal communities in soil are diverse and include free-living and symbiotic fungi, as well as plant and animal pathogens, and unicellular yeasts.
Fig. A: Simple model of fungal action on naturally-occurring and/or anthropogenically-derived organic and inorganic substrates. (1) Organic and inorganic transformations mediated by enzymes and metabolites, e.g. protons (H\(^+\)), carbon dioxide (CO\(_2\)), and organic acids, and physicochemical changes occurring as a result of metabolism; (2) uptake, metabolism or degradation of organic substrates; (3) uptake, accumulation, sorption, metabolism of inorganic substrates; (4) production of organic metabolites, exopolymers, and biomass; (5) production of inorganic metabolites, secondary minerals and transformed metal(loids); and (6) chemical interactions between organic and inorganic substances, e.g. complexation and chelation (from Gadd 2004).

Fungi encounter metals as normal components of the natural environment, as well as those introduced or redistribute by human activities. Like other organisms, fungi possess a variety of properties that can influence interactions with metals, although metal toxicity can be influenced by the physico-chemical attributes of the environment; fungi possess a variety of intrinsic and inducible properties that can ensure survival. It seems fungi can be isolated from any habitats polluted by toxic metals (Gadd, 2007). Fungi have also the role in plant growth promotion and soil health. They produce a large number of secondary metabolites (IAA, siderophores, ammonia, organic acids, antibiotics, extracellular enzymes etc.), enhance the plant growth, crop productivity and soil fertility (Field et al., 1993; Archer et al., 1994; Akthar and Mohan, 1995; Feijoo and Lema, 1995; Prasertsan et al., 1997; Radzio and Kuck, 1997; Wongwicharn et al., 1999; Palma et al., 1999; Xu et al., 2000, Coulibaly, 2002).

2.3 Heavy Metal Pollution

Heavy metal is a dangerous group of soil pollutants. The contamination by heavy metals causes a serious problem because they cannot be naturally degraded like organic pollutants and they accumulate in different parts of the food
The Pollution of the biosphere by the toxic metals has accelerated dramatically since the beginning of industrial revolution (McIveen and Negusanti, 1994). Metals such as lead, arsenic, cadmium, copper, zinc, nickel, and mercury are continuously being added to our soils through various agricultural activities such as agrochemicals usage and long-term application of urban sewage sludge in agricultural soils, industrial activities such as waste disposal, waste incineration and vehicle exhausts, as well as from anthropogenic sources. All these sources cause accumulation of metals and metalloids in our agricultural soils and pose threat to food safety issues and potential health risks due to soil-to-plant transfer of metals. Co-existence and persistence of heavy metals in soils as multiple contaminants and human exposure to them through ingestion of heavy metal contaminated food or uptake of contaminated drinking water can lead to their accumulation in humans, plants and animals. They can also cause a considerable detrimental effect on soil ecosystems, environment and human health due to their mobilities and solubilities which determine their speciation (Kabata-Pendias, 1992; Del Val et al., 1999). In some cases, the 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 bodies, particularly in developing countries (Yanez et al., 2002; Khan, 2005). Metal toxicities have received widespread attention due to increasing number of toxic metals being released into the environment, their extended persistence and toxicity to a wide variety of organisms. Cadmium (Cd) is ubiquitous in the human environment and has been recognized as one of the most deleterious heavy metal pollutants (Robards and Worsfold, 1991; Christine, 1997). It may easily transfer from soil to food plants through root absorption and accumulate in their tissues (Oliver, 1997; Ortega-Larrocea et al., 2007). In this way, Cd may enter the food chain and affect human health (Adriano, 1986; Smith, 1996; Jose et al., 2002; Yao et al., 2003).

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, wood-preserving facilities, and petroleum refineries (USEPA, 1990; Allen et al., 1998). These Cr wastes create a serious threat to water quality and the environment. Chromium (VI) is toxic to biological systems due to its strong
oxidizing potential that can damage cells (Ko-tas and Stasicka, 2000). Inside cells, Cr (III) formed from Cr (VI) can complex with organic compounds, interfering with metallo–enzyme systems at high concentrations (Kotas and Stasicka, 2000). Chromium (VI) has been shown to have carcinogenic and allergenic effects in humans and animals, while Cr (III) is considered a trace element essential for living systems (Costa, 1997; Nies, 1999).

The chemical form of chromium determines its availability for microorganisms. The difference in toxicity between compounds of tri- and hexavalent chromium is determined by their different chemical properties (Czekala et al., 1996). Because toxicity of hexavalent chromium is from 100 to 1000 times higher than trivalent (onta and Hattori, 1983; Wyszowska et al., 2001). Many other peoples also reported the 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).

2.4 METAL-FUNGI INTERACTION

2.4.1 Fungi in Metal Polluted Soils and Metal Rich Environments

Fossil fuel combustion, mineral mining and processing, and the production 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 the biota (Gadd and Griffiths, 1978; Gadd, 1992a, 2000c, 2005 and 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 processes 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. Researchers have revealed that heavy metal toxicity reduces microbial numbers and mitigates their activity and greatly affects microbe-mediated processes in soil ecosystems, such as organic matter decomposition (Brookes and McGrath, 1984; Chander and Brookes, 1991; Aoyama and Nagumo, 1997a and 1997b; Kuperman and Carreiro, 1997; Khan and Scullion, 2000; Olayinka and
Babalola, 2001). The frequency of tolerant microbial population may increase with an increase in toxic metal levels (Olson and Thornton, 1982; Huysman et al., 1994; Kunito et al., 1997). Resistant fungal species are mostly present at low frequencies in non-metal polluted soils, but can become dominant under toxic metal stress (Kunito et al., 1998).

Mycelial fungi can develop a significant biomass in soil and may sequester considerable amounts of metals (Massaccesi et al., 2002). Several observations have shown that microbial population responses to toxic metals are characterized by a shift from single-celled bacteria and streptomycetes to fungi (Mineev et al., 1999; Chander et al., 2001a and 2001b; Kostov and van Cleemput, 2001; Olayinka and Babalola, 2001; Khan and Scullion, 2002). The different responses of bacteria and fungi to toxic metals were reflected in an increase in the relative fungal: bacterial ratio (estimated using phospholipid fatty acid analysis) with increased metal concentrations (Rajapaksha et al., 2004). All nutritional groups of fungi (saprotrophs, biotrophs, and necrotrophs) can be affected by toxic metals. Toxic metal (cadmium, chromium, copper, nickel, lead and zinc) pollution of soil led to a significant decrease in the number of AM fungi and low colonization of plant roots, and as a result, to changes in the species diversity of mycorrhizal fungi (Mozafar et al., 2002; Moynahan et al., 2002, Repetto et al., 2007).

Zygomycetes were more tolerant to cadmium than ascomycetes and conidial fungi (Plaza et al., 1998). The most frequent soil saprotrophic microfungi isolated from heavily metal polluted habitats in Argentina, Czech Republic and Ukraine were species of Penicillium, Aspergillus, Trichoderma, Fusarium, Rhizopus, and Mucor, as well as Paecilomyces lilacinus, Acrostalagmus luteo-albus (as ‘Nectria inventa’). Cladosporium cladosporioides, Alternaria alternata, and Phoma fimeti (Kubatova et al., 2002; Massaccesi et al., 2002). Melanized fungi (e.g. Cladosporium spp., Alternaria alternata, Aureobasidium pullulans), are often isolated from soil samples treated with toxic industrial effluents containing high concentrations of copper and mercury (Zhdanova et al., 1986) and may also be dominant members of the mycobiota of metal-contaminated phylloplanes (Mowll and Gadd, 1985).

Aspergillus strains were more cadmium tolerant than strains of Penicillium (Plaza et al., 1998), but Penicillium species are often reported to be dominant in
copper-contaminated environments. In Brazilian soils with copper concentrations 25–11 500 mg kg\(^{-1}\) the predominance of a *Penicillium* species tolerant to a copper concentration of 750 mg kg\(^{-1}\) in soil was found (Ribeiro *et al*., 1972). A very high predominance of *Penicillium* species (80 % of isolations) was observed in freshly excavated archeological soil, containing 500 mg kg\(^{-1}\) copper and 500 mg kg\(^{-1}\) lead, at the site of a Bronze Age ancient Greek copper-smelting furnace (Olviya, Ukraine) (Gadd, 2007).

### 2.4.2 Heavy Metal Effect on Microbial Population

Different biomass measurements or plate counting techniques have also indicated that heavy metals have negative effect on bacteria and fungi in soil differently (Maliszewska *et al*., 1985; Hiroki, 1992; Müller *et al*., 2001; Khan and Scullion, 2002; Rajapaksha *et al*., 2004). Bond *et al.* (1976) found no effect on CFUs for bacteria and fungi after addition of 10\(\mu\)g Cd g\(^{-1}\) to Douglas fir forest litter microcosms. Freedman and Hutchinson (1980b) did find a decrease in fungal CFUs near the Sudbury smelter, but the decrease was not significantly different from the non-polluted soil samples. Zibliske and Wagner (1982) found bacterial CFUs to be less affected by addition of Cd, Cu, and Cr than soil ATP, which is an indicator of the total microbial biomass.

The fungus CFUs are less sensitive measurement than actual fungal biomass, measured as fungal length, was shown by Nordgren *et al.* (1983). While no effect on CFUs was found, even in areas with more than 10000\(\mu\)g Cu g\(^{-1}\) soil, and approximately the same level of Zn, fungal total length and FDA –active fungal length was affected at much lower level, 1000 \(\mu\)g Cu g\(^{-1}\) +1500 \(\mu\)g Zn g\(^{-1}\) soil.

A reduction in abundance and biomass of fungi and bacteria due to heavy metals has been detected in numerous investigations. Fungi appear to be generally more tolerant than bacteria. Among bacteria, gram negative appear to be more tolerant than gram positive ones, while selective groups, like *Azotobacter* and nitrifiers, have been reported to be especially sensitive to heavy metal pollution (Pancholy *et al*., 1975; Maliszewska *et al*., 1985).
Arnebrant *et al.* (1987) found no effect of pollution level on total fungal CFU in the Gusum area. However, the number of fungi capable of growth on malt agar amended with 200 mg Cu/litre differed according to the pollution level. The percentage of such tolerant fungi increased from 3.1% in control sites to 24% in intermediately polluted sites. Similar results were reported by Jordan and Lechevalier (1975), who found 24% Zn tolerant fungi in Zn polluted soil, compared to 4.5% in a control soil. Yamamoto *et al.* (1985) added Cu to a paddy field soil and found no differences in the number of fungi growing on non amended media or media with 100 mg Cu/litre between control samples and soil samples amended with 1600 μg Cu g⁻¹ soil. The use of 1000 mg Cu/litre in the media, however, clearly differentiated between different soil treatments.

Nordgren *et al.* (1985) found only minor effects on microfungal species diversity, except at contamination levels of about 10000 μg Cu g⁻¹ soil in the Gusum area. A shift in the species composition was, however, evident at Cu levels over 1000 μg Cu g⁻¹. Other studies have also found an altered microfungal species composition due to metal pollution, for example Zn (Jordan and Lechevalier, 1975), Cd, Cu, Pb and Zn (Hartman, 1976) and Cu (Kendrick, 1962). Carter (1978) reported a significant change in fungal community structure, while Freedman and Hutchinson (1980b) found no change at the same site.

Usually a decrease in commonly isolated genera such as *Penicillium*, oidiodyamrun and mortierella are found in metal polluted soils (Jordan and Lechevalier, 1975; Nordgren *et al.*, 1985; Arnebrant *et al.*, 1987; Williams *et al.*, 1977). However, Yamamoto *et al.* (1981) found a significant flora, with a dominant of *Penicillium* spp. in soil polluted by drainage.

Large variations in metal tolerance exist within single fungal genera (e.g. Arnebrant *et al.*, 1987). Carter (1978) found waksmanii to be abundant only in polluted soils and Arnebrant *et al.* (1987) found some species of *Mortierella* and *Penicillium* only in polluted soil samples, although other genera disappeared in sites with high metal concentrations.

Ruhling (1983) found no indication of differences between mycorhizal and decomposer fungi in their reaction to heavy metals. It has been suggested that mycorhizal fungi may modify metal toxicity to their host plant.
genera have been found on metal containing sites, for example Laccaria, Thelephora, Paxillus, and Boletus on arsenic-containing mine waste (Pyatt, 1973; Benson et al. 1980). Pisolithus on Ni tailings (Malloch and Duja, 1979), Suillus, Amanita, Thelephora and Paxillus on Zn and Cd contaminated sand (Colpaert and Van Assche, 1987), and Laccaria and Paxillus on U mill tailings (Kalin and Stokes, 1981).

Heavy metals appear to induce a shift towards more gram negative bacteria compared to gram-positive. Thus, Doelman and Haanstra (1979c) found more gram-negative bacteria tolerant to Pb and Barkay et al. (1985) found more pseudomonas species in sludge amended soils with increased levels of Cd. Similar trends in soils to Cd, Co, Cu, Hg, Ni, and Zn were found by Duxbury and Bicknell (1983). Barkay et al. (1985) found no differences in species composition and diversity for the total bacterial community in control and sludge amended soils. However, the Cd tolerant bacterial community had a higher diversity in sludge amended soils, with a predominance of Pseudomonas spp.

Baath et al. (2005) reported the heavy metal effect on microbial population in forest site in Norway. They observed that the high Pb concentrations in the soil resulted in increased tolerance to Pb of the bacterial community, measured using both thymidine incorporation and plate counts. Furthermore, changes in tolerance were correlated to changes in the community structure. The bacterial community of the most contaminated soils showed higher specific activity (thymidine and leucine incorporation rates) and higher culturability than that of control soils. Fungal colony forming units (CFUs) were 10 times lower in the most Pb-enriched soils, the species composition was widely different from that in control soils, and the isolated fungi had high Pb tolerance. The most commonly isolated fungus in Pb-enriched soils was Tolypocladium inflatum. Comparison of isolates from Pb-enriched soil and isolates from unpolluted soils showed that T. inflatum was intrinsically Pb-tolerant, and that the prolonged conditions with high Pb had not selected for any increased tolerance.

Oliveira et al. (2006) investigated total heavy metal content and its effects on soil microbiological characteristics in soil from an area known long-term pollution problem. The total heavy metal concentrations of contaminated soils samples were 109 and 1558 mg/kg for Hg and As, respectively. Key microbiological parameters measured included dehydrogenase activity. ATP content and number of culturable
bacteria, actinomycetes, fungi and asymbiotic nitrogen fixers. Quantitative analysis of soil microbial populations shows a marked decrease in total culturable numbers of the different microbial groups of the contaminated soil samples. Certain groups of soil microbes were particularly sensitive to long term contamination (asymbiotic nitrogen-fixer and heterotrophic bacteria).

Joshi (2008) also reported the heavy metal effect on fungal and bacterial population. He observed that the bacterial population was higher at the unpolluted site. Bacterial population showed a significant negative correlation with lead, zinc, copper, cadmium and sulphur. Similarly, fungal population was higher at the unpolluted site. A total of 29 fungal species were isolated from the phylloplane of A. nepalensis (polluted site 16 species; unpolluted site 28 species). Some fungal forms like Mortierella sp., Fusarium oxysporum and Aureobasidium pullulans were dominant in the polluted site. Numbers of phylloplane fungi and bacteria were significantly reduced in the polluted site.

Shukla et al. (2009) revealed the microbial population in tannery effluent containing Cr (IV) during its composting phases. They revealed that the profile of microbial communities indicated that population of anaerobic, aerobic and nitrifying bacteria increased quickly at the initial phase, and reached a peak level of $4.2 \times 10^6$, $9.78 \times 10^8$ and $9.32 \times 10^9$ CFU g$^{-1}$, respectively at 21 d; while population of actinomycetes and fungi was found maximum i.e. $3.29 \times 10^7$ and $9.7 \times 10^6$ CFU g$^{-1}$, respectively, after 35 d of composting. Overall bacterial population dominated over the actinomycetes and fungi during the composting process. Cr (VI)) was transformed to Cr ((III)) due to the microbial activity during the process.

2.4.3 Effect of Metal Contamination on Soil Microbiological Activities

Toxic effects of heavy metals on soil microorganisms have been extensively studied in the past (Baath, 1989; Van and Doelman, 1997; Giller et al., 1998), and almost every group of organisms has been studied in this respect. The biological effects of this heavy metal pollution have been studied for 10 years in the forests surrounding the brass mill in a small town of south Sweden. Considerable damage to the vegetation has been reported (Folkesson, 1981), Strong suppression of soil
respiration, decomposition, and soil enzymatic activities has been found (Tyler, 1975a; Tyler, 1975b; Gibson and Mitchell, 2005; Kao et al., 2006).

Although the toxic effect of heavy metals on soil microorganism activity is well known, little is known about the effects on different organism groups. The influence of heavy metal addition on total, bacterial, and fungal activities was studied by Rajapaksha et al. (2004) for up to 60 days in a laboratory experiment using forest soil contaminated with different concentrations of Zn or Cu. The effects of the metals varied between the different activity measurements. During the first week after metal addition, they found decrease by 30% in total activity (respiration rate) at the highest level of contamination and then remained stable during the 60 days of incubation. Fungal activity (acetate-in-ergosterol incorporation rate) initially increased with the level of metal contamination, being up to 3 and 7 times higher than that in the control samples during the first week at the highest levels of Zn and Cu addition, respectively. The positive effect of metal addition on fungal activity then decreased, but fungal activity was still higher in contaminated than in control soil after 35 days. This is the first direct evidence that fungal and bacterial activities in soil are differently affected by heavy metals.

2.4.3.1 Growth and metabolic activity

After heavy metals enter the fungal cell, they affect both individual reactions and complex metabolic processes. Cd and Hg are in general the most toxic metals almost for all Fungi (Lilly et al., 1992). Addition of 0.05–0.25mM mercury to growing cultures of Phanerochaete chrysosporium led to decrease of growth rate and in higher Hg concentrations lysis of the mycelium occurred, accompanied by the decrease in protein contents of the mycelia (Dhawale et al., 1996; Baldrian et al., 2000).

2.4.3.2 Enzymatic activities

Heavy metals in general are powerful inhibitors of enzymatic reactions (Vallee and Ulmer, 1972). Mercury exerts its toxic effect mainly by binding to SH groups present in the active or regulation sites of enzyme and causing their
irreversible inactivation. Copper and cadmium—in addition to binding to aromatic amino acid residues in enzyme molecules, can also cause oxidative damage of proteins by the induction of oxidative stress associated with the production of reactive oxygen species like hydroxyl or superoxide radicals (Stohs and Bagchi, 1995). Exposure to Cd reduced activities of soil alkaline phosphatase, arylsulphatase and protease (Wilke, 1988; Renella et al., 2005). Enzyme activities may also be reduced by binding of Cd2C to sulphydryl groups (Sanadi, 1982; Gülser and Erdogan, 2008).

2.4.3.3 Carbon and Nitrogen Mineralization

Heavy metals are the main concern in regarding biosolid use on land, and in some cases are toxic to humans and animals. However, microbial activities of soil, such as biomass, respiration and N mineralization, were direct responses to the application of biosolid containing heavy metals reported by previous studies (Giller et al., 1998; Vig et al., 2003). Application of biosolid containing heavy metals to soil has reserve effects in quality and quantity on microbial biomass (Fließbach et al., 1994; Kuperman and Carreiro, 1997; Hernaández et al., 2002), C and N mineralization (Chander et al., 1995; Khan and Scullion, 2002; Aka and Darici, 2004), enzyme activity (Kuperman and Carreiro, 1997) and microbial community structures (Frostegard et al., 1993; Aceves et al., 2007).

2.5 METAL TOXICITY FOR SOIL FUNGI

2.5.1 Metal Resistance and Tolerance

Toxic metals, especially cadmium, copper, mercury, manganese, and zinc, are increasingly being released into the environment from industrial wastewater and other human activities. Some, for example, copper, iron, manganese and zinc, are essential micro-nutrients for most, if not all, living organisms. However, when the concentrations of beneficial metals in the environment are excessively high, or when metals with no known essential biological functions are present, for instance, mercury, lead, or cadmium, can become toxic (Gadd, 1986; Dedyukhina and Eroshin, 1991; Errasquin, and Vazquez, 2003). Many microbial species tolerate arsenic, but the underlying mechanisms of this in filamentous fungi are not fully understood (Tamas
The description of arsenic tolerant organisms isolated from contaminated sites has revealed no hyper-tolerant life forms (Cullen and Reamer, 1989; Meharg and Macnair, 1992; Sharples et al., 2000; Ma et al., 2001; Canovas et al., 2003).

Metals can variously influence soil fungi, to diminish their populations, impoverish the diversity and to change fungal morphology and physiological activity: to affect the growth rate, reproduction processes, enzyme production, etc. (Gadd, 1992 and 1993; Martino et al., 2000). It has been reported that *Penicillium* fungi response to heavy metals varies in a very wide range. There were detected rather sensitive and extremely resistant fungi of this genus. *P. ochrochloron* is reported to grow in a saturated solution of copper sulphate (Stokes and Lindsay, 1979). In fungi metal effects can vary not only among organisms, also among different vegetative and reproductive forms of the same organisms (Sabie and Gadd, 1990). Fungal survival in the presence of toxic metals mainly depends on intrinsic biochemical and structural properties, physiological and/or genetical adaptation including morphological changes, and environmental modification of metal speciation, availability and toxicity, the relative importance of each often being difficult to determine (Gadd and Griffiths, 1978; Gadd 1990 and 1992b).

Heavy metal resistance in fungi has been investigated in greater 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 resistance fungi isolated from polluted environment have also been reported (Ashida, 1965; Gadd, 1993), but the mechanism of resistance in most cases was not studied. Resistance to heavy metals in microorganism can be due to any one of the two broad mechanisms. Transport blocks which restricts the entry of toxic metals, and Intracellular sequestration into vacuoles or binding to specific proteins, viz. Metallothioneins as described by Rao et al. (1997).
Environmental factors
(Physico-chemical and biologically derived)

Extracellular fungal products
(e.g. polysaccharide, melanin and other pigment, siderophores, organic acids
other metabolites and gases e.g. H₂S)

Cell wall components
(e.g. structural component, melanin and other pigments,
wall and cell surface associated enzymes)

Plasma membrane
(transport processes including influx, efflux, exchange phenomena and
alterations in membrane permeability)

Cytosol
(metallothioneins, metal γ-glutamyl peptides, other proteins, peptides,
and amino acids, precipitation, transformations)

Vacoule
(compartmentation, precipitation, poly-phosphate, transport across tonoplast)

Other-organelles
Mitochondria (oxidative detoxification)
endoplasmic reticulum (metalloid reduction)

**Fig. B:** Diagrammatic representation of metal tolerance in fungi
2.5.2 Physiological Responses of Fungi to Toxic Metals

Toxic metals can inhibit growth and spore germination of fungi, affect reproduction and metabolic activity, and reduce the ability of mycorrhizal fungi to colonize host plant roots (Gadd, 1993a; Amir and Pineau, 1998; Fay and Mitchell, 1999; Hartley-Whitaker et al., 2000a,b; Jentschke and Godbold, 2000; Mozafar et al., 2002; Moynahan et al., 2002; Baldrian, 2003). Effects of toxic metals on fungal growth have shown intra and interspecific variability and dependence on metal species and speciation (Plaza et al., 1998; Gadd, 1993a). Nickel was reported to be more toxic to fungal growth and ectomycorrhiza formation than chromium (Aggangan et al., 1998). Copper was found to be toxic and cadmium very toxic to cultures of 15 decomposer basidiomycetes (Holland, 1995). However, a similar toxicity of both metals was shown on a solid medium with cadmium and copper reducing radial growth of most strains of aquatic hyphomycetes by 50% at concentrations between 150 and 400 mM (Miersch et al., 1997). Radial extension rates of Trichoderma virens did not significantly differ during growth on tap water agar containing glucose and 0.1mM copper or cadmium (Ramsay et al., 1999). For T. virens and Clonostachys rosea colonizing spatially discrete toxic metal containing domains, colonization distance, hyphal extension rates and the efficacy of carbon substrate utilization decreased considerably with increasing concentrations of copper and cadmium (Fomina et al., 2003).

Levinskaite (2001) investigated the influence the cadmium, nickel and zinc on the development of fungi Penicillium atramentosum 25SL and P. funiculosum 6AL. all tested strains of fungal development (spore germination, germ tube growth, radial growth rate and conidiogenesis) were affected. The both fungi at their tube emergence stage were most susceptible to the metals than at the other periods of their development. He found P. funiculosum 6AL, more resistant towards the tested metals at all stages of development.

Baldrian and Gabriel (2002) studied the intraspecific variability in growth response to cadmium (Cd) on agar media and in liquid culture among fourteen strains of a wood-rotting fungus Piptoporus betulinus. The variability of Cd tolerance was found to be very high. On agar media the addition of Cd to nutrient media resulted in reduction of relative growth rate and increased lag time. While the reduction of growth rate was already apparent at 10 mM Cd, the lag time was significantly
increased in higher Cd concentrations. Five strains of *P. betulinus* failed to grow at 250 mM Cd and none grew at 500 mM metal. Biomass production in liquid culture was less sensitive to addition of Cd than the growth rate on solid media. At 100 mM Cd the radial growth rate of the mycelium was reduced to 27%, whereas the dry mass of mycelium was 77% of the respective control value. A group of four Cd-sensitive strains was found, showing low metal tolerance both on solid media and in liquid cultures. Although the isolates originated from sites with different Cd pollution level, no correlation between level of Cd-pollution and resistance (ED50) was found. The growth rate of fourteen tested strains displayed lower variability than biomass production, showing that radial growth rate is more species-specific and therefore more valuable for interspecific comparisons of growth response.

Plaza et al. (1998) investigated the effect of Cd on the mycelial growth of some potentially pathogenic soil fungi. Sixty four strains from twenty five fungal species were tested for their susceptibility to Cd. Final colony diameter, radial growth rate and final mycelial dry mass (for Cd: 1-200ppm) were measured. The intra- and intraspecific variability in the results was obtained. Among the keratinolytic fungal group, the genera *Arthrographis, Trichophyton and Chrysosporium* (including the *Chrysosporium anamorph of Aphanoascus reticulisporus*) were the most resistant to Cd. *T. mentagrophytes* was the most sensitive of all *Trichophyton* species tested. Among the nonkeratinolytic fungal group, the genera *Pseudallescheria, Absida* and *Rhizopus* were highly resistant to Cd than strains of *Penicillium*. Zoagymycetes were more resistant to Cd than Ascomycetes with fungi Imperfecti. Non keratinolytic fungi showed higher resistant to Cd than keratinolytic fungi. The two last differences resulted from extremely high EcD50 values for *R. oryzae* and *A. corymbifera* A decrease in metal toxicity is correlated with an increase available carbon source (Ramsay et al., 1999; Fomina et al., 2003). For *Stereum hirsutum* and *Trametes versicolor*, cadmium and mercury toxicity was lower in rich, complex media (Baldrian and Gabriel, 1997). This was also reported for *T. virens* grown with copper, cadmium and zinc where radial extension rate was commensurate with the availability of carbon, revealing a decrease in metal toxicity with increasing levels of glucose (Ramsay et al., 1999). A decrease in growth rate in the presence of toxic metals is sometimes accompanied by an increase in the lag phase (or growth delay) (Gadd and Griffiths, 1980b; Baldrian and Gabriel, 2002; Baldrian, 2003). A considerable
increase in the lag period was observed for *T. virens* and *C. rosea* grown with copper and cadmium (Fomina et al., 2003). Toxic metal treatment was reported to reduce the sporulating ability of *Aspergillus niger* and AM fungi (Magyarosy et al., 2002; Liao et al., 2003). Spore germination was found to be more sensitive to Ni\(^{2+}\), Co\(^{2+}\), Fe\(^{2+}\), Mn\(^{2+}\) and Mg\(^{2+}\) than mycelial growth (Amir and Pineau, 1998). However, a proportion of the spores of metal-sensitive strains of *Curvularia* sp. and *Fusarium* sp. were able to germinate and grow moderately well in the presence of relatively high metal concentrations (Amir and Pineau, 1998). Toxic metals can be potent inhibitors of enzymatic reactions. Cadmium, copper, lead, manganese, nickel and cobalt decreased cellulase and amylase production by several fungi, with reduced enzyme activity correlating with increasing metal concentration (Falih, 1998a and 1998b; Jarosz-Wilkolazka et al., 2002; Baldrian and Gabriel, 2003; Vivas et al., 2006; Zeng et al., 2006 and 2007; An and Kim, 2009).

### 2.5.3 Morphological Strategies in Response to Toxic Metals

Fungal morphology can be altered by toxic metals, and changes in mycelial density have often been observed (Ramsay et al., 1999; Fomina et al., 2005b). For example, *Schizophyllum commune*, *Daedalea quercina* and *Paxillus involutus* exhibited increased hyphal branching in response to cadmium (Darlington and Rauser, 1988; Lilly et al., 1992; Gabriel et al., 1996). *S. commune* also developed loops and connective filaments under cadmium stress (Lilly et al., 1992). Changes in mycelial morphology have also been observed in *Stereum hirsutum* and *Trametes versicolor* cultivated with cadmium and mercury (Baldrian and Gabriel, 1997), in *Mucor rouxii* in the presence of a high copper concentration (Gardea-Torresdey et al., 1997), and in ectomycorrhizal fungi during growth in metal-containing (copper, aluminium, zinc) media (Jones and Muehlchen, 1994). It was also found that biomass distribution within *Trichoderma viride* colonies was altered by toxic metals, with biomass concentrated in the periphery of the colonies in the presence of copper and towards the interior of the colonies in the presence of cadmium (Ramsay et al., 1999; Gadd et al., 2001).

Gadd et al. (2001) examined the nutritional influence on the fungal colony growth and biomass distribution in response to toxic metals. In low substrate solid medium, 0.1mM Cd and Zn caused a decrease in radial expansion of both
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*Trichoderma viride* and *Rhizopus arrhizus*. However, as the amount of available carbon source (glucose) increased, the apparent toxicity of the metals decreased. These metals also affected the overall length of the fungal mycelium and branching patterns. In low nutrients conditions, *T. viride* showed a decrease in overall mycelial length and number of branches in response to Cu, resulting in an extremely sparsely branched colony. Conversely, although, Cd also reduced overall mycelial length to about one third of the control length, the number of branches decreased only slightly which resulted in a highly branched colony with many aberrant features. Cu and Cd induced similar morphological changes in *R. arrhizus*. A large scale mycelial mapping technique showed that disruption of normal growth by Cu and Cd resulted in altered biomass distribution within the colony. When grown on metal free low substrate medium, These results imply that such alterations of growth and resource allocation by Cu and Cd may influence success in locating nutrients as well as survival, and that these metals have individual and specific effects on the growing fungus.

During growth of fungi in metal-containing agar tiles, a wide range of morphological changes and growth responses occurred (Fomina *et al.*, 2000, 2003). Changes in strategies can be represented by changes in branching patterns or different degrees of commitment to radial (explorative) or tangential (exploitative) growth (Rayner *et al.*, 1995). Penetration of hyphae into metal-containing domains was often followed by the formation of very dense mycelia or mycelial ‘bushes’ (Fomina *et al.*, 2003). Such hyphal aggregation could be an example of the phalanx growth form with profusely branching hyphae facilitating the colonization of a substrate, and the production of high local concentrations of extracellular enzymes, antibiotics and other metabolites. In the case of a toxic metal-containing domain, aggregated mycelia could produce high local concentrations of extracellular products such as complexing agents (e.g. organic acids, siderophores, polyphenolic compounds), metal precipitating agents (e.g. oxalate), and polysaccharides and pigments with metal-binding abilities (Gadd, 1993a; Dutton and Evans, 1996; Morley *et al.*, 1996; Baldrian, 2003). After fungi enter toxic metal-containing domains under poor-nutritional conditions, they often produced long sparsely-branched or branchless hyphae representing an explorative growth strategy with a large hyphal growth unit and infrequent branching (Fomina *et al.*, 2003). Many other workers also reported recently the heavy metals’
effect on radial growth of soil fungi and observed decrease and increase of mycelial size (Meharg, 2003; Ali, 2007; Finlay, 2008; Purchase et al., 2009).

2.5.4 Mechanism of Metal Resistance and Tolerance

Metals and their compounds can interact with fungi in various ways depending on the metal species, organism and environment, while metabolic activity can also influence speciation and mobility. Many metals are essential for fungal growth and metabolism (e.g. sodium, potassium, copper, zinc, cobalt, calcium, magnesium, manganese, Fe), but all can exert toxicity when present above certain threshold concentrations (Gadd, 1993a). Other metals (e.g. cadmium, Mercury, lead) have no known biological function but can still be accumulated by fungi (Gadd, 1993a). Metal toxicity is greatly affected by the physicochemical nature of the environment and the chemical behaviour of the particular metal species in question. 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 and 1993a; Avery et al., 1996; Howlett and Avery, 1997). Fungi possess many properties that influence metal toxicity, including the production of metalbinding proteins, organic and inorganic precipitation, active transport and intracellular compartmentalization, while major constituents of fungal cell walls (e.g. chitin, melanin) have significant metal binding abilities (Gadd and Griffiths, 1978; Gadd, 1993a). All these mechanisms are highly dependent on the metabolic and nutritional status of the organism, as this will affect expression of energy-dependent resistance mechanisms, as well as synthesis of wall structural components, pigments and metabolites, which gratuitously affect metal availability and organism response (Gadd, 1992b and 1993a; Ramsay et al., 1999). Fungi are able to 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 neoformed minerals (e.g. oxalates); and (3) extracellular metal sequestration by, e.g.exopolysaccharides and other extracellular metabolites Gadd, 1993a and 2001a, b, c; Macreadie et al., 1994; Blaudez et al., 2000a; Perotto and Martino, 2001; Baldrian, 2003). Metal tolerant fungi can survive
due to their abilities of intracellular chelation by, for example metallothioneins and phytochelatins, and metal localization/sequestration within vacuoles. Fungal vacuoles have an important role in the regulation of cytosolic metal ion concentrations and the detoxification of potentially toxic metals (White and Gadd, 1986; Gadd, 1993a; 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, 1993a; Okorokov, 1994), Ni$^{2+}$ (Joho et al., 1995) and the monovalent cations K$^+$, Li$^+$ and Cs$^+$ (Okorokov et al., 1980; Perkins and Gadd, 1993a and 1993b). Recently many workers also explained about the metal resistance in soil fungi (Meharg, 2003, Bucková et al., 2007; Gonçalves et al., 2007; Richie et al., 2007; Xiao et al., 2008).

Survival mechanisms found in non-mycorrhizal fungi seem to be used in ectomycorrhizal fungi as well. These include reduction of metal uptake into the cytosol by extracellular chelation through extruded ligands and binding to cell wall components. Intracellular chelation of metals by a range of ligands (glutathione, metallothioneins), or increased efflux from the cytosol or into sequestering compartments are also key mechanisms conferring tolerance. Free-radical scavenging capacities through the activity of superoxide dismutase or production of glutathione are another defence mechanism against toxic effects of metals (Bellion et al., 2006).

### 2.5.5 Metal Solubilization

Solubilization of insoluble 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 into biogeochemical cycles.

Soil fungi including mycorrhizas may increase inorganic-nutrients availability to the plants and other microorganisms by increasing the mobility of essential metal cations and other anions e.g. sulfate (Gharieb et al., 1998). Fungal solubilization of insoluble metal compounds, including certain oxides, phosphates, sulphides and mineral ores, occurs by several mechanisms including:

- Protonation of anion of the metal compound, decreasing its availability to the cation. With the proton-translocating ATPase of the plasma membrane. The production of

Organic acid anions are frequently capable of soluble complex formation with metal cations, thereby increasing mobility (White et al., 1997). Such complexation is dependent on the relative concentrations of the anions and metals in solution, pH, and the stability constant 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 iron (III). Siderophores are the most common means of acquisition of iron by bacteria and fungi and are effective in a wide range of soils, including calcareous soil. The most common fungal siderophore is ferrichrome (Crichton, 1991). Acidification can lead to metal release via a number of obvious routes, e.g., competition between protons and the 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 can 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).

![Fig. C: Action of free-living and mycorhizal fungi on insoluble metal minerals in the terrestrial environment resulting in release of mineral components; metal(s), anionic substances, trace organics and other impurities, which can be taken up by the biots, as well as forming secondary minerals with soil components or fungal metabolites/biomass, and also be sorbed or otherwise removed by organic and inorganic soil components. The dashed arrows imply secondary mineral formation from excreted metabolites, as well as fungal action on non-biogenic minerals. Losses to groundwater are not shown, from Gadd 2004.](image-url)
However, in many fungi an important leaching mechanism occurs through the production of organic acids (e.g. oxalic acid, citric acid) (Adams et al., 1992; Francis et al., 1992; Denevreb et al., 1996; Sayer et al., 1997; Gadd, 1999 and 2000b; Sayer and Gadd, 2001; Jarosz Wilkolazka and Gadd, 2003; Fomina et al., 2005a). Organic acid excretion by fungi is inter- and intraspecific, and can be strongly influenced by the presence of toxic metal minerals (Sayer et al., 1995; Sayer and Gadd, 2001; Fomina et al., 2004 and 2005c). Oxalic acid can leach those metals that form soluble oxalate complexes, including aluminium and Fe (Strasser et al., 1994; Devevre et al., 1996). Notably, oxalic acid was the only tested agent to give a clear solubilization zone for pyromorphite (Fomina and Gadd, 2007).

Fig. D: Simple biogeochemical model for metal mineral transformations in the mycorrhizosphere (the role of the plant and other microorganisms contributing to the overall process is not shown). (1) Proton-promoted (proton pump, cation-anion antiport, organic anion efflux, dissociation of organic acids) and ligand-promoted (organic acids) dissolution of metal minerals; (2) release of anionic (e.g. phosphate) nutrients and metal cations; (3) nutrient uptake; (4) intra-and extracellular sequestration of toxic metals: biosorption, transport, compartmentation, precipitation, etc; (5) immobilization of metals as oxalates; (6) binding of soluble metal species to soil constituents, e.g. clay minerals, metal oxides, humic substances (From Formina et al., 2006a).
Sayer et al. (1997) reported the ability of soil fungus *Aspergillus niger* to tolerate and solubilize seven naturally occurring metal bearing minerals, limescale and lead phosphate was investigated. *Aspergillus niger* was able to solubilize four of the test insoluble compounds when incorporated into solid medium: Cuprite (CuO$_2$), galena (PbS), rhodochrosite (Mn(CO$_3$)$_x$) and limescale (CaCO$_3$). *A. niger* was able to grow on all concentrations of all the test compounds, whether solubilization occurred or not, with no reduction in growth rate from the control. In some cases stimulation of growth occurred, most marked with phosphate containing mineral, apatite. Fomina et al. (2005) investigated the ability of ericoid mycorrhizal (ErM) and ectomycorrhizal (EcM) fungi to solubilize different toxic metal (Cd, Cu, Pb, Zn)-containing minerals.

### 2.5.6 Metal Immobilization

Toxic metal species, including radionuclides, can be bound, accumulated, and precipitated by fungi. Fungal biomass can act as a metal sink, either by: (1) metal biosorption to biomass cell walls, pigments and extracellular polysaccharides; or (2) intracellular accumulation and sequestration; or (3) precipitation of metal compounds onto and/or around hyphae. In addition to immobilizing metals, this also reduces the external free metal concentration, and may therefore drive the equilibrium to release more metal ions into the soil solution (Gadd, 1993a and 2000a; Sterflinger, 2000). Fungi can be highly efficient accumulators of soluble and particulate forms of metals (e.g. nickel, zinc, silver, copper, cadmium, lead), especially from dilute external concentrations (Gadd, 1993a, 2000b, 2000c, 2001b and 2001c; Baldrian, 2003). Binding of metal ions onto cell walls and other external surfaces can be an important passive process in both living and dead fungal biomass (Gadd, 1990 and 1993a; Sterflinger, 2000).

Melanin and chitin in fungal hyphal walls strongly influences their ability to act as biosorbents (Gadd and Mowll, 1985; Manoli et al., 1997; Fomina and Gadd, 2002b). Melanin-containing chlamydospores of Aureobasidium pullulans can absorb three times more copper than hyaline cells (Gadd and Mowll, 1985). Fungi have also been shown to accumulate radionuclides, not only from aqueous solution (Gadd and White, 1989, 1990 and 1992) but also from radioactive substrata.

Most studies on mycorrhizas indicate that extramatrical mycelium provides the major metal binding sites, and that most metals are bound to hyphal wall
components or, in interhyphal spaces, to extracellular polysaccharide (Jones and Hutchinson, 1988; Denny and Wilkins, 1987b; Colpaert and van Assche, 1992 and 1993; Turnau et al., 1996; van Tichelen et al., 2001; Meharg, 2003; Christie et al., 2004; Krupa and Kozdroj, 2004).

Mechanisms for metal immobilization include intracellular uptake with complexation to ligands, such as sulphur-containing peptides (e.g. metallothionein) (Gadd, 1993a; Sarret et al., 1998 and 2002; Fomina et al., 2005). Some fungi can also precipitate metals in amorphous and crystalline forms, such as oxalates and other secondary mycogenic minerals (Gadd, 1999; Burford et al., 2003a and 2006).

2.5.7 Metal Transformations

Transformation is the mechanisms by which fungi (and other microorganisms) effect changes in metal speciation and mobility are important components of biogeochemical cycles for metals, as well as all other elements including carbon, nitrogen, sulphur and phosphorus (Gadd, 1999, 2001b, 2004, 2006 and 2007a, c). Rocks and minerals, including mineral components of soil, contain considerable quantities of metals that are biologically unavailable. Some of the mechanisms (mobilization and immobilization) by which fungi and other microorganisms solubilize metals. These may increase metal bioavailability and potential toxicity.

Fungi as well as other microorganisms, can effect chemical transformations of metals by e.g. 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 the reduction of Cu (II) to Cu (I) by cell wall associated materials in Debaryomyces hansenii (Wakatsuki et al., 1988 and 1991).

Methylation of Hg, and other metals and metalloids can be catalyzed by several fungi, and may be viewed as a detoxification mechanism since methylated species are usually more volatile and may be lost from the environment (Landner, 1971; Yannai et al., 1991; Gadd, 1992a, b).
2.6 Bioremediation

Fungal involvement in element cycling at local and global scales has important implications for living organisms, plant production and human health. Some of the processes detailed previously have the potential for treatment of contaminated land and waters (Thomson-Eagle and Frankenberger, 1992; Gadd, 2000a, 2000b, 2001a, 2001b, 2001c, 2002, 2004 and 2007b; Hochella, 2002; Fomina et al., 2005b). Solubilization provides a route for heavy metal removal from industrial effluents and byproducts, low-grade ores, and metal-bearing minerals, which is relevant to bioremediation of soil matrices and solid wastes, and metal recovery and recycling (Burgstaller and Schinner, 1993; Kadoshnikov et al., 1995; Gadd, 2000b; Gadd and Sayer, 2000; Brandl, 2001; Kartal et al., 2006; Tang and Valix, 2006).

Living or dead fungal biomass and fungal metabolites have been used to remove metal or metalloid species, compounds and particulates, radionuclides and organometal compounds, from solution by biosorption (Gadd and White, 1989, 1990, 1992 and 1993; Gadd, 1990; Kadoshnikov et al., 1995; Wang and Chen, 2006). These processes are best suited for use in bioreactors (Gadd, 2000b). Regarding metal mobilization, extracellular ligands excreted by fungi have been used, especially from Aspergillus and Penicillium spp., to leach metals such as zinc, copper, nickel and cobalt from a variety of solid materials, including low-grade mineral ores (Brandl, 2001; Mulligan and Galvez-Cloutier, 2003). Mycorrhizal associations may also be used with plants for metal clean up in the general area of phytoremediation (Pichtel and Salt, 1998; Pichtel et al., 2000; Pichtel and Liskanen, 2001; van der Lelie et al., 2001; Gohre and Paszkowski, 2006).

The potential impact of mycorrhizal fungi on bioremediation may be conditional and dependent on the metal tolerance of fungal strains, their mycorrhizal status, and the nutritional status of contaminated soils (Meharg, 2003). Several studies have shown mycorrhizas can reduce plant metal uptake (Tullio et al., 2003).

2.6.1 Biosorption

Biosorption is the process by which metals are sorbed or complexed to either living or dead biomass (Volesky and Holan, 1995). Biosorption might be an important natural process for 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 biosorative capacity 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 that a barrier of microorganisms with biosorptive abilities could be established in subsurface environments in order to remove metals from groundwater flowing through the biobarrier. Although small-scale bioremediation of mine drainage with biosorption has been documented (Ledin and Pedersen, 1996) to date, 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 search for novel microorganisms with unique biosorption capacities (Pradham and Levine, 1995; Hu et al., 1996; Vesper et al., 1996).

2.6.1.1 Biosorbents

Adsorptive removal of heavy metals from aqueous effluents which have received much attention in recent years is usually achieved by 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; Monsen and Adhoun, 2002; Igwe et al., 2005).

Some biosorbents can bind and collect a wide range of heavy metals with no specific priority, whereas others are specific for certain types of metals (Hosea et al., 1986; Volesky and Kuyucak, 1988). When choosing the biomass for metal biosorption experiments, its origin is a major factor to be taken into account. Biomass can come from

(i) industrial wastes which should be obtained free of charge;
(ii) organisms easily available in large amounts in nature; and
(iii) organisms of quick growth especially cultivated or propagated for biosorption purposes.

Biosorbents are prepared from the naturally abundant or waste biomass of mainly algae, fungi or bacteria that have been killed by washing biomass with acids or bases, or even both, before final drying and granulation (Brierley, 1990; Kratochvil et al., 1997).
2.6.1.2 Binding sites

Fig. E shows the general structure of the main types of microbial cell walls. Numerous chemical groups have been suggested to contribute to biosorption metal binding by either 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 certain metal by a certain biomass depends on factors such as:

(a) The number of sites in the biosorbent material

(b) The accessibility of the sites

(c) The chemical state of the site (i.e. availability)

(d) and affinity between site and metal (i.e. binding strength)

Fig. E: Cell wall structure in A: algae (example: brown algae), B: Gram-positive bacteria (in part after Beveridge (Beveridge 1986) and Remacle (Remacle, 1990), C: Gram negative bacteria (in part after Beveridge (Beveridge, 1986) and Remacle (Remacle, 1990) D: fungi (example: type V, e.g. Euascomycetes) (in part after Moore (Moore-Landecker, 1996)
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. Difference in cell wall composition among different groups of micro-organisms, viz. algae, bacteria, cyanobacteria and fungi and the intra group differences can thus cause significant differences in the type and amount of metal ion binding to them (Horikoshi et al., 1981; Friis and Myers-Keith, 1986; Muraleedharan, 1991). The various groups involved in metal binding have been discerned using the modification/ blocking of the groups (Tobin, 1990). Whether any group is important for biosorption of certain metal by a certain biomass depends on factors such as the quantity of sites in the biosorbent material, the accessibility of the sites, the chemical states of the site (i.e. its availability), and the affinity between the site and the metal (i.e. the binding strength).

2.6.1.3 Metal uptake by living cells

*Penicillium* can remove a variety of heavy metals from aqueous solutions. Spores of *Penicillium italicum* were shown to accumulate copper (Somers, 1963; Kapoor and Viraraghavan, 1995). The metal accumulation by growing cells varied with the cell age. The maximum metal uptake took place during the lag period or the early stages of growth and declined as cultures reached a stationery phase. *Aspergillus niger*, *P. spinolosum* and *Trichoderma viride* showed a similar uptake pattern (Townsley and Ross, 1985 and 1986, Kapoor and Viraraghavan, 1995). Recently many workers also reported metal uptake by the living cells. (Bayramoglu, 2006; Zafar et al., 2007; Melgar et al., 2007; Akhtar et al., 2007; Pakshirajan and Swaminathan, 2009)

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

2.6.1.4 Metal uptake by dead cells

The biosorption capacity of dead cells may be greater, equivalent to or less than that of living cells. Use of dead biomass in industrial applications offers certain advantages over living cells. Systems using living cells are likely to be more sensitive to metal-ion concentration (toxicity effects) and adverse operating conditions (pH and temperature). Furthermore, constant nutrient supply is required for systems using living cells (increased operating cost for waste streams devoid of nutrients) and recovery of metal and regeneration of biosorbent is more complicated for living cells. Cells can be killed by physical treatment methods using heat treatment (Siegel et al., 1987), autoclaving, and vacuum drying (Tobin et al., 1984; Huang et al., 1988) or chemicals like acids, alkalis, detergents (Tsezos and Volesky, 1981; Ross and Townsley, 1986; Huang et al., 1988; Rao et al., 1993; Brady et al., 1994; Kapoor and Viraraghavan, 1995). Recently many workers also reported metal uptake by the dead cells. (Naeem et al., 2006; Chen et al., 2007; Akhtar et al., 2007; Bishnoi et al., 2007; Tsekova et al., 2007)

2.6.2 Mechanism of biosorption

Veglio and Beolchini (1997) described the biosorption mechanisms on the basis of cells metabolism, (Metabolism dependent and Metabolism independent) and according to the location where the metal removed from the solution is found.

The process of biosorption may be studied as follows.
(a) Extracellular accumulation/precipitation,
(b) Cell surface sorption/precipitation,
(c) Intracellular accumulation.

2.6.2.1 Extracellular accumulation/precipitation

Some prokaryotic (bacteria, Archaea) and eukaryotic (algae, fungi) microorganisms can produce or excrete extracellular polymeric substances (EPS), such as polysaccharides, glucoprotein, lipopolysaccharide, soluble peptide etc. These substances possess a substantial quantity of anion functional groups which can adsorb metal ions. Research work published on metal biosorption with EPS mainly focus on
the bacterial organism, such as *Bacillus megaterium*, *Acinetobacter*, *Pseudomonas aeruginosa*, sulphate-reducing bacteria (SRB), Cyanobateria or activated sludge (Liu et al., 2001), whereas EPS study for fungi and algae is limited (Wang and Yang, 1996; Pirog, 1997; Suh et al., 1998b; Flemming and Wingender, 2001) also discovered that initial rate of Pb\(^{2+}\) uptake by live cells of *S. 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 the live cells are higher than those in the dead cells, due to the presence of EPS for live *A. pullulans*.

### 2.6.2.2 Cell surface sorption/precipitation

The cell wall tends to be the first cellular structure to come in contact with metal ions, excluding a possible existing extracellular layer mainly related to bacterial cells. Two basic mechanisms of metal uptake by cell wall are as follows: stoichiometric interaction between functional groups of cell wall composition, including phosphate, carboxyl, and amine as well as phosphodiester; and 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 uptake by organism (Volesky, 1990a, b; Liu et al., 2002b).

Kapoor and Viraraghavan (1997) showed that, in dried fungal biomass of *Aspergillus niger*, amine and carboxyl groups were important functional groups involved in lead, cadmium and copper biosorption and they reported that phosphate groups and the lipids 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 ions displaced (H\(^{+}\)+Mg\(^{2+}\)+Ca\(^{2+}\)) accounted for only a small portion of the metal ions taken up in the biosorption of metal ions (Sr\(^{2+}\), Mn\(^{2+}\), Zn\(^{2+}\), Cd\(^{2+}\), Cu\(^{2+}\), Pb\(^{2+}\)) 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 the ion exchange was the main mechanism for metal ion uptake by brown algae.

Precipitation and redox reactions in 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., 2003a; Xie et al., 2003a), Pt$^{4+}$ (Xie et al., 2003b), Au$^{+}$ (Lin et al., 2005), Ag$^+$ (Lin et al., 2001) and Rh$^{3+}$ (Lin et al., 2001), were unexceptionally bound to the cell wall of the yeast and then in situ reduced into the corresponding metal particle.

Biosorption often involves in many kinds of mechanisms. Kratochvil et al. (1998) proved that the maximal uptake of Cr (VI) by protonated Sargassum biomass at pH 2 was due to simultaneous anion exchange and the reduction of Cr (VI) to Cr(III).

**2.6.2.3 Intracellular accumulation/precipitation**

Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cells' metabolism. This implies that this kind of biosorption may take place only with viable cells (Veglio and Beolchini, 1997). After entering into the cell, the metal ions are compartmentalized into different subcellular organelles (e.g. mitochondria, vacuole etc.). Vijver et al. (2004) summarized the metal ion accumulation strategies, especially internal compartmentalization strategies. The mechanism mainly relates to a specific metal binding protein, metallothioneins (MT), which has a low molecular weight and is cysteine-rich, usually occurring 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, Zn etc. (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 (Peregol 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 sulphur and nitrogen as well as detoxification of metal ions. GSH in S. cerevisiae may account for 1% of the cell dry weight (Gharieb and Gadd, 2004). The role of vacuole in the detoxification of metal ions was investigated, and the results showed that vacuolatedeficient strain displayed much higher sensitivity and the biosorption capacity for Zn, Mn, Co and Ni decreased (Ramsay and Gadd, 1997).

Many genes involved in the uptake or detoxification or tolerance to metal ions have been identified (Rosen, 2002). For example, the S. cerevisiae Arr4p plays an important role in the tolerance to metal ions (As$^{3+}$, As$^{5+}$, Co$^{2+}$, Cr$^{3+}$, Cu$^{2+}$, VO$_4^{3-}$)
(Shen et al., 2003). Based on the understanding of metal uptake mechanism, genetic technologies, including the cell surface display technology 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) have constructed a cell surface-modified yeast S. cerevisiae which displays histidine hexa-peptide, the engineered yeast that can chelate copper ion, and possesses the property of self-aggregation, which indicates the potential application for bioremediation of heavy metal pollution.

2.6.3 Factors affecting heavy metal biosorption

Bioremoval of a pollutant using microorganisms is affected by several factors. These factors include the specific surface properties of the microorganism and the physicochemical parameters of the solution such as temperature, pH, metal ion concentration, metal solubility, metal valency, concentration of complexing agents and particle size (Brown and Lester, 1979).

2.6.3.1 Temperature

Butter et al. (1998) showed temperature variations from 15-35°C did not affect the cadmium uptake by dead Streptomyces biomass. Also, Kasan (1993) found that the complexation/removal of the toxic metals Cr, Pb, and Zn, by the use of living activated sludge was independent of the temperature.

2.6.3.2 pH

It is known that pH has a very significant effect on the metal removal from solutions. Taking into account this importance of the pH on metal ion biosorption several studies are reported in literature, which have investigated the effect of pH on biosorption. Most investigators have reported negligible sorption in their studies at pH values lower than 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 the cell walls, which results in lower metal uptake.
2.6.3.3 The Effect of Biomass Concentration on Biosorption

The biosorbent concentration has been shown as one of the important factor in the biosorption process. In the literature there are examples of the effect of biomass concentration on heavy metal biosorption. It has been found that the metal uptake was increased when the biomass concentration decreases (Esposito et al., 2001). Such behaviours have been explained that an increase in biomass concentration leads to interference between the binding sites (Veglio et al., 1997; Esposito et al., 2001).

2.6.3.4 The Effect of Initial Metal Concentration on Biosorption

Another factor that affects biosorption process is the initial metal concentration. It has been reported that generally the adsorption rate increased with increasing initial metal concentration. For example, adsorption of Fe(II), Pb(II) and Cd(II) by *S. leibleini* has increased with increasing initial metal ion concentrations up to 150 mg/L, at high concentration the adsorption rates have not been changed (Ozer et al., 1999).

2.6.3.5 The Effect of Pretreatment on Biosorption

Living cells have been pretreated using physical and chemical methods to increase the metal biosorption capacity. Physical pretreatment methods have included heat treatment, autoclaving, freeze, drying and boiling. Chemical pretreatment methods such as contacting especially fungal cells with acids, alkaline and organic chemicals that increase the biosorption capacity have been reported in the literature (Huang, et al., 1990; Brady et al., 1994; Wase and Forster, 1997; Kapoor and Viraraghavan, 1998; Zhao and Duncan, 1998; Sag and Kutsal, 2000).

2.6.3.6 Adsorbent and Adsorbate Properties

Physico-chemical properties (e.g. metal ion concentration, metal solubility, and metal valency) of adsorbate are important factors for biosorption. Holan and Volesky (1994) stated that metal sorption increases with increasing valence and atomic number. Also, Brown and Lester (1982) found that the sequence of solubilities of cadmium, manganese, cobalt and nickel is the reverse of the sequence of metal affinities for adsorption onto cells of *K. aerogenes* indicating that the more soluble metals displayed the lowest removals.
2.6.4 Biosorption potential of fungal biomass

In the last two decades there is a growing interest in evaluation of metal biosorption potential among scientific communities. Considerable amount of data have been generated on various microbial biomasses including fungi. Some of the findings on fungal biomass are mentioned below.

Niu et al. (1993) studied the removal of lead ions from aqueous solutions by adsorption on nonliving *Penicillium chrysogenum* biomass. Biosorption of the Pb\(^{2+}\) ions was strongly affected by pH. Within a pH range of 4 to 5, the saturated sorption uptake of Pb\(^{2+}\) was 116 mg/g dry biomass, higher than that of activated charcoal and some other microorganisms. At pH 4.5, *P. chrysogenum* biomass exhibited selectivity for Pb over other metal ions such as Cd\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), and As\(^{3+}\). Sorption preference for metals decreased in the following order: Pb > Cd > Cu > Zn > As. The sorption uptake of Pb\(^{2+}\) remained unchanged in the presence of Cu\(^{2+}\) and As\(^{3+}\), it decreased in the presence of Zn\(^{2+}\), and increased in the presence of Cd\(^{2+}\).

Volesky and May-Phillips (1995) examined abundant and common yeast biomass for its capacity to sequester heavy metals from dilute aqueous solutions. Live and non-living biomass of *Saccharomyces cerevisiae* differs in the uptake of uranium, zinc and copper at the optimum pH 4-5. Culture growth conditions can influence the biosorbent metal uptake capacity which normally was: living and non-living brewer's yeast: U > Zn > Cd > Cu; non-living baker's yeast: Zn > (Cd) > U > Cu; living baker's yeast: Zn > Cu approximately (Cd) > U. Dead cells of *S. cerevisiae* removed approximately 40% more uranium or zinc than the corresponding live cultures.

Lo et al. (1999) studied the removal of lead from aqueous solutions by adsorption on filamentous fungal biomass. The fungal biomass exhibited the highest lead adsorption capacity at pH 6. The maximum lead biosorption capacity of *Mucor rouxii* estimated 769 mg/g dry biomass, significantly higher than that of most microorganisms. Biomass of *Mucor rouxii* showed specific selectivity for Pb\(^{2+}\) over other metals ions such as Zn\(^{2+}\), Ni\(^{2+}\) and Cu\(^{2+}\).

Bai and Abraham (2001) reported that the optimum pH for biosorption of Cr (IV) was found to be 2.0. Higher adsorption percentage was noted at lower initial concentrations of Cr ions, while the adsorption capacity of the biomass increased with increasing concentration of ions. The adsorption capacity increase with increase in temperature and agitation speed and the optimum were determined as 45°C and 120
rpm. Pradhan and Rai (2001) reported that the biosorption of Cu, Zn and Cd by *Microcystis sp.* in single, bi and trimetallic combination. Highest biosorption of Cu followed by Zn and Cd in single as well as in mixture containing two or three metals was noticed. The order of inhibition of Cu, Zn and Cd biosorption in bi and trimetallic combinations was suggestive of screening or competition of the binding sites on the cell surfaces.

Yan and Viraraghavan (2001) studied the biosorption capacity of *Mucor 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 multi-component metal solution containing Cd, Ni and Zn, 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 from artificial wastewaters of heavy metals (Cd(II), Pb(II) and Cu(II)) onto the dry fungal biomass of *Phanerochaete chryosporium* in the concentration range of 5-500 mg/l. The maximum absorption of different heavy metal ions on the fungal biomass was obtained at pH 6.0. The experimental biosorption data for Cd(II), Pb(II) and Cu(II) ions were in good agreement with those calculated by the Langmuir model.

Tsekova and Petrov (2002) assessed the ability of mycelia of *Rhizopus delemar* (both free and immobilized on polyurethane foam) to remove heavy metals from single-ion solutions as well as from a mixture of them. Immobilized mycelia showed some times increase in uptake compared with that of free cells. Metal ions accumulation from a mixed solution was decreased slightly for cobalt and iron and considerable for copper ions. Heavy metal uptake was examined in the immobilized column experiments and more than 92% heavy metal removal (mg heavy metals removed/mg heavy metals added) from a mixed solution was achieved during the 5 cycles.

Magyarosy *et al.* (2002) isolated a strain of *Aspergillus niger* from a metal contaminated soil which was able to grow in the presence of cadmium, chromium, cobalt, copper and usually high levels of nickel on solid (8.0mM) and in liquid (6.5mM) media. This fungus removed >98% of the nickel from liquid medium after 100h of growth but did not remove the other metals, as determined by inductively coupled plasma spectroscopy.
Yan and Viraraghavan (2003) reported that the dead biomass of *Mucor rouxii* adsorbed metal ions in the order of Pb^{2+}, Zn^{2+}, Cd^{2+} and Ni^{2+}, with the biosorption capability of 25.22, 16.62, 8.36 and 6.34 mg/g at pH 5.0 respectively. At pH 6.0, the capacity of the dead biomass increased to 53.75, 53.85, 20.31 and 20.49 mg/g, respectively. For bi- or multi-metal ion adsorption, biosorption capacity of individual metal ion was reduced in the presence of other metal ions, but total biosorption capacity increased, indicating the capability of *Mucor rouxii* biomass in adsorbing multi-metal ions.

Tan and Cheng (2003) used alkaline pretreatment for *Penicillium chrysogenum* to remove proteins and nucleic acids from cells, and this treatment increased the adsorption capacities, Cr^{3+} from 18.6 mg/g to 27.2 mg/g for Ni^{2+} from 13.2 mg/g to 19.2 mg/g, for Zn^{2+} from 6.8 mg/g to 24.5 mg/g. Yakup et al., (2004) investigated effect of pH and maximum adsorption of metal ions on the calcium alginate and both live and inactivated immobilized fungal preparations of *Fungalium trogii* at pH 6.0. The biosorption of Hg^{2+}, Cd^{2+} and Zn^{2+} ions on the Ca-alginate beads and on both immobilized forms was studied in aqueous solution in the concentration range of 30-600 mg/l. The metal biosorption capacities of the 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 form were 333.0, 164.8, and 42.1 mg/g respectively. The same affinity order on a molar basis was observed for single or multi-metal ions.

Akar and Tunali (2006) examined the Pb(II) and Cu(II) biosorption characteristics of *Aspergillus flavus* fungal biomass. They used heat inactivated (killed) biomass of *Aspergillus flavus*. The maximum biosorption values were found to be 13.46 ± 0.99 mg/g for Pb(II) and 10.82 ± 1.46 mg/g for Cu(II) at pH 5.0 ± 0.1 with an equilibrium time of 2 h. The results indicated that A. flavus is a suitable biosorbent for the removal of Pb(II) and Cu(II) ions from aqueous solution. Ahmad et al. (2006) studied metal tolerant fungal isolates including *Aspergillus* and *Penicillium* for biosorption capacity. Two isolates, *Aspergillus niger* and *Penicillium* sp. were tested for their Cr, Ni and Cd biosorption potential using alkali treated, dried and powdered mycelium. Biosorption experiment was conducted in 100 ml of solution at three initial metal concentrations i.e., 2, 4 and 6 mM with contact time (18 hr) and pretreated fungal biomass (0.1g) at 25 degrees C. Biosorption of all metals was found...
higher at 4 mM initial metal concentration as compared to biosorption at 2 and 6 mM concentrations. At 4 mM initial metal concentration, chromium biosorption was 18.05 and 19.3 mg/g of *Aspergillus* and *Penicillium* biomasses, respectively. Similarly, biosorption of Cd and Ni ions was also maximum at 4 mM initial metal concentration by *Aspergillus* (19.4 mg/g for Cd and 25.05 mg/g of biomass for Ni) and *Penicillium* (18.6 mg/g for Cd and 17.9 mg/g of biomass for Ni).

Pal *et al.* (2006) screened cobalt-resistant fungi belonging to *Aspergillus*, *Mortierella*, *Paecilomyces*, *Penicillium*, *Pythium*, *Rhizopus* and *Trichoderma*, isolated from serpentine soil of Andaman (India). Eleven out of total 38 isolated fungi which tolerated > 6.0 mM Co²⁺ were evaluated for cobalt biosorption using dried mycelial biomass. Maximum Co²⁺-loading (1036.5 microM/g, 60 min) was achieved with *Mortierella* SPS 403 biomass, which removed almost 50% of 4.0 mM cobalt from the aqueous solution. The metal biosorption capacity of the isolate was accelerated with increasing cobalt concentration, while it was reverse with increase of initial biomass. The optimum pH and temperature for Co²⁺ removal were 7.0 and 30 degrees C, respectively. However, Co²⁺-uptake was inhibited in presence of other metals (Pb, Cd, Cu, Ni, Cr and Zn).

The untreated, heat- and alkali-treated *Lentinus* sajor-caju mycelia were used for the recovery of uranium from aqueous solutions by Bayramoğlu (2006). He reported that the alkali treated form had a high biosorption capacity (378 mg/g) than those of the untreated (268 mg/g) and heat-treated fungal mycelia (342 mg/g). Optimum biosorption was observed at pH 4.5 for all the tested fungal preparations and was independent of temperature (5-35degreesC). Naeem *et al.* (2006) studied Proton, Cd, Pb, Sr, and Zn adsorption onto the fungal species Saccharomyces 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 pKa values (with 1 sigma uncertainties) 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 follows the following trend: Pb > Zn > Cd > Sr. They 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 arsenic from an aqueous solution by Non-viable fungal biomass of *Aspergillus niger*, coated with iron. *A. niger* biomass coated with iron oxide showed maximum removal (approximately 95% of As(V) and 75% of As(III)) at a pH of 6. No strong relationship was observed between the surface charge of the biomass and arsenic removal.

Melgar et al. (2007) investigated biosorption of the metals like zinc, copper, mercury, cadmium or lead by living or non-living biomass of *A. macrosorpus* from an acid solution, acid solution supplemented with potassium and phosphorus, and an alkaline solution. Results of metal uptake showed the maximum percentage uptake of all metals was found to occur at alkaline pH (Cu 96%, Pb 89%). With living biomass, metal biosorption was greater and faster in K/P-supplemented acid medium than in non-supplemented acid medium. Zafar et al. (2007) observed the biosorption strategy among Metal-resistant fungi isolated from wastewater-treated soil belonged to genera *Aspergillus, Penicillium, Alternaria, Geotrichum, Fusarium, Rhizopus, Monilia* and *Trichoderma*. *Aspergillus* and *Rhizopus* isolates were tested for their metal biosorption potential for Cr and Cd in vitro. Biosorption experiments were conducted with initial metal concentrations of 2, 4, 6 and 8 mM with a contact time of 4 h and wet fungal biomass (1-5 g) at 25 degrees C. Maximum biosorption of Cr and Cd ions was found at 6 mM initial metal concentration. *Aspergillus* sp.1 accumulated 1.20 mg of Cr and 2.72 mg of Cd per gram of biomass. Accumulation of these two metals by very tolerant *Aspergillus* sp.2 isolate was at par with relatively less tolerant *Aspergillus* sp.1 isolate. *Rhizopus* sp. accumulated 4.33 mg of Cr and 2.72 mg of Cd per g of biomass. The findings indicated promising biosorption of cadmium and chromium by the *Rhizopus* and *Aspergillus* spp. from aqueous solution. There is little, if any, correlation between metal tolerance and biosorption properties of the test fungi. Tsekova et al. (2007) reported the biosorption of copper and cobalt, both singly and in combination (in equimolar concentrations), by the resting cells of *Penicillium brevicompactum*. The adsorption of binary mixtures of heavy metal solutions on the fungal biomass was found to be of competitive type where the adsorption capacity for any single metal decreased in the presence of the other. The cobalt ions showed a higher affinity for *Penicillium brevicompactum* than the copper ions.
Bishnoi et al. (2007) reported biosorption efficiency in the powdered *Trichoderma viride* biomass entrapped in polymeric matric of calcium alginate compared with cell free calcium alginate beads. Biosorption of Cr (VI) was pH dependent and the maximum adsorption was observed at pH 2.0. The maximum adsorption capacity of 16.075 mg/g was observed at dose 0.2 mg in 100 ml of Cr (VI) solution. The experimental results were fitted satisfactory to the Langmuir and Freundlich isotherm models. The hydroxyl (-OH) and amino (-NH) functional groups were responsible in biosorption of Cr (VI) with fungal biomass spp.

Akhtar et al. (2007) reported removal and recovery of uranium from dilute aqueous solutions by indigenously isolated viable and non-viable fungus (*Trichoderma harzianum*) and algae (RD256, RD257) was studied by performing biosorption-desorption tests. Fungal strain (*T. harzianum*) was found comparatively better (612 mg U g⁻¹ candidate for uranium biosorption than algae. 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 0.1M Hydrochloric acid which regenerated the uranium-free cell biomass facilitating the sorption-desorption cycles for better economic feasibility. Mukhopadhyay et al. (2007) studied a kinetic model for biosorption of copper and it was developed considering the possibility of different forms of functional groups being present on the surface of the biomass prepared from *Aspergillus niger*. Copper sorption by *A. niger* was influenced by the biomass dose, initial metal ion concentration, and pH of the solution. The retention capacity of the biomass was determined at pH 6.0 to be equal to 23.62mg/g of biomass.

Akar et al. (2007) investigated the lead (II) biosorption potential of *Aspergillus parasiticus* biomass in a batch system. The initial pH, biosorbent dosage, contact time, initial metal ion concentrations and temperature were studied to optimize the biosorption conditions. The maximum lead (II) biosorption capacity of the fungal biosorbent was found as 4.02 x 10⁻⁴ mol g⁻¹ at pH 5.0 and 20 degrees C. The biosorption equilibrium was reached in 70 min. Das and Guha (2007) described removal of chromium from aqueous solution by biomass of different moulds and yeasts. They found biomass of *Termitomyces clypeatus* (TCB) the most effective of all the fungal species tested. The sorption of hexavalent chromium by live TCB depends on the pH of the solution, the optimum pH value being 3.0. The amino, carboxyl, hydroxyl, and phosphate groups of the biomass were involved in chemical
interaction with the chromate ion forming a cage like structure depicted by scanning electron microscopic (SEM) and Fourier transform infrared spectroscopic (FTIR) results. Desorption and FTIR studies also exhibited that Cr\(^{+6}\) was reduced to trivalent chromium on binding to the cell surface.

Chen et al. (2007) used waste biomass of *Saccharomyces* as biosorbent to adsorb 10 kinds of metal ions, and their maximum biosorption capacity (\(q_{\text{max}}\)) was determined by the Langmuir isotherm model. They reported the values of \(q_{\text{max}}\) decreased in the following order (in millimole per gram): 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 suggested that the greater the covalent index value of metal ion was, the greater the potential to form covalent bonds with biological ligands, such as sulphydryl, amino, carboxyl, hydroxyl groups, etc. on the biomass surface, and the higher the metal ion biosorption capacity was. Classification of metal ions, for divalent ion or for soft-hard ion could improve the linear relationship (\(R^2 = 0.89\)). Fan et al. (2008) 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 ions concentration, biomass dose, contact time, temperature and co-ions on the biosorption were studied. The results of the kinetic studies of all metal ions at different temperature showed that the rate of adsorption followed the pseudo second-order kinetics well. The thermodynamics constants DeltaG\(_\text{degrees}\), DeltaH\(_\text{degrees}\) and DeltaS\(_\text{degrees}\) of the adsorption process showed that biosorption of Cd\(^{2+}\), Zn\(^{2+}\) and Pb\(^{2+}\) ions on *Penicillium simplicissimum* were endothermic and spontaneous.

Pan et al. (2009) investigated the effects of single and multiple heavy metals on the growth and uptake of consortium of two types of fungal strains, *Penicillium sp. A1* and *Fusarium sp. A19*. These fungal strains were tested to be tolerant to several heavy metals. A1, A19, and their combination (A1+A19) were inoculated on potato dextrose agar (PDA), Czapek Dox agar (CDA), and potato dextrose broth (PDB) containing Cu\(^{2+}\), Cd\(^{2+}\), Pb\(^{2+}\), and Zn\(^{2+}\). Experimental results showed that the combined inoculation of A1 and A19 had profound effects on the growth of the two fungi in PDA and CDA under the treatments with Cu\(^{2+}\) and mixed Cd\(^{2+}\)+Zn\(^{2+}\). The amount of metals through bioaccumulation by A1, A19, and A1+A19 was significantly higher than that through biosorption by these fungi. The highest amount of Cd, Cu, and Zn
accumulated by fungal biomass was obtained in the presence of Cd$^{2+}$+Cu$^{2+}$+Zn$^{2+}$ in PDB. Compared with the individual A1 or A19 used in PDB, A1+A19 accumulated higher amount of Cu and Pb in the presence of Cd$^{2+}$+Cu$^{2+}$+Pb$^{2+}$ and higher amount of Pb in the presence of Cd$^{2+}$+Cu$^{2+}$+Zn$^{2+}$+Pb$^{2+}$. The results indicated that there was no simple relationship between the metal biosorption by fungal biomass and the fungal metal tolerance. The biomass of A1+A19 cultivated in PDB absorbed higher amount of metals than A1 or A19 in the presences of single metals and their combinations. The results suggested that the applicability of growing fungi tolerant to heavy metals provided a potential biotechnology for treatment of wastewaters with heavy metal pollutions.

Amini et al. (2009) studied the effects of biosorbent *Aspergillus niger* dosage, initial solution pH and initial Ni (II) concentration on the uptake of Ni (II) by NaOH pretreated biomass of *A. niger* from aqueous solution were investigated. Batch experiments were carried out in order to model and optimize the biosorption process. The influence of three parameters on the uptake of Ni$^{2+}$ was described using a response surface methodology (RSM) as well as Langmur and Freundlich isotherm models. Optimum Ni$^{2+}$ uptake of 4.82 mg Ni$^{2+}$g$^{-1}$ biomass (70.30%) was achieved at pH 6.25, biomass dosage of 2.98 gl$^{-1}$ and initial Ni(II) concentration of 30.00 mgl$^{-1}$ Ni$^{2+}$. Langmuir and Freundlich were able to describe the biosorption isotherm fairly well. However, prediction of Ni$^{2+}$ biosorption using Langmuir and Freundlich isotherms was relatively poor in comparison with RSM approaches. The biosorption mechanism was also investigated by using Fourier transfer infrared (FT-IR) analysis of untreated, NaOH pretreated, and Ni$^{2+}$ loaded *A. niger* biomass. Prigione et al., (2009) characterized a tanning effluent from a mycological point of view and tested different fungal biomasses for the removal of Cr$^{3+}$ from the same tanning effluent in which, after the conventional treatments, Cr$^{3+}$ amount was very low but not enough to guarantee the good quality of the receptor water river. The experiments gave rise to promising results with a percentage of removed Cr(III) up to 40%. Moreover, to elucidate the mechanisms involved in biosorption process, the same biomasses were tested for Cr$^{3+}$ removal from synthetic aqueous solutions at different Cr$^{3+}$ concentrations.

Pakshirajan and Swaminathan (2009) studied biosorption of copper (II) and cadmium (II) by live *Phanerochaete chrysosporium* immobilized by growing onto
polyurethane foam material in individual packed bed columns over two successive
cycles of sorption-desorption were investigated in this study. Initial pH and
concentrations of the metals in their respective solutions were set optimum to each of
those: 4.6 and 35 mg x l^-1 in case of copper and 5.3 and 11 mg x l^-1 for cadmium. The
breakthrough curves obtained for the two metals during sorption in both the cycles
exhibited a constant pattern at various bed depths in the columns. The maximum yield
of the columns in removing these metals were found to be, respectively, 57% and
43% for copper and cadmium indicating that copper biosorption by the immobilized
fungus in its column was better than for cadmium. Recovery values of the sorbed
copper and cadmium metals from the respective loaded columns by using 0.1 N HCl
as eluant was observed to be quite high at more than 65% and 75%, respectively, at
the end of desorption in both the cycles.

2.7 Biochemical Traits of Soil Fungi Relevant to Plant Growth
Promotion

Soil microorganisms are critical to the maintenance of soil function in both
natural and managed agricultural soils because of their involvement in such key
processes as soil structure formation, decomposition of organic matter, toxin removal,
and cycling of carbon, nitrogen, phosphorus and sulphur. In addition soil,
microorganisms play key role in suppressing soil borne plant diseases, in promoting
plant growth (Garbeva et al., 2004). Importance and role of soil fungi in plant
growth promotion and soil health are well known. They produce a large number of
secondary metabolites (IAA, siderophores, ammonia, organic acids, antibiotics,
extracellular enzymes etc.), enhance the plant growth, crop productivity and soil
fertility (Field et al., 1993; Archer et al., 1994; Akthar and Mohan, 1995; Feijoo and
Lema, 1995; Prasertsan et al., 1997; Radzio and Kuck, 1997; Wongwicharn et al.,
1999; Palma et al., 1999; Xu et al., 2000, Coulibaly, 2002). However such studies are
limited to certain group of fungi such as mycorrizae and few free living phosphate
solubilising fungi (Gaur, 1990; Khan et al., 2007). Biochemical and physiological
attributes of free living soil fungi relevant to plant growth and soil functions are
summarized below.
2.7.1 Phosphate solubilization

Phosphorus is one of the major nutrients, second only to nitrogen in requirement for plants. A greater part of soil phosphorus, approximately 95–99% is present in the form of insoluble phosphates and cannot be utilized directly by the plants (Vassileva et al., 1998). To increase the availability of phosphorus for plants, large amounts of fertilizer are being applied to soil (Omar, 1998). Therefore, very little amount of the applied phosphorus is available to plants, making continuous application necessary (Abd Alla, 1994). However, phosphorus deficiencies are widespread on soil throughout the world and phosphorus fertilizers represent major cost for agricultural production. Many soil fungi and bacteria are known to solubilize inorganic phosphates (Asea et al., 1988; Illmer and Schinner, 1992). Phosphate solubilizing microorganisms (PSMs) play an important role in supplementing phosphorus to the plants, allowing a sustainable use of phosphate fertilizers. Many bacterial, fungal, yeast, and actinomycetes species capable of solubilizing sparingly soluble phosphorus in pure culture have been isolated and studied (Goldstein, 1986; Halder et al., 1991; Abd-Alla, 1994; Whitelaw, 2000). Application of PSMs in the field has been reported to increase crop yield. Several mechanisms like lowering of pH by acid production, ion chelation and exchange reactions in the growth environment have been reported to play a role in phosphate solubilization by PSMs (Goldstein, 1986; Halder et al., 1991; Abd-Alla, 1994; Whitelaw, 2000). Among PSMs, fungi perform better in acidic soil conditions (Ahmad and Jha, 1968). Species of Aspergillus, Penicillium and yeast have been widely reported solubilizing various forms of inorganic phosphates (Asea et al., 1988; Whitelaw, 2000). Fungi have been reported to possess greater ability to solubilize insoluble phosphate than bacteria (Nahas, 1996).

In India, it is estimated that about 260 million tons of phosphatic rock deposits are available and this material should provide a cheap source of phosphate fertilizer for crop production (FAI, 2002). Soil microorganisms that solubilize mineral phosphates can significantly affect phosphorus cycling in both natural and agricultural ecosystems. Phosphorus absorption by plants can be increased by the presence of symbiotic organisms such as mycorrhizal fungi (Azcon-Aguilar et al., 1986) or by the inoculation with the soil mineral phosphate solubilizing fungi particularly black Aspergilli (Vassilev et al., 1997; Narsian and Patel, 2000; Goenadi et al., 2000;
Reddy et al., 2002) and some species of *Penicillium* (Asea and Kucey, 1988; Cunningham and Kuiack, 1992; Whitelaw et al., 1999). The black *Aspergilli* include *Aspergillus tubingensis*, *Aspergillus niger*, *Aspergillus awamori* and *Aspergillus aculeatus*. AM fungi have been found to be essential components of sustainable soilplant systems (Smith and Read, 1997; van der Heijden et al., 1998; Schreiner et al., 2003) and particularly important in counteracting desertification of Mediterranean ecosystems (Carpenter and Allen, 1988; Brundrett, 1991). They increase plant uptake of phosphorus (Duponnois et al., 2005), micronutrients (Bu’rkert and Robson, 1994) and nitrogen (Barea et al., 1991). They enhance water absorption (George et al., 1992) and act as antagonists against some plant pathogens (Dehne, 1982; Lendzemo et al., 2005). AM fungal symbiosis changes root functions (i.e. root exudation) (Graham et al., 1981; Marshner et al., 1997), modifies carbohydrate metabolism of the host plant (Shachar-Hill et al., 1995) and interacts with rhizosphere populations (Hayman, 1983; Azaizeh et al., 1995; Andrade et al., 1997 and 1998). The structure and functionalities of these AM associated microbial communities differ from those of the rhizosphere (Duponnois et al., 2005) and this microbial compartment has been named “mycorrhizosphere” (Linderman, 1988; Caravaca et al., 2004 and 2005a).

In other studies ectomycorrhizal fungi have been shown to solubilize a range of sparingly-soluble metal-phosphate and metal-sulphate complexes including gypsum (CaSO_4·2H_2O), CaHPO_4, Ca_3 (PO_4)_2 and Ca_5 (PO_4)_3OH (Lapeyrie et al., 1987; Lapeyrie et al., 1991; Nguyen et al., 1992; Gharieb and Gadd, 1999). An isolate of *Penicillium bilaii* previously reported to solubilize mineral phosphates and enhance plant uptake of phosphate was studied by Cunningham and Kuiack (1992). They recorded the influence of the medium composition on phosphate solubility and medium acidification by Using agar media with calcium phosphate and the pH indicator alizarin red S. Singal et al. (1994) found *Aspergillus japonicus* and *A. foetidus* to solubilize five types of Indian rock phosphates at pH 8 and 9. Both the
Aspergilli were found to be good pyrite solubilizers and could grow over a wide pH range. Solubilization of rock phosphates was the result of organic acid release and pyrite oxidation. Vassileva et al. (1998) reported the rock phosphate solubilizing capacity of encapsulated spores of Aspergillus niger due to its citric acid production ability in agar, calcium alginate and k-carrageenan. They obtained the highest average soluble P concentration of 0.20 g/l batch⁻¹ with agar-cell beads as compared with other encapsulated systems.

Reddy et al. (2002) tested three isolates of Aspergillus tubingensis and two isolates of Aspergillus niger isolated from rhizospheric soils and tested for solubilization of different rock phosphates. They found all the Aspergillus isolates capable of solubilizing all the natural rock phosphates. A. tubingensis (AT1) showed maximum percent solubilization in all the rock phosphates tested in this study when compared to other isolates. This isolate also showed highest phosphorus (P) solubilization when grown in the presence of 2% of rock phosphate. A. tubingensis (AT1) seems to be more efficient in solubilization of rock phosphates compared to other isolates reported elsewhere. Gibson and Mitchell (2004) assessed the influence of nutrient variation on solubilizing ability of the fungi (Four ericoid mycobionts) grown on solid agar plates supplemented with zinc phosphate (0.25 %) containing different forms of nitrogen (nitrate, ammonium or alanine) and different concentrations of carbon (glucose) and phosphorus (K₂HPO₄). They observed the phosphate solubilization by measuring the zones of solubilization appearing beneath the growing colonies. All four mycobionts were found capable of zinc phosphate solubilization in the presence of all three nitrogen sources and in media containing no nitrogen. All but one of the mycobionts were capable of solubilizing calcium phosphate (CaHPO₄), while no solubilization was observed in media containing aluminium phosphate (AlPO₄), iron phosphate (FePO₄ x 4H₂O) or copper phosphate (Cu₃O₈P₂ x 2H₂O) under conditions which were found to be optimal for zinc phosphate solubilization.

Xu et al. (2004) carried out a series of batch experiments to investigate the phosphorus release from rock phosphate and iron phosphate by low-molecular-weight organic acids. Results showed that citric acid had the highest capacity to solubilize P from both rock and iron phosphate. P release from iron phosphate was positively correlated with Fe-organic acid stability constants except for aromatic acids. Increase
in the concentrations of organic acids enhanced P solubilization from both rock and iron phosphate almost linearly. Barroso and Nahas (2005) reported the status of soil phosphate fractions and the ability of fungi to solubilize hardly soluble phosphates. Of the 481 fungi isolated, 33 showed the ability to solubilize the inorganic phosphates in culture. Of these, 14 were considered to be high or very high solubilizers based on a solubilization capacity >1000 mg PO\textsubscript{4}\textsuperscript{3-} ml\textsuperscript{-1}. Isolate F111 was the only one that dissolved all the insoluble phosphates used. Nine isolates solubilized both Al-P and Ca-P, and four other isolates only solubilized Ca-P.

Gibson and Mitchell (2005) reported the heavy metal effect on phosphatase enzyme activity of Ericoid endomycorrhizal fungi (two isolates of Hymenoscyphus ericae growing on Cu-contaminated mine spoil). Wall-bound phosphatase activity, excluding PDEase of one H. ericae-type endophyte, was generally unaffected after the isolates had been grown on medium containing 0.25 mM Cu. Extracellular PDEase of the two H. ericae-type endophytes from mine spoil sites was stimulated by 0.25 mM Cu in the growth medium. Cu concentrations up to 5.0 mM in the assay medium did not inhibit wall-bound phosphatase activity whereas three of the isolates showed a stimulation of extracellular activity with increasing Cu. Edson et al. (2006) studied the Communities of P-solubilizing bacteria, fungi and arbuscular mycorrhizal fungi, from two areas of Atlantic forest, in Paraty – RJ, Brazil, one with a secondary forest and the other with a grass pasture. The bacteria were identified as Enterobacteriaceae and Bacillus sp., while the P-solubilizing fungi were identified as Aspergillus sp. Glomus macrocarpum and Glomus etunicatum were the dominant mycorrhizal fungi in the secondary forest and grass pasture area, respectively.

Barroso et al. (2006) reported the influence of several carbon and nitrogen sources on the solubilization of CaHPO\textsubscript{4} (Ca-P) and AlPO\textsubscript{4} (Al-P) by Aspergillus niger. Phosphate solubilization was related to acid production, pH drop and fungal growth in the culture medium. They carried out this study under abiotic conditions showed that organic acids solubilize more Ca-P than Al-P. Evaluating the effect of the nitrogen source, the solubilization of Ca-P or Al-P decreased in the following order: glycine > NH\textsubscript{4}Cl > NaNO\textsubscript{3} and NH\textsubscript{4}NO\textsubscript{3} > urea > (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, respectively. Ammoniacal nitrogen (NH\textsubscript{4} +) sources were the most effective in the production of acids and in lowering of the pH. Fomina et al. (2006) investigated zinc phosphate transformations by Paxillus involutus/pine ectomycorrhizas using zinc-resistant and
zinc-sensitive strains of the ectomycorrhizal fungus under high- and low-phosphorus conditions to further understand fungal roles in the transformation of toxic metal minerals in the mycorrhizosphere. They concluded that zinc phosphate solubilization and zinc and phosphorus uptake by the association depend on ectomycorrhizal infection, strain of the mycobiont, and the phosphorus status of the matrix.

Gupta et al. (2007) reported phosphate solubilization properties among fungi and bacteria obtained from heavy metal mines of Orissa (India). They screened among 62 fungi and 253 bacteria for phosphate solubilization properties, 12 fungi and 19 bacteria were found to solubilise tricalcium phosphate (TCP). *Penicillium* sp. 21 solubilised and released 81.48 mg PmL⁻¹ whereas *Penicillium* sp. 2 showed better efficiency of rock phosphate solubilization and produced 4.87 mg PmL⁻¹ into the liquid culture. Bacterial strains were comparatively poor solubilisers of TCP and rock phosphate in solid and liquid culture. Phosphate solubilising fungi were acid producers and more efficient than bacterial isolates. *Penicillium* sp. 21 and *Penicillium* sp. 2 were confirmed the best for TCP and rock phosphate solubilization. Vyas et al. (2007) reported the *Eupenicillium parvum* as a phosphate solubilizer fungul strain from tea rhizosphere and they studied its qualitative and quantitative capability of phosphate solubilization in vitro condition. The fungus developed a phosphate solubilization zone on modified Pikovskaya agar, supplemented with tricalcium phosphate. Quantitative estimation of phosphate solubilization in Pikovskaya broth showed high solubilization of tricalcium phosphate and aluminium phosphate.

Ahuja et al. (2007) tested a phosphate solubilizing fungus, Paecilomyces marquandii AA1 from phosphate deficient soil on Pikovskaya's medium buffered with Tris-HCl pH 8. The organism could release phosphate from both buffered and unbuffered medium and solubilized rock phosphates from various places. Wahid and Mehana (2002) reported about the three phosphate solubilizer fungal isolates, *Aspergillus niger*, *A. fumigatus* and *Penicillium pinophilum* isolated from the rhizosphere of different plants grown in Ismailia and South Sinai Governorates. The fungal isolates effectively solubilized rock phosphate or tricalcium phosphate in Pikovskaya's liquid medium. In pot and column experiments, they significantly reduced pH and increased available phosphorus in the soil treated with either rock phosphate or superphosphate. Wakelin et al. (2007) reported the phosphate solubilizing fungi *Penicillium radicum*, *Penicillium bilaiiae* (strain RS7B-SD1), and
an unidentified *Penicillium* sp. designated strain KC6-W2 for their ability to increase the growth and phosphorus (P) nutrition of wheat, medic, and lentil in three soils of neutral to alkaline pH reaction. *Penicillium* sp. KC6-W2 was found the strongest plant growth promoting (PGP) strain which stimulated significant increases in shoot growth and dry mass in seven of the nine experiments conducted. Bojinova *et al.* (2008) reported the phosphate solubilizing capability of *Aspergillus niger*. The phosphorus concentration (as P$_2$O$_5$), citric acid production, glucose concentration and pH in the cultural medium were monitored. They recorded that Phosphate dissolution was not strongly correlated both with the citric acid production and the incubation period. When the fungi were grown without water soluble phosphorus compounds the MP solubilization had higher values. A maximum of 94.80% total P$_2$O$_5$ extraction was achieved.

### 2.7.2 Organic acid

Although many organic acids are made by living cells, few are produced commercially. Citric, gluconic, itaconic, and lactic acids are manufactured via large-scale bioprocesses. Oxalic, fumaric, and malic acids can be made through fungal bioprocesses, *Aspergillus* species, *A. terreus*, is used to make itaconic acid. Chelating properties of citric acid in conjunction with the increased solubility of most metal compounds at acidic pH may allow *A. niger* to grow in environments where metals are present at very low concentrations or in an insoluble state. Second, acidification would inhibit the growth of competitors, as a majority of rapidly growing bacterial species and many fungi cannot grow below pH 3. The three fungal species produce a variety of organic acids, which may reflect different strategies to compete with other microorganisms. Fungi classified as Zygomycetes, including *R. oryzae*, have developed a different strategy for acidifying the environment by producing lactic acid to compete with fungi unable to metabolize lactic acid (Jan and Lene Lange, 2004).

Low molecular weight organic acids (LMWOA) are recognized to be of major importance in a number of soils and plant processes (Ryan *et al*., 2001). Within plants and soil microorganisms organic acids perform a number of pivotal metabolic roles including the provision of C for respiration and biomass production, making nutrients available (e.g. Fe-citrate) and maintaining internal charge balance (Jones, 1998). Further, both plants and microorganisms can excrete LMWOAs into the soil to
increase the solubility of nutrients (e.g. P), detoxify metals (e.g. Al₃C), and aid in chemotactic responses and the formation of symbiotic associations (Dakora and Philips, 2002). Several LMWOAs are found in soil solution with the most commonly identified ones being oxalate, citrate, acetate and formate (Fox, 1995; Jones, 1998). Normally, soil solution concentrations are in the micromolar range (Jones, 1998). There are several primary sources of LMWOAs in soil and among these root exudation is regarded as one of the most important (Jones, 1998). Moreover, it is well documented that bacteria and saprophytic fungi can produce significant amounts of LMWOAs in soils (Fox, 1995). Likewise, mycorrhizal fungi may be an important source particularly in forest soils. Cromack et al. (1979) and Griffiths et al. (1994) have shown that large amounts of Ca-oxalate can accumulate in so called 'mycorrhizal mats'. In laboratory experiments it has been demonstrated that mycorrhizal pine seedlings exude more oxalate than non-mycorrhizal ones (Ahonen-Jonnarthe/a/., 2000).

Adsorption of organic acids to mineral surfaces, e.g. Al and Fe oxyhydroxides, is another factor which may lower soil solution concentrations, but will also prevent biodegradation (Jones et al., 2003) The relative importance of production and removal factors, as well as their regulatory mechanisms, remains largely unknown. Enhanced root and mycorrhizal exudation in response to certain nutrient demands (e.g. P) have been proposed (Jones, 1998; Dakora and Philips, 2002), but controversy still exists (Jones et al., 2003). Oxalic is a leaching agent for solubilizing heavy metals, for instance from bauxite, clay, kaolin, nepheline, quartz sand, sewage sludge and spodumene (Gadd et al., 2005b). Oxalic acid is a common metabolite excreted by several fungi under specific conditions. Among other species such as Aspergillus ficuum, Penicillium oxalicum, sclerotium rolfsii, gleophyllum trabeum and white or brown rot fungi (Gadd, 2007). The fungus Aspergillus niger is a best producer of oxalic acid (Gadd, 1999; Khan, 2005).

Oxalic acid production in fungi is often related to metal detoxification and pathogenesis; the latter due to affects that include acidification of host tissues, sequestration of metal ions such as calcium, manganese, magnesium, and iron, and possible inhibition or disruption of host defense responses (Godoy et al., 1990; Gadd 1999; Cessna et al., 2000; Jarosz-Wilkolazka and Gadd, 2003).
2.7.3 Indole acetic acid (IAA) production

Growth hormones are organic substances produced naturally in higher plants, controlling growth or physiological functions and are able to mediate intercellular communications in minute amounts. Five (Pincus and Thiamann, 1948) major types of hormones regulate plant development namely: Auxins, Gibberellin, Cytokinin, Ethylene and Abscisic acid (Arshad et al., 1990). Indole Acetic Acid (IAA) has been found to be of universal occurrence and is a major plant hormone. It is one of the most common and extensively used auxin. (Wilkins, 1972) In the modern biotechnological methods i.e., especially tissue culturing, IAA stimulates differentiation of root/shoot in an undifferentiated callus. Plants (Leopold, 1980) propagated by cuttings are difficult to root. IAA initiates and hastens the root development and often results in larger and more vigorous roots. A significant increase in root length, root number and mycorrhizal infections has also been reported by IAA application. Seedless fruits (El-Aishy, 1976; Thiamann, 1972 and Firdaus, 1987a) of full marketable size have also been successfully prepared by exogenous application of various hormones including IAA in the case of cucumber and watermelons (Das, 1956).

Indole-3-acetic acid and its analogue is the primary active auxin in most plants. It is synthesized from tryptophan, primarily in leaf primordion and young leaves and developing seeds. More than one pathway of IAA synthesis in plants has been demonstrated. Auxin plays an important role in cell enlargement, cell division, root initiation etc (Glick, 1995). Many fungi can produce auxins in axenic cultures (Gruen, 1959; Buckley and Pugh, 1971). Most species use tryptophan to produce indole-3-acetic acid (IAA), mainly through the indole-3-pyruvic acid and tryptamine pathways (Tudzynski and Sharon; 2002.). The physiological role of auxins in fungi is not well understood. In most studies, auxin was added to the culture medium, and the effects on fungal growth and development were determined. It is not known whether exogenous IAA and endogenous IAA cause the same phenotype. One of the roles suggested for fungus-produced IAA is to mediate fungal-plant interaction. High concentrations of IAA can inhibit the hypersensitive response (Robinette and Matthysse, 1990; Jouanneau, 1991) and may suppress expression of plant defense genes (Yamada, 1985; Shinshi, 1987).
Tuomi et al. (1993) found the molds *Botrytis cinerea*, *Cladosporium cladosporioides*, and the yeast *Aureobasidium pullulans*, isolated from the leaves of three short-rotation *Salix* clones, to produce indole-3-acetic acid (IAA). They suggested that the effect of fungal IAA production on plants was limited to the rhizosphere and that *B. cinerea*, which was a known pathogen, induced ABA production by the mother plant as a response to physiological stress. Robinson et al. (1998) characterized the biosynthesis of indole-3-acetic acid by the mycoherbicide *Colletotrichum gloeosporioides* f. sp. *aeschnomene*. Auxin production was tryptophan dependent. Compounds from the indole-3-acetamide and indole-3-pyruvic acid pathways were detected in culture filtrates. Indole-3-acetamide was the major pathway utilized by the fungus to produce indole-3-acetic acid in culture.

In a study of various phytopathogenic fungi, Furukawa et al. (1996) found that fungi that belong to the genus *Rhizoctonia* produce IAA efficiently from tryptophan. *R. solani* Kiihn MAFF- 305219, in particular, produced large amounts of tryptophol (Tol), which was assumed to be a specific by-product of the indole-3-pyruvate (IPy) pathway, in addition to IAA. They suggested that both IAA and Tol are synthesized from tryptophan through the IPy pathway in *Rhizoctonia*.

Hasan (2002) screened the fungi, mostly *Aspergillus flavus*, *A. niger*, *Fusarium oxysporum*, *Penicillium corylophilum*, *P. cyclopium*, *P. funiculosum* and *Rhizopus stolonifer* for their capability to produce gibberellin and IAA. All fungal species have the ability to produce gibberellin (GA) but *F. oxysporum* was found to produce both GA and indole-acetic acid (IAA). The optimum period for GA and IAA production by *F. oxysporum* was 10 days in the mycelium and 15 days in the filtrate at 28 degrees C.

### 2.7.4 Siderophore production

To sequester and solubilize ferric iron, many microorganisms utilize an efficient system consisting of low-molecular mass (<1000 Da) compounds with high iron affinity termed 'siderophores' (Guerinot et al., 1990; Neilands, 1995). According to the generally accepted definition, siderophores are ferric-specific microbial iron-chelator compounds whose biosynthesis is regulated by the availability of iron in the surrounding medium and under conditions of high iron concentrations; the production of these compounds is repressed. Therefore, a certain variation can be expected depending on the particular species under investigation (Neilands, 1984; Guerinot,
1994). Studies of microorganism siderophore producers have received much attention because of the clinical applications and potential utilization of these chelators in agriculture (Neilands, 1993 and 1995). Most species of the genus *Aspergillus* are known to produce several hydroxamate-type siderophores and many reports on the isolation and characterization of siderophores have been published (Hider, 1984; Dube et al., 2000). Moreover, *Aspergillus* fungi are producers of organic acids such as citric and oxalic acids which have been reported to act as siderophores in other microorganisms (Guerinot et al., 1990; Carson et al., 1992; Winkelmann, 1992; Dutton and Evans, 1996).

Some siderophores (e.g., aerobactin) have lower and others (e.g., enterobactin) have higher affinities (Ratledge and Dover, 2000). Siderophores are typically produced by bacteria, fungi, and monocotyledonous plants in response to iron stress (Ratledge and Dover, 2000). Typically, microbial siderophores are classified as catecholates, hydroxamates, and *α*-carboxylates, depending on the chemical nature of their coordination sites with iron (Winkelmann, 1991 and 2002).

Hydroxamates are produced by fungi and bacteria, whereas catecholates are produced exclusively by bacteria and comprise catechol and hydroxy groups as ligands. *α*-carboxylates are produced by the group of fungal zygomycetes (mucorales) and a few bacteria, such as *Rhizobium* meliloti and *Staphylococcus hyicus*, and coordinate iron through hydroxy and carboxyl groups (Drechsel et al., 1995b; Baakza et al., 2004). Although plentiful in the environment, iron exists in nature predominantly in the insoluble ferric iron oxidation state, which is not readily bioavailable for microbial assimilation. Successful adaptation to this environmental context has provided almost all fungi with an iron uptake strategy that involves reduction of Fe$^{3+}$ to Fe$^{2+}$ via two general mechanisms: (i) iron reduction before uptake or reductive iron assimilation and (ii) iron uptake before reduction or nonreductive iron assimilation (de Luca and Wood, 2000).

In brown-rot fungi, at least three strategies could be involved in this process. One mechanism is the secretion of low molecular weight Fe$^{3+}$-chelating hydroxamate siderophores (Milagres et al., 2002; Arantes and Milagres, 2006). A second possible route to acquire iron could be the release of organic acids, as wood decaying fungi are also known to produce oxalic and citric acids. A final iron acquisition strategy could
rely on secretion of catechols, associated with the extracellular nonenzymatic ferric-reducing activity (Goodell et al., 1997; Arantes and Milagres, 2006).

Korat et al. (2001) examined 18 filamentous fungi belonging to Zygomycotina (10 mucorales) and Ascomycotina (4 Aspergillus spp. and 4 Penicillium spp.), all produced siderophores in iron deficient m9 and grimm-allen media except Rhizopus sp. and Aspergillus flavus. The siderophore produced by the 9 mucorales were carboxylates, while those produced by 3 aspergilli and 4 penicillia were hydroxamates. They found highest (72.2×10^{-4}\text{ gml}^{-1}) and lowest (14.9×10^{-4}\text{ gml}^{-1}) siderophore producers Rhizopus oryzae and Synchephalastrum sp. respectively.

Gupta et al. (2001) examined the heavy metal effect on siderophore production by Pseudomonas aeruginosa. Different metal ion compounds (ZnSO_{4}, MnCl_{2}, FeCl_{3}, and MnSO_{4}) of different ions concentrations were used and observed their effect. Individually ZnSO_{4} (12\mu m) promoted sideraphore production but suppressed the growth and protein content of test organism, MnCl_{2} and FeCl_{3} (12\mu m) enhanced the growth, whereas MnCl_{2} and MnSO_{4} (12\mu m) induced protein contents of test organism. Vandana and Satyanarayana (2002) screened seven ectomycorrhizal fungi, Laccaria Laccata, L. fraterna, Pisolithus tinctorious, Paxillus involutus, Rhizopogon vulgaris and R. luteolus for production of siderophores by chrome azurol s universal plate assay. The secretion of siderophore was seen in all except L. fraterna and Paxillus involutus. They observed that the fungi produced only hydroxamate type siderophores.

Baakza et al. (2004) examined siderophore producing potential of 20 fungal isolates (same 10 species from each marine and terrestrial habitat) and compared. Except marine Aspergillus flavus, all isolates produced siderophores as evidenced by positive reaction in FeCl_{3} test, CAS assay and CAS agar plate test. The results indicated widespread occurrence of siderophores in both the habitats. They revealed that mucoraceous fungi produced carboxylate, while others produced hydroxamate siderophores. Among all the isolates, Cunninghamamella elegans (marine form) was maximum siderophore producer (1987.5Ag/ml) followed by terrestrial form of C. elegans (1248.75 Ag/ml). They observed four terrestrial isolates (Aspergillus niger, Aspergillus ochraceous, Penicillium chrysogenum, Penicillium citrinum) were ahead in siderophore production, while, the other four marine isolates. Pérez-Miranda et al. (2007) screened about 48 microorganisms for siderophore production on popular CAS
assay, based on the utilization of chrome azurol S. As a result, they determined 36 microorganisms as siderophore producers out of 48 isolates from three sampling sites.

2.7.5 Extracellular enzymes

The enzymes are essential proteins for the metabolic system of all living organisms and have an important role in the degradation of organic matter, in host infection and food spoilage. In the metabolic pathways, they act in organized sequences of catabolic and anabolic routes (Lehninger, 1988). Enzymes may also act in the control of biochemical processes in the living cells. They may be isolated from plants, animals and microorganisms. The microbes are considered good source of industrial enzymes for the great diversity of enzymes that have been found (Lima et al., 1986). The enzymes are used in large scale in textile (amylase, cellulose, oxidoreductase), food (pectinase, protease, cellulose, oxidoreductase), detergents (protease, lipase, cellulose, oxidoreductase), paper (xylanase, oxidoreductase and lipase), and leather (protease, lipase) industries (Nielsen et al., 1998).

Extracellular enzymes may be produced in liquid or solid media. The use of solid media permits a fast screening of large populations of fungi, allowing the detection of specific enzymes and helping in the chemotaxonomical differentiation of many microorganisms (Alves et al., 2002). Filamentous fungi such as *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei* are able to produce and secrete large concentrations of enzymes (e.g. amylases, proteases, cellulases) into the environment. Efficient fermentation technologies have been developed for antibiotic, organic acid and native enzyme production from filamentous fungi (Wiebe, 2003). Soil microbial communities are important contributors to the decomposition of organic matter. Saprophytic fungi play a major role in decomposition because they must rely on dead organic matter as their source of carbon and energy. Mycorrhizal fungi, which obtain carbon primarily from their host plants (Smith and Read, 2002) contribute to decomposition as they can access organic sources of nitrogen (Bending and Read, 1996). The chemical composition of soil organic matter is an important factor controlling the activity of soil microbes and decomposition processes (Waldrop et al., 2006).

Lignin is an energy-rich, recalcitrant polyphenolic macromolecule and its decomposition is accomplished in large part by the activity of extracellular enzymes that catalyze the oxidation of phenylpropane alcohols from the lignin polymer (Kirk
and Farrell, 1987). Lab studies have found saprotrophic fungi and ectomycorrhizal fungi (Colpaert and van Laere, 1996; Courty et al., 2006) possess the physiological capacity to produce phenol oxidase and peroxidase, two enzymes important in lignin depolymerization (Kirk and Farrell, 1987). The inhibition of lignin degradation may be due to changes in the composition or abundance of the soil fungal community, or it may be due to repressed fungal enzyme production or activity (Kirk and Farrell, 1987; Fog, 1988). α-Amylase has been derived from several fungi, yeasts, bacteria and actinomycetes, however, enzymes from fungal and bacterial sources have dominated applications in industrial sectors (Pandey et al., 2000). Fungal sources are confined to terrestrial isolates, mostly to Aspergillus species and to only one species of Penicillium, P. brunneum (Haska and Ohta, 1994; Pandey et al., 2000). These enzymes are common in fungi, and Aspergillus sp and Rhizopus sp are often used as sources of industrial amylases (Boyce, 1986; Moreira et al., 1999).

Many workers also reported the production of extracellular enzymes like amylase, chitinase, cellulose and lipase from fungi. (Moreira et al., 1999; Tikhonov et al., 2002; Peixoto et al., 2003; Michael et al., 2004; Velázquez-Cedeño et al., 2004; Nampoothiri et al., 2004; Patidar et al., 2005; Kunamneni et al., 2005; Voigt et al., 2005; Kathiresan and Manivannan 2006; Guimaraes et al., 2006; Mahmood et al., 2006; Szczesna-Antczak et al., 2006; Rodrigues et al., 2007; Villena and Gutiérrez-Correa 2007; Savitha et al., 2007).

### 2.7.6 Role of extracellular enzyme production by fungi

Secretion of hydrolytic enzymes, particularly chitinases and glucanases, is a feature common to many effective biological control agents. Strains of Trichoderma, for example, are efficient producers of lytic enzymes, and many are used in commercial enzyme manufacturing. Using molecular biology techniques, the genes encoding chitinases, glucanases and proteinases have been cloned and sequenced from Trichoderma species. Mutant strains with disrupted activity of ech 42, a chitinase-encoding gene, were shown to be less effective as biocontrol agents against Rhizoctonia solani and Botrytis cinerea compared with wild-type strains (Baek et al., 1999; Woo et al., 1999). Conversely, the overexpression of several genes encoding enzymes such as chitinase (ech 42, chit 33) (Baek, et al., 1999; Limo’n, et al., 1999), endoglucanase (egl1) (Migheli et al., 1998), and proteinase (prb1) (Flores et al.,
1997), in transformed *Trichoderma* spp. improved the antagonistic potential of the agent against pathogens such as *Rhizoctonia* and *Pythium*, both in vitro and in vivo. A mutant strain of *T. harzianum*—with an enhanced ability to hydrolyze pustulan (a polymer of b-1, 6-glucans) showed enhanced production of chitinase and b-1, 3- and b-1, 6-glucanases, and produced more extracellular proteins and other compounds (Rey *et al.*, 2001). Specificity of hydrolytic enzyme activity was also suggested in a study by Woo *et al.*, (1999), where an endochitinase-deficient mutant displayed differing levels of biocontrol activity against different pathogens. Secretion of an exo-b-1, 3-glucanase by *Ampelomyces quisqualis* was found to play a role during the later stages of hyperparasitism of powdery mildew (Rotem *et al.*, 1999).

### 2.7.7 Production of antibiotic compounds by fungi

The production of antibiotic compounds is characteristic of many effective fungal and yeast biocontrol agents, and can be shown both in vitro and in vivo. Species of *Trichoderma* and *Gliocladium*, as well as the yeast *Pseudozyma*, which are currently all registered biological control products, are known to produce several secondary metabolites with broad-spectrum antimicrobial activity. For example, gliotoxin and gliovirin are well-described antibiotics (among others) produced by *Trichoderma* (*Gliocladium*) virens (Howell, 2003). The antibiotics produced by *Pseudozyma flocculosa* are a mixture of fatty acid-containing derivatives that affect membrane permeability of the target organisms, thereby inhibiting their growth (Avis and Be’langer, 2001). Conclusive evidence for the role of antibiotics in biocontrol-mediated disease suppression has required the development of antibiotic-minus mutant strains with a subsequent evaluation of their efficacy. Mutants of *T. Virens* unable to synthesize gliotoxin and gliovirin were shown to have lost their capacity to control root-infecting fungi such as *Pythium* (Howell, 2003). In some instances, disease-suppressive activity was directly correlated with the timing and amount of antibiotic produced (Wilhite and Straney, 1996).

### 2.8 Microbial Co-operation in the Rhizosphere

Many microbial interactions, which are regulated by specific molecules/signals (Pace, 1997), are responsible for key environmental processes, such as the biogeochemical cycling of nutrients and matter and the maintenance of plant
health and soil quality (Barea et al., 2004). Many studies have demonstrated that soil-borne microbes interact with plant roots and soil constituents at the root–soil interface (Lynch, 1990; Linderman, 1992; Glick, 1995; Kennedy, 1998; Bowen and Rovira, 1999; Barea et al., 2002b).

Microbial activity in the rhizosphere influence rooting patterns and the supply of available nutrients to plants, thereby modifying the quality and quantity of root exudates (Bowen and Rovira, 1999; Gryndler, 2000; Barea, 2000). Because of current public concerns about the sideeffects of agrochemicals, there is an increasing interest in improving the understanding of co-operative activities among rhizosphere microbial populations and how these might be applied to agriculture (Kennedy, 1998; Bowen and Rovira, 1999; Barea et al., 2004; Lucy et al., 2004). Barea et al. (2004) concluded that detrimental microbes included both the major plant pathogens and the minor parasitic and non-parasitic deleterious rhizosphere bacteria and fungi. Beneficial saprophytes, from a diversity of microbial groups, are able to promote plant growth and health. These include (i) decomposers of organic detritus, (ii) the plant growth promoting rhizobacteria (PGPR), and (iii) fungal and bacterial antagonists of root pathogens. Some of these micro-organisms, the endophytes, colonize the root tissues and promote plant growth and plant protection. Beneficial, plant mutualistic symbionts include the N2-fixing bacteria and the arbuscular mycorrhizal fungi. Some micro-organisms can benefit plants in several ways. For example, Trichoderma species control fungal pathogens by acting both as a microbial antagonist and by inducing localized and systemic plant defence responses (Harman et al., 2004). Endophytic bacteria and fungi (Sturz and Novak, 2000; Surette et al., 2003; Landa et al., 2004; Sessitsch et al., 2004) act both as growth promoters and as biocontrol agents.

Indigenous and/or introduced AM fungi can benefit annual crops, such as cereals and legumes, vegetable crops, temperate fruit trees or shrubs, tropical plantation crops, ornamentals, and spices (Azco´n-Aguilar and Barea, 1997; Vestberg et al., 2002). Microbial populations in the rhizosphere are known either to interfere with or to benefit the establishment of mycorrhizal symbioses (Gryndler, 2000). A typical beneficial effect is that exerted by the ‘mycorrhiza-helper-bacteria’ (MHB), a term that was coined by Garbaye (1994) for those bacteria known to stimulate mycelial growth of mycorrhizal fungi and/or enhance mycorrhizal formation. This


appplies both to Ectomycorrhiza (Garbaye, 1994; Founoune et al., 2002; Frey-Klett et al., 2005) and to AM associations (Azcoñ- Aguilar and Barea, 1995; Gryndler, 2000; Barea et al., 2004; Johansson et al., 2004). Kucey (1987) conducted Greenhouse and field experiments to test the effect of a P-solubilizing isolate of Penicillium bilaji on the availability of Idaho rock phosphate (RP) in a calcareous soil. Under controlled greenhouse conditions, inoculation of soils with P. bilaji along with RP at 45 mug of P per g of soil resulted in plant dry matter production and P uptake by wheat (Triticum aestivum) and beans (Phaseolus vulgaris).

De Freitas et al. (1997) reported that the stimulatory effect on chickpea plants was more pronounced with A. awamori than P. citrinum, though Penicillium strains exhibited more P-solubilization activity under in vitro conditions as compared to Aspergillus strains. They also reported that growth and yield in pot experiments. Like P-solubilizing activity, the IAA production was also found to be more in Penicillium strains than Aspergillus strains under in vitro conditions. Reyes et al. (2002) reported their assessment of Maize root colonization and phosphate solubilizing activity of the fungus Penicillium rugulosum in a greenhouse trial using soil-plant microcosms. In the absence of P fertilization, inoculation with any P. rugulosum isolate significantly reduced the size of the total and P-solubilizing bacterial community present in maize rhizosphere. All P. rugulosum strains were able to stimulate the growth of maize plants as indicated by 3.6 to 28.6% increases in dry matter yields. In the presence of rock phosphate, P uptake by maize plants inoculated with the two mutants Mps^+ and Mps^- was not always in agreement with their P-solubilizing phenotypes. Strain IR-94MF1 and transformant w-T3 increased P assimilation by the plants fertilized with Navay rock phosphate by 26 and 38%, respectively. In this treatment, w-T3 showed its highest significant maize rhizosphere colonization. With the simple superphosphate treatment, w-T3 increased P uptake in plants by 8% over the uninoculated control and also decreased significantly the community size of total bacteria, total fungi, and P-solubilizing fungi in the rhizosphere.

Babana and Antoun (2006) studied the TPR-solubilizing microorganisms (TSM) with high P-mobilization activities. They used the rhizosphere of three wheat cultivars (Alkama Beri, Hindi Tossom and Tetra) to isolate TSM, only bacterial isolates were selected. TPR-solubilizing fungi were only obtained by soil enrichment in liquid medium containing TPR as sole P source. In the rhizosphere a significant
correlation was observed between the total microbial population and the number of microorganisms solubilizing. Significant interactions were observed between TSM inoculation and P-fertilization for root colonization with AM, plant height at 30 days and root dry matter yield. The bacterial isolate *Pseudomonas* sp. BR2, which appeared to be a mycorrhiza helper bacterium, significantly enhanced wheat seedling emergence very early (5 days after planting) under field condition, and caused 128% increase in root dry matter yield. The two TPR-solubilizing fungal isolates *Aspergillus awamori* Nakazawa C1 and *Penicillium chrysogenum* Thom C13 also caused respectively 60 and 44% increases in root dry matter yields. Pengnoo *et al.* (2007) studied the infertile and strongly acidic soils widely distributed throughout Southern Thailand. They reported the role of phosphate solubilizing microorganisms in the well growth of some native plants of this soil, containing low quantity of available phosphorous.

Morales *et al.* (2007) studied the effect of inoculation with *Penicillium albidum*, a phosphate-solubilizing fungus, on the growth of red clover (*Trifolium pratense* L.) in Volcanic soils. They reported that *Penicillium albidum* contributed to growth and nutrition of the red clover through the induction of root development and enhancing phosphate mobilization from the soil and into the plant. It was concluded that *Penicillium albidum*, under greenhouse conditions, in soils deficient in available P could increase the inoculation potential for volcanic soils n Chile.

Xiao *et al.* (2009) observed the three phosphate-solubilizing fungi; identified as *Penicillium expansum*, *Mucor ramosissimus*, and *Candida krissii*, were isolated from phosphate mines (Hubei, People’s Republic of China) and characterized. All the isolates demonstrated diverse levels of phosphate-solubilizing capability. Acidification of culture medium seemed to be the main mechanism for rock phosphate solubilization. Indeed, citric acid, oxalic acid, and gluconic acid were shown to be present in the culture medium inoculated with these isolates. Moreover, the isolates produced acid and alkaline phosphatases in culture medium, which may also be helpful for RP solubilization. All the isolates promoted growth, soil available phosphorus, phosphorus, and nitrogen uptake of wheat seedling in field soil containing rock phosphate under pot culture conditions, thus demonstrating the capability of these isolates to convert insoluble form of phosphorus into plant
available form from rock phosphate, and therefore hold great potential for development as biofertilizers to enhance soil fertility and promote plant growth.

Many others workers also reported the composite effect of bacteria and fungi on crops like chick pea, moon bean, maize, wheat etc, (Zaidi et al., 2003; Rosas et al., 2006 and 2009; Hameeda, et al., 2008; El-Tarabily et al., 2008,). Mittal et al. (2008) carried out various pot experiments in green house, maximum stimulatory effect on chickpea plants growth was observed by inoculation of two A. awamori strains. They reported 7–12% increase in shoot height, nearly three-fold increase in seed number and two-fold increase in seeds weight as compared to the control (un-inoculated) plants. Inoculation of four strains of P. citrinum exhibited lesser stimulatory effect. It showed 7% increase in shoot height, two-fold increase in seed number and 87% increase in seeds weight as compared to the control plants. Varsha and Patel (2009) reported that after the various rhizosphere soil properties studied, a highly significant positive correlation was established between PSF and soil available P as well as pH. A significant positive association observed between total fungal population and organic matter as well as soil available P. Both abundance and number of PSF were more pronounced in descending order in plant covers: oilseeds, flowers, orchards, vegetables, pulses and cereals.