2. REVIEW OF LITERATURE

2.1 Soil system

Soil is a fundamental resource necessary for meeting the diverse needs of different life forms including humans (Laishram et al., 2012). It is a complex living entity containing diverse group of microorganisms, which play significant role in nutrient cycling, fertility and plant growth (Jha, 1990). Raman (1911, 1928) defined soil as the “upper weathering layer of the solid earth crust”. Joffe (1936), however, termed it as a natural body differentiated into horizons of mineral and organic constituents, usually unconsolidated of variable depths, which differ from parent material below in morphological, physical, and biological characteristics and is influenced by the prolonged interaction between microorganisms, organic matters and minerals. Consequently, developing an interlinked and interdependent balanced living soil system (Maharana and Patel, 2013b; Tapadar and Jha, 2015).

It is composed of minerals, organic matter, water and air, which influence physico-chemical properties of the soil. The soil and its horizons (O: litter layer; A: top soil; B: zone of accumulation; C: parent material; R: bed rock) develop due to action of climate and soil organisms on the parent rock (Brady and Weil, 2002). Soil texture i.e. the relative proportion of sand (0.05-2.0 mm), silt (0.002-0.05 mm) and clay (<0.002 mm) regulates other properties of soil (McCauley et al., 2005). Soil colloids (finest clay and soil organic matter) has large surface area resulting in increased physical and chemical activity (McCauley et al., 2005).

Arrangement of soil particles into larger clusters i.e. aggregates increases stability, porosity; improve soil-water movement, fertility and carbon sequestration (Nichols et al., 2004). Movement of water and air in the soil depends upon texture and porosity of soil. Well aggregated loamy soils are best in nature for supplying water and air to plants because of the presence of balanced soil macro and micro pores (Gurevitch et al., 2002). Soil colloidal surfaces are both positively and negatively charged. Negative charge is, however, predominant resulting in more cation exchange capacity (CEC) of soil than anion exchange capacity (AEC). Soil organic matter responsible for stability of soil aggregates accounts for cation exchange capacity (CEC) of soil (Gobat et al.,
Okalebo et al. (1993) mentioned that soil rich in organic matter has high CEC, and, therefore, retain more nutrients to support soil microorganisms and plants (Courtright et al., 2001).

Soil pH is a critical soil chemical property that regulates other important soil properties. pH also affects the soil nutrients (C, N, P, K, Ca, Mg, S) by altering its bioavailability (Andersson et al., 2000; Kemmitt et al., 2006; Aciego and Brookes, 2008). In acidic soil, the bioavailability of soil nutrients get reduced, while, bioavailability of heavy metals (Flis et al., 1993) get increased resulting in detrimental effect on plant and soil microorganisms. Soil pH is among the most influential factors affecting the soil microbial community (Rousk et al., 2009). Microorganisms are key biological component of the soil system which significantly alter the soil microhabitats in terms of physical and chemical characteristics (Ike-Izundu, 2007; You et al., 2014). They help in transformation (Lamers et al., 2012), cycling of nutrients and availability of minerals to plants, increase soil nutrient status, produce vitamins, hormones and phytostimulators. Excessive salinity has adverse effects on the soil physico-chemical and biological characteristics (Tejada et al., 2006). Soil organic matter is intrinsically associated to biological functioning. Different forms of soil organic matter supports diverse population of soil organisms and consequently, maintains a complex food web. Soil provides food, energy and all the nutrients required to run metabolic process within the body of soil organisms and plants.

2.2 Degradation of soil

Soil degradation is defined as the alteration in physical, chemical and biological properties of soil resulting in decline in of soil health, consequently, leading to temporary or permanent lowering of productivity of soil. The decline in soil health/quality due to anthropogenic activities is a serious global environmental problem (Kapalanga, 2008). Nevertheless, it has not been properly addressed (Bhattacharyya et al., 2015).

A range of factors involved in the soil degradation include natural, industrial, urban, mining disturbances and disturbances due to land management practices. There are two processes involved in soil degradation i.e. intrinsic and extrinsic (Young, 1998). The intrinsic processes cause degradation of physical, chemical and biological
characteristics of soil while, the extrinsic ones include loss of accessibility, natural disaster, climate fluctuations, inadequate agricultural policies, illiteracy etc. Scott (2007) classified soil disturbance into five classes based on the type and degree of disturbance to topsoil and subsoil (Class 0 to 5). Class 0 represents the undisturbed soil, whereas, classes 1-5 represents the disturbed soil. Class 1 represents the soil in which topsoil is compacted (not churned) due to reduction of macropore space resulting in reduced movement of air and water in the soil. Class 2 represents the soil in which topsoil is churned and partly to completely puddle. The subsoil is compacted, but not churned. Class 3 represents the soil in which part of the topsoil is removed and puddle, while the part that is not removed is mixed up with subsoil and puddle. Class 4 represents the soil in which topsoil is completely removed and subsoil is puddle. Class 5 represents the soil in which top soil is saturated and subsoil with high water table. The soils of class 4 and 5 are highly disturbed and the condition is categorized as severe.

Bai et al. (2008) have reported that all over the world 20% of the cultivated, 30% of the forest and 10% of grassland soils are undergoing degradation. Degradation of land has affected >2.6 billion people of the world (Adams and Eswaran, 2000). According to the National Bureau of Soil Survey and Land Use Planning (Anonymous, 2004) ~146.8 Mha degraded soil is present in India. A rapid increase in industrialization, urbanization and infrastructure development has accelerated the degradation of soils. Opencast mining activities disturb the physical, chemical and biological properties of the soil and consequently alter the socioeconomic features of the area where it is practiced. Negative effects of mining include water scarcity due to lowering of water table, soil contamination, heavy metal pollution, acid mine drainage and finally loss of flora and fauna. Overburden removal from mine areas results in significant loss of vegetation and rich topsoil (Sahu and Dash, 2011; Anonymous, 2011; Maharana and Patel, 2013ab). Coal is an important mineral resource used in different sectors. India is the third largest producer of coal after China and USA. The open cast coal mine overburden dumping soils are deficient in plant nutrients, lack biologically rich top soil (Mummey et al., 2002), which obstructs revegetation and restoration (Tapadar and Jha, 2015). Coal mine overburden soils have unbalanced macro and micropores resulting in inappropriate air and water availability for biotic components. This also
leads to acid mine drainage (AMD) and heavy metal contamination in the soil (Sheoran et al., 2011, Mohammed et al., 2011). Acid mine drainage (AMD) increases the severity of pollution by increasing solubility and consequently bioavailability of heavy metals (Tapadar and Jha, 2015).

Soil microbial community is the most significant component of the soil system and its composition and activities are affected by soil physico-chemical characteristics (Jha, 1990). Distribution and activities of bacteria are affected by soil pH (Nicol et al., 2008; Jiang et al., 2015). The soil microbial community structure alters due to change in land use, edaphic characteristics, nutrient quantity and quality (Stevenson et al., 2014) and change in carbon inputs into soil (Prevoust-Boure et al., 2011). Heavy metal toxicity causes pressure on microorganisms and thereby changes the diversity of soil microflora (Šmejkalová et al., 2003). Kikovic (1997) reported decreased microbial density while Dias et al. (1998) observed reduction in carbon biomass due to heavy metal contamination.

Soil heavy metal pollution has been receiving increasing attention in developing countries. The remediation of such heavy metal polluted coal mine dumping soils deficient in essential nutrients is very important because these usually cover large areas that are rendered unsuitable for agricultural and other human uses (Khan, 2005; Tapadar and Jha, 2013).

2.3 Heavy metal/metalloid toxicity in soil

Heavy metals are elements with metallic properties and density higher than 5 g cm$^{-3}$ (Denton, 2007). They do not get degraded rather get accumulated in the food chain consequently forming complex toxic compounds causing oxidative stresses altering protein structure and severely influencing biological functions (Rajbanshi, 2008; Lenart and Wolny-Koladka, 2013). Heavy metal cations are required in cell metabolism as trace elements to perform biochemical reactions (Ahemad, 2012). Many enzymes contain specific heavy metal species as cofactors for proper functioning and displacement of respective heavy metal ion by another result in the inhibition or loss of enzyme activities (Gill, 2014). Thus, they are indispensible and essential for the biochemistry of life (Lemire et al., 2013). Heavy metal at higher concentrations forms non-specific complexes consequently manifesting toxicity
There are certain non-essential heavy metals viz. tellurium (Te), mercury (Hg), silver (Ag), cadmium (Cd) etc. which are highly toxic and have microbicidal activity at very low concentrations (Nies, 1999; Harrison et al., 2004). Heavy metal toxicity also occurs in plant cells at higher concentrations (Hossain et al., 2012). There are two categories of heavy metals viz. redox active (Fe, Cu, Cr, etc.) and redox inactive (Cd, Zn, Ni, Al, etc.) (Gill, 2014). The redox active heavy metals are directly involved in the redox reaction in cells and result in production of ROS (Schützendübel and Polle, 2002). Redox inactive heavy metals on the other hand use indirect mechanism and cause oxidative stress affecting antioxidant system blocking electron transport chain and induction of lipid peroxidation. Heavy metal binds strongly to nitrogen and sulphur atoms and cause enzyme inactivation by binding to cysteine residues (Gill, 2014). Misfolding and inhibition of activity of proteins and enzymes occur due to binding of Cd to sulphhydryl groups (Dalcorso et al., 2008; Hall, 2002). The basic principle of heavy metal toxicity is primarily identical in soil microorganisms as well as plants. Lemire et al. (2013) categorized the mechanism of heavy metal toxicity into five types: i) reactive oxygen species (ROS) and antioxidant depletion ii) protein dysfunction and loss of enzyme activity iii) impaired membrane function iv) interference with nutrient assimilation, and v) genotoxicity. These mechanisms are not exclusive and might vary based on the chemistry of the metal (Fig. 1).

2.3.1 Reactive oxygen species (ROS) and antioxidant depletion

There are reports of increased production of intracellular ROS upon exposure to metal ions and metalloids viz. Cr (VI), As (III), Fe (II), Cu (II) etc. (Lemire et al., 2013). Imlay (2003) reported that exogenous addition of H$_2$O$_2$ or agents producing superoxide to *Escherichia coli* cause DNA damage and inhibit key enzyme activities vital for cell growth and survival (Fig. 1b). Mutants of *Sacharomyces cerevisiae*, *E. coli* and *Pseudomonas aeruginosa* deficient in ROS scavenging enzymes and antioxidants exhibit Cr, As, Te, Fe and Cu toxicity (Touati et al., 1995; Parvatiyar et al., 2005; Sumner et al., 2005).

For the production of ROS, three mechanisms are proposed (Lemire et al., 2013). First, Fenton like reactions (Fig. 2 play important role in toxicity of redox active metal
(Fe, Cu, Cr, Co, V, Ni) by producing ROS (Ercal et al., 2001). Factors like pH, coordinating ligands and relative reduction potential influence the Fenton chemistry of heavy metals and consequently determine production rate of ROS.

\[
M^{(n)} + O_2 \rightarrow M^{(n-1)} + O_2
\]

\[
2 O_2^- + 2 H^+ \rightarrow H_2O_2 + O_2
\]

\[
M^{(n-1)} + H_2O_2 \rightarrow M^{(n)} + HO` + OH^-
\]

**Figure 2: Fenton like reactions of metals (Adapted from Ercal et al., 2001).**

Second, heavy metals might disrupt the cellular donor ligands that coordinate iron (Fe). The primary targets for metals are 4Fe-4S clusters of metalloproteins, which are important in oxidation reduction reactions of mitochondrial electron transport chain. (Middaugh et al., 2005; Macomber et al., 2011; Calderón et al., 2009; Xu and Imlay, 2012) resulting in release of Fe and consequently of more Fenton reaction mediated ROS formation into cytoplasm (Lemire et al., 2013). Third, *in-vitro* experiments suggest thiol mediated reduction of heavy metals that generates ROS (Valko et al., 2005; 2006). Valko et al., (2005) reported thiol mediated reduction of Cr (VI) that generates other Fenton active metal species resulting in increased ROS production. The thiol groups (-C-SH or R-SH) of proteins, more specifically of amino acids are oxidized by metals and form energetically stable covalent bonds with sulphur (S), resulting in the formation of disulphides in proteins and consequently depleting cellular antioxidant reserves especially glutathione (GSH) (Lemire et al., 2013). Harrison et al. (2009) reported depletion of cellular thiols of *E. coli* upon exposure to toxic doses of Ag(I), Cd(II), Co(II), Zn(II), Cr(VI), As(III) and Te(IV). The depletion of glutathione leads protein targets vulnerable to attack by metal species or ROS. Heavy metal stress increased level of methyl glyoxal (cytotoxic compound) in plants because of inactivation of glyoxylase system that is involved in reducing oxidative stress (Gill, 2014).

### 2.3.2 Protein dysfunction and loss of enzyme activity

Heavy metals catalyze site specific damage to cellular proteins and enzymes leading to loss of catalytic activity and degradation of proteins (Fig. 1a). The amino acids susceptible to metal catalyzed oxidation remain present adjacent to metal binding sites (Stadtman,1993). These amino acids particularly lysine, arginine, histidine and
proline produce carbonyl derivatives by metal catalyzed oxidation of their side chains as they are more vulnerable.

Figure 1: Mechanism of heavy metal toxicity. a) Protein dysfunction b) ROS production and depletion of antioxidant depletion c) Impairment of membrane function d) Interference with membrane uptake e) Genotoxicity. (Adapted from Lemire et al., 2013).
Stadtman (1993) used number of carbonyl groups as indicator of degree of oxidative damage of protein. Sumner et al. (2005) on the basis of their experiment on *Saccharomyces cerevisiae* reported elevated carbonyl level within a minute after exposure to Cr (VI) metal species. Mononuclear metalloenzymes such as peptide deformylase and threonine dehydrogenase etc. are sensitive to ROS (Anjem and Imlay, 2012). A family of bacterial Fe-S dehydratases are vulnerable to toxic metals leading to site specific inactivation (Macomber and Imlay, 2009; Calderón et al., 2009; Lemire et al., 2013). Imlay (2003) reported Cu induced destruction of 4Fe-4S clusters in enzymes responsible for biosynthesis of dihydroxy-acid dehydratase and isopropylmalate isomerase (IPMI) resulting in the growth arrest (Lemire et al., 2013). Macomber and Imlay (2009) distinguished ROS toxicity from 4Fe-4S due to the presence of oxygen (O) dependent mechanism. Xu and Imlay (2012) reported the damage of Fe-S containing enzyme dehydratase independent of ROS. In contrast, cysteine desulphurase (IscS), Fe-S cluster scaffold protein Sulf A involved in Fe-S cluster repair process are not affected by metal (Lemire et al., 2013). Calderón et al. (2009) reported that metalloid oxyanion Te (IV) cause indirect oxidation of Fe-S clusters of proteins through ROS intermediates. Enzyme inhibition also results due to ionic mimicry (Lemire et al., 2013). Erskine et al. (1997) reported displacement of Zn (II) by Pb (II) from active site of δ-aminolevulenic acid dehydratase (ALAD) resulting into inhibition of enzyme activity. Ciriolo et al. (1994) reported similar results in case of *S. cerevisiae*. They found inhibition of superoxide dismutase (Cu-Zn SOD) activity due to replacement of Cu (I) by Ag (I). They further suggested that loss of SOD activity did not inhibit growth directly. Sometimes, substitution of metal at non-catalytic metal binding sites also cause destruction of active sites consequently inhibiting enzyme activity (Lemire et al., 2013). Macomber et al. (2011) reported loss of activity of fructose-6-biphosphate aldolase (FbaA) in *E. coli* due to substitution of Ni (II) for Zn (II). Rivetta et al. (1997) reported inactivation of RuBisCO due to replacement of Mg$^{2+}$ with other divalent metal cations.

### 2.3.3 Impaired membrane function

Cell membrane has been get damaged due to oxidation and cross-linking of heavy metals with protein thiols, inhibition of H$^+$-ATPase and alteration of structural and functional properties of lipids (Merag, 1993). The bacterial membrane adsorbs metal...
cations due to presence of electronegative polymer groups (Zhang and Rock, 2008) on them (Fig. 1c). It is also demonstrated that membrane is the site of bactericidal toxicity caused by heavy metals. Many experimental findings suggest that Ag blocks the electron transport chain (Lok et al., 2006; Bragg and Rainnie, 1974) causing both NQR (NADPH-quinone oxidoreductase) dependent and independent membrane dysfunction. In Vibrio harveyi, Ag (I) inhibits NQR activity (respiratory chain enzyme) by altering transmembrane Na⁺ potential (Lemire et al., 2013) while in Vibrio cholerae membrane potential is altered due to proton leakage through it. Lipid peroxidation, an oxidative degradation of cell membrane lipids is another mode of membrane dysfunction (Lemire et al., 2013). This is confirmed by increased concentration of thiobarbituric acid-reactive substances (TBARS), a byproduct of lipid peroxidation, in the cell extracts of E. coli on exposure to Cu (Hong et al., 2012). Similar results were also found in S. cerevisiae (Howlett and Avery, 1997). Evidences, however, suggest that lipid peroxidation occur mainly in case of polyunsaturated fatty acids (Zhang and Rock, 2008).

2.3.4 Interference with nutrient uptake
Heavy metal blocks the uptake of nutrients resulting in starvation and arrest of cell growth (Fig. 1d). Fauchon et al. (2002) reported inhibition of sulphate uptake in S. cerevisiae due to the presence of Cr (VI) in the growth media. Both sulphate and chromate has same affinities for sulphate transporters (Sul1 and Sul2) causing depletion of intracellular sulphate metabolite pools upon exposure to Cr (VI) (Pereira et al., 2008). Fe starvation is observed in Pseudomonas aeruginosa, due to presence of Ga (III), which represses Fe-responsive transcriptional regulator PvdS, that leads to decrease in expression of genes involved in Fe (III) uptake (Kaneko et al., 2007).

2.3.5 Genotoxicity
DNA damage occurs due to exposure to heavy metals (Lemire et al., 2013) (Fig. 1e). Fe-mediated Fenton chemistry cause lethal DNA damage in E. coli (Linley et al., 2012) and the damages are further accelerated by the mutation that disrupts Fe-homeostasis, thereby releasing Fenton active Fe in the cell (Touati et al., 1995; Keyer and Imlay, 1996).
2.4 Soil degradation vs. enzyme activity

Physical, chemical, microbial and enzymological components of the soil together contribute to maintain soil health. Healthy soils are indicator of healthy terrestrial ecosystems and necessity for recovery from disturbances (Ellert et al., 1997). Monitoring of soil degradation is needed to formulate conservation strategies for the sustainable use of limited land resources. Keeping in view the severity of the problem different assessment and monitoring methods have been developed (Kapalanga, 2008). Soil enzyme activities are indicators of the soil quality/health because of their intimate relationship with soil physico-chemical and microbial characteristics, besides high sensitivity and ease of measurement (García-Ruiz et al., 2008). They facilitates key biochemical reactions essential for decomposition of organic matter, stabilization of soil structure, formation of organic matter, and nutrient cycling in the soil system (Sinsabaugh et al., 1991; Das and Varma, 2011). Soil harbor different categories of enzymes like amylase, β-glucosidase, cellulase, dehydrogenase, phosphatase, protease, urease etc. that determine its intrinsic metabolic processes (McLaren, 1975; Das and Varma, 2011). Dehydrogenase, phosphatase and urease activities are primarily considered as indicators of soil biological activities (Das and Varma, 2011). Dehydrogenase, which depends on soil type and edaphic and microbial factors, is associated with respiration pathways of soil microorganisms (Kandeler, 1996). Studies on the activities of dehydrogenase in the soil is very important as it indicates the potential of the soil to support biochemical processes which are essential for maintaining soil fertility (C-cycling) as well as health (Das and Varma, 2011). Phosphatase plays important role in P-cycling and is correlated to P stress and growth of the plant (Speir and Ross, 1978). Acid phosphatase secretion is increased in plant roots, in case of P-deficiency in soil, to enhance the solubilization and availability of phosphate (Karthikeyan et al., 2002). Urease plays important role in soil N-cycling. This enzyme is responsible for the hydrolysis of urea fertilizers applied to the soil into NH₃ and CO₂ with the concomitant rise in soil pH (Andrews et al., 1989) consequently causing N loss due to NH₃ volatilization (Simpson and Freney, 1988). There are strong evidences that soil microbes and enzyme activities are sensitive to disturbances caused by mining (Chen et al., 2005; Yang et al., 2006; Gao et al., 2009). The mining activity along with post mining practice affects the enzyme
activity (Baldrian et al., 2008). Baldrian et al. (2008) also reported increase in enzyme activity during initial phases of primary succession on spoil heaps. Disturbed open cast coal mine dumping soils showed less enzyme activity as compared to undisturbed forest soils (Maharana and Patel, 2013b). Gogoi et al. (2012) reported lowest activity (dehydrogenase, phosphatase and urease) in un-reclaimed coal mine disturbed soil compared to reclaimed spoil soil. Thus, determination of activities of enzymes like dehydrogenase, phosphatase and urease help one to understand the quality of soil at coal mine dumpings.

2.5 Rhizosphere

Hiltner (1904) coined the term rhizosphere, which is a zone of increased microbial activity facilitated by exudates containing diverse array of nutrients, stimulants and attractants (Ahemad, 2014; Bhattacharyya and Jha, 2015). Rhizosphere is the narrow zone of soil surrounding the root system that is directly influenced by exudates and associated microorganisms (Ahemad, 2014; Bhattacharyya and Jha, 2015). Plant root exudates contain amino acids, organic acids, sugars, vitamins, purines, nucleosides, enzymes, inorganic ions and gaseous molecules (Dakora and Phillips, 2002). Ahemad (2014) stated that there are reports of exudates which can repel microorganisms rather than attracting them. The chemical composition of exudates is regulated by physiological status of the plants and associated microorganisms (Khang et al., 2010). Bacteria inhabiting the rhizosphere are known as rhizobacteria (Kloepper et al., 1991) and those beneficial to plants are termed as plant growth promoting rhizobacteria (PGPR) (Bhattacharyya and Jha, 2012). Mycorrhizal fungi and rhizobacteria are integral part of the rhizosphere microbiota. They enhance plant growth by improving nutrient status, release of growth promoting substances and increase tolerance to biotic and abiotic stresses.

2.5.1 Arbuscular mycorrhizal fungi (AMF)

Arbuscular mycorrhizal (AM) fungi belonging to phylum Glomeromycota, (Table 1) has more than 246 species which are endosymbionts of diverse groups of plants falling within Bryophytes, Pteridophytes, Gymnosperms and Angiosperms (Ike-Izundu, 2007; Schüßler and Walker, 2010). Mycorrhiza is neither root nor the fungus,
rather a structure developed from these two partners. Both the organisms involved in the interaction benefit from the association.

**Table 1: Classification of AM fungi**

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
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<tbody>
<tr>
<td>Glomeromycota</td>
<td>Glomerales</td>
<td>Glomeraceae</td>
<td>Glomus, Funneliformis, Rhizophagus, Septoglomus, Sclerocystis Claroideoglomus</td>
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<tr>
<td></td>
<td>Claroideoglomeraceae</td>
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<tr>
<td></td>
<td>Diversisporales</td>
<td>Gigasporaceae</td>
<td>Gigaspora, Scutellospora, Cetraspora, Dentisculata, Intraornatospora, Paradentisculata, Racocetra Acaulospora Pacispora Diversispora, Corynbiglomus, Redeckera, Tricispora, Otospora Sacculosporaceae</td>
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<tr>
<td></td>
<td>Diversisporales</td>
<td>Pacisporaceae</td>
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<td></td>
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<td>Diversisporaceae</td>
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<td></td>
<td>Diversisporales</td>
<td>Sacculosporaceae</td>
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<td></td>
<td>Paraglomerales</td>
<td>Paraglomeraceae</td>
<td>Paraglomus</td>
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<tr>
<td></td>
<td>Archaeosporales</td>
<td>Geosiphonaceae</td>
<td>Geosiphon</td>
</tr>
<tr>
<td></td>
<td>Archaeosporales</td>
<td>Ambisporaceae</td>
<td>Ambispora</td>
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<td></td>
<td>Archaeosporales</td>
<td>Archaeosporaceae</td>
<td>Archaeospora</td>
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</table>

There are seven categories of mycorrhiza (arbuscular, ecto, ectendo, arbutoid, monotropoid, ericoid and orchidaceous mycorrhizae), depending on the fungus and host involved. Arbuscular and ericoid are the most widespread and ecologically and economically important types (Makdoh, 2014). The AM fungal evolution dates back to 460 million years ago from the fossil records of Ordovician age, which suggests their crucial role in colonization of terrestrial plants (Brundrett, 2002; Khan, 2006; Ike-Izundu, 2007). AM fungi are universal and form symbiotic association with 80-90% of terrestrial plants in natural, agricultural and forest ecosystems (Brundrett, 2002). The obligatory biotrophic AM fungi do not maintain their viability and multiplication in absence of host (Khan, 2005). Prolonged absence of host leads to increased proportion of empty hyphae suggesting senescence phase in the mycelium (Khan, 2005). AM fungi improve plant growth by different mechanisms like improved uptake of nutrients, release of growth promoting substances, tolerance to drought, heavy metal toxicity, salinity, transplantation shock, improved systemic resistance, synergistic interaction with other soil microorganisms, increased formation
and stabilization of soil aggregates (Wright and Upadhaya, 1998; Gaur and Adholeya, 2004; Turk et al., 2006; Ahanthem and Jha, 2006).

Arbuscular mycorrhiza increase absorption capacity of roots by bringing morphological and physiological modifications in plants. They increase surface area of roots and help in exploration of larger volume of soil for nutrients, increase longevity of absorbing roots, improve storage of soluble nutrients, play important role in nutrient cycling etc. The hyphal network transports nutrients to greater distance (Jacobsen, 1995) by increasing root length and changing the root architecture (Berta et al., 1995). Phosphorus is critical for plant growth and development because it constitutes about 0.2% of dry weight. In soil, though it may be present in relatively large amounts but much of it is poorly available to plants because of its low mobility and low solubility of phosphates of iron, aluminum, and calcium, leading to soil solution concentrations of 10 mM or less and very low mobility (Smith et al., 2011). AM fungi increase phosphorus uptake as this element is extremely immobile in the soil and help crops to acquire biomass even in nutrient deficient conditions. They produce phosphatase that hydrolyses and release phosphorus from organic phosphorus complexes consequently increasing its availability (Turk et al., 2006).

The mycorrhizal symbiosis is characterized by exchange of nutrients primarily phosphorus (P) and nitrogen (N), from the fungus for carbon (C) from the host (Fellbaum et al., 2012). The host transfers around 20% of its carbon (C) fixed by photosynthesis to the fungus (Wright et al., 1998). This dependency between fungus and host suggests that the host is under control of the symbiosis and nutrient transport is driven by host demand (Fellbaum et al., 2012). Recent studies (Fellbaum et al., 2012; Casieri et al., 2013; Wyatt et al., 2014) revealed that both the partners are able to detect variation in the resources supplied by their respective partners and consequently help them to adjust their own resource allocation. This ensures ‘fair trade’ between the symbiostic partners (Kiers et al., 2011). AM fungi simultaneously interact with multiple hosts belonging to different species by a common mycelial network (CMN) and, therefore, do not rely on a single host for their C supply and simultaneously allocate nutrients to different plants. AM fungi, therefore, regulate the plant community dynamics (Brundrett, 2002).
Evidence suggests that AM fungi enhance the ability of plants to adapt to salt stress (Jahromi et al., 2008) by improving mineral nutrient absorption, maintaining ion balance, protecting enzyme activities, and facilitating water uptake (Turk et al., 2006). The accumulation of sugars due to AM symbiosis is a positive response to salt stress because it can prevent structural alteration in soluble protein, maintain the osmotic equilibrium in plant cells, and protect membrane integrity (Turk et al., 2006).

AM fungi also play important role and contribute to plant growth and establishment in nutrient deficient heavy metal contaminated soils (Gaur and Adholeya, 2004). Apart from nutrient acquisition, heavy metal tolerant AM fungi improve the soil quality, water availability and block translocation of heavy metals to shoots by binding with them in roots, thereby help in revegetation of degraded coal mine spoil soils (Marx and Altman, 1979). AM fungi adapted to local soil conditions are able to stimulate plant growth better than non-indigenous isolates. Evolution of indigenous ecotypes of AM fungi results from long-term adaptation to soils with extreme properties. Chen et al. (2004) reported accumulation of zinc in Glomus mosseae (1200 mg Kg\(^{-1}\)) and G. versiforme (600 mg Kg\(^{-1}\)). Sambandan et al. (1991) isolated 15 AM fungal species from heavy metal polluted soils of Tamilnadu and found G. geosporum as the most dominant species. G. mosseae was also reported from the roots of Frageria vesca growing in Zn polluted soil in southern Poland (Turnau et al., 2001). Scutellospora dipurpurascens was reported from rhizosphere of Agrostis cappilaris growing on Cu polluted soils (Griffioen et al., 1994). It is very essential to find out the appropriate indigenous heavy metal tolerant AM fungal isolates for the success of remediation of polluted soil (Gaur and Adholeya, 2004). AM fungal population and functioning even though are affected by mechanical and chemical disturbances of the soil, the indigenous heavy metal tolerant AM fungal isolates, however, stimulate plant growth better than the non-indigenous isolates (Sylvia and Williams, 1992). The indigenous AM fungal isolates can be used as a part of the strategy for the revegetation or phytoremediation of heavy metal contaminated soils (Gaur and Adholeya, 2004; Khan, 2005).

2.5.1.1 Glomalin
Glomalin, a brown-red coloured glycoprotein, produced by AM fungi within the hyphal walls (Driver et al., 2005), which get deposited in the soil after senescence
(Rillig et al., 2003). Gadkar and Rillig (2006) reported the homology of glomalin with plant heat shock protein 60. This compound constitutes an important reservoir of soil C and N (Treseder and Turner, 2007).

Glomalin is generally a loose term used for specific soil protein or group of proteins. Rillig (2004) proposed a new classification of glomalin according to which the glomalin should be used only for the putative gene product of AMF and advocated use of GRSP for other fractions of soil proteins. The GRSP fractions are further categorized into four response variables based on the methods of extraction and estimation (Table 2). The AMF origin of GRSP is strongly supported by the monoclonal antibody immunoreactive fractions, decomposition and in vitro culture evidences (Singh, 2012). Rilllig (2004) classified soil glomalin as BRSP (Bradford Reactive Soil Protein), EE-BRSP (Easily Extractable BRSP), IRSP (Immunoreactive Soil Protein), EE-IRSP (Easily Extractable IRSP), GRSP (Glomalin Related Soil Protein) and Glomalin (reserved for gene product) (Table 2).

Glomalin production depends upon the composition of AM fungal community, root length, plant nutrient balance by allocation of photosynthetic products to AM fungi (Treseder and Turner, 2007). Decomposition of glomalin is controlled by soil nutrient availability for microbial activity and clay content, which provide physical protection to the glycoprotein (Nichols and Wright, 2005). Glomalins get accumulated in the soil because of their recalcitrance and long residence time there (Janos et al., 2008) and represent a major component of soil organic matter (Wright et al., 2006). Lovelock et al. (2004) reported non-linear relationship between glomalin (EE-IRSP) and hyphal lengths of AM fungi. Variation in hyphal diameters, different turnover rate of glomalin and hyphae in soil, predation of microarthropods on hyphae and types of AM fungi etc. (Lovelock et al., 2004; Treseder and Turner, 2007). Lovelock et al. (2004) reported higher levels of glomalin production (EE-IRSP) by Acaulospora morrowiae (0.036 µg ml⁻¹) compared to G. intraradices, G. etunicatum and Gigaspora rosea (0.0068, 0.022 and 0.026 µg ml⁻¹ respectively) indicating allocation of less resource by Glomus sp. for glomalin production. Glomus usually invests more in intraradical root structures as compared to extraradical hyphae as opposite to Gigaspora, Acaulospora and Scutellospora (Klironomos et al., 1998; Dodd et al., 2000; Treseder, 2005). The relative abundance of genus Glomus declines with
limiting host plant carbon (C) allocation to fungal symbiont (Treseder, 2005). This ecological characteristic is consistent with the less glomalin production by *Glomus* per unit biomass, since glomalin requires notable investment of C (Treseder and Turner, 2007).

**Table 2: Categories of soil protein and glomalin on the basis of methods of extraction and estimation (Adapted from Singh, 2012).**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Old Name</th>
<th>New Name</th>
<th>Reason for Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TG (Total Glomalin)</td>
<td>BRSP (Bradford Reactive Soil Protein)</td>
<td>Bradford method measures all protein sources; may be non-specific.</td>
</tr>
<tr>
<td>2</td>
<td>EEG ( Easily Extractable Glomalin)</td>
<td>EE-BRSP ( Easily Extractable BRSP)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>IRTG (Immunoreactive Total Glomalin)</td>
<td>IRSP (Immunoreactive Soil Protein)</td>
<td>Potential for antibody cross reactivity or sensitivity issues</td>
</tr>
<tr>
<td>4</td>
<td>IREEG (Immunoreactive Easily Extractable Glomalin)</td>
<td>EE-IRSP ( Easily Extractable IRSP)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Glomalin</td>
<td>GRSP (Glomalin Related Soil Protein)</td>
<td>To clearly separate soil derived protein from putative gene product</td>
</tr>
<tr>
<td>6</td>
<td>Glomalin (Sensu Stricto)</td>
<td>Glomalin(s)</td>
<td>The name Glomalin restricted only for the gene product</td>
</tr>
</tbody>
</table>

Rillig and Steinberg (2002) have reported the influence of soil structure on the glomalin production which they attributed to variation in water potential and gas diffusion among different soil types. Rillig *et al.* (2003) reported quick turnover of glomalin (BRSP) in afforested soil compared to agricultural and native forest soil. This may be either due to poor N in the afforested soil and was replenished by the quick turnover of N rich glomalin or might be due to variation of decomposability of glomalin in different ecosystem types. Plant cover affect the available photosynthate to AM fungi, subsequently alter glomalin production. Rillig *et al.* (1999) reported alteration of soil glomalin concentration by the influence of plant community composition, availability of inorganic resources to plants, elevated CO₂ concentration, high soil fertility (N,P).
Soil organic matter viz. fulvic and humic acid can potentially bind heavy metals to alleviate soil heavy metal toxicity. Gonzalez-Chavez et al. (2004) reported the stabilization of heavy metals like Pb, Cd, Zn, Cu, Fe and Mn by GRSP to remediate polluted soil. Heavy metal sequestering ability of GRSP is further confirmed by Cornejo et al., 2008 and Vodnik et al., 2008. Wu et al. (2013) on the basis of \textit{in situ} field experiments reported the sequestration of Pb and Cd by GRSP. They found that GRSP bound Pb and Cd accounted for 0.21-1.78\% and 0.38-0.98\% of the total Pb and Cd content respectively in the soil. However, when compared on a soil organic matter (SOM) basis, only 4\% of the Pb or Cd was bound to the GRSP fraction of the SOM compared to 40-54\% of the Pb or Cd bound to humic and fulvic acids in the SOM fraction. Nevertheless, GRSP is relatively much stable than SOM in the soil carbon pool owing to its low turnover (Wu et al., 2013).

\subsection{2.5.2 Plant growth promoting rhizobacteria (PGPR)}

Plant growth-promoting rhizobacteria (PGPR) are free-living soil bacteria that colonize the rhizosphere of plant consequently enhance growth and yield of plants (Majeed et al., 2015). According to Kloepper (1994) PGPR efficiently colonize the rhizosphere, survive, multiply and compete with other microbiota and promote plant growth. PGPRs are classified as biofertilizers, phytostimulators, rhizoremediators and biopesticides on the basis of their functions (Somers et al., 2004). On the basis of proximity to roots and intimacy of association, however they are described into two categories viz. extracellular (ePGPR) and intracellular (iPGPR) (Ahemad and Kibret, 2014). The ePGPR viz. \textit{Pseudomonas}, \textit{Serratia}, \textit{Bacillus}, \textit{Azotobacter}, \textit{Azospirillum}, \textit{Arthrobacter}, \textit{Burkholderia}, \textit{Flavobacterium}, \textit{Chromobacterium}, \textit{Caulobacter}, \textit{Micrococcus} etc. remain present in the rhizosphere or the spaces between cells of root cortex, whereas, iPGPR viz. \textit{Allorhizobium}, \textit{Azorhizobium}, \textit{Bradyrhizobium}, \textit{Mesorhizobium}, \textit{Rhizobium} etc. exist within root cells in specialized nodular structures (Figueiredo et al., 2011; Bhattacharyya and Jha, 2012). PGPR promote plant growth directly by facilitating resource acquisition or indirectly by inhibiting plant pathogens or other agents with inhibitory effects on plant growth and development (Glick, 2012). Plants cannot directly utilize vast atmospheric reserve of nitrogen though it is important for their growth and development (Ahemad and Kibret, 2014). Plants predominantly use nitrogen in the form of ammonia and nitrate.
Microorganisms like bacteria and actinomycetes fix atmospheric nitrogen using enzyme nitrogenase (Kim and Rees, 1994).

Nitrogen fixing bacteria such as *Rhizobium, Mesorhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium and Sinorhizobium* form nodules in the roots of leguminous plants where they convert atmospheric nitrogen into ammonia (Van Rhijn and Vanderleyden, 1995). There are reports about the presence of nitrogen fixing endophytes within the non-legumes (Mirza *et al.*, 2001). Free living *Azospirillum, Azotobacter, Enterobacter* improves growth and yield of wheat and maize by fixing $N_2$ (Lugtenberg and Kamilova, 2009). Yanni *et al.*, (1997) isolated nodulating *Rhizobium leguminosarum* bv. *trifolii* from roots of rice plants in Egypt where clover is used for crop rotation for generations. Inoculation of rice with *R. trifolii* notably increased total N content and consequently grain yield (Yanni *et al.*, 1997, 2001; Biswas *et al.*, 2000). PGPR has also the potential of heavy metal bioremediation (Khan, 2005; Ahemad, 2012).

### 2.5.2.1 Biosurfactants

Biosurfactants are diverse categories of surface active compounds produced extracellularly, intracellularly or as part of the cell membranes by microorganisms. They are consisting of polar hydrophilic and non-polar hydrophobic moiety (Pacwa-Plociniczak *et al.*, 2011). Their low toxicity, high specificity and ecofriendly nature make them most sought for alternative agents to be applied for bioremediation, health care, food processing and oil industries as compared to the chemical surfactants. There are two categories of surface active compounds of microbial origin viz. biosurfactants and bioemulsifiers. Biosurfactant reduce surface tension at the air-water interface. While, emulsifiers reduce the interfacial tension between solid-liquid or immiscible liquids. Generally, biosurfactants show emulsifying ability, whereas, bioemulsifiers do not necessarily alleviate surface tension (Batista *et al.*, 2006). The major classes of biosurfactants based on their chemical composition, molecular weight, microbial origin, physico-chemical properties and mode of action glycolipids, lipopeptides, lipoproteins, phospholipids, fatty acids, polymeric surfactants and particulate surfactants (Singh and Cameotra, 2004). Biosurfactants increase desorption of heavy metals, first, by complexation of the metals present in the
solution, consequently, increase desorption by decreasing the solution phase activity of heavy metal. Secondly, direct interaction of biosurfactant to metal at solid solution interface under reduced interfacial tension (Singh and Cameotra, 2004). The di-rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* BS2 was used for heavy metal mobilization in contaminated soil (Juwarkar *et al*., 2008).

2.6 Remediation of heavy metal contaminated coal mine spoil soils

Soil remediation refers to ‘the return of soil to a condition of ecological stability together with the establishment of plant communities it supports or supported to conditions prior to disturbance’ (Allen, 1988). Remediation of metal contaminated soil faces challenge because unlike organic pollutants, heavy metals do not get degraded (Lasat, 2000). Several efforts have been made till to date to remediate and restore heavy metal polluted open cast coal mine spoils (Khan, 2005; Lone *et al*., 2008; Jabeen *et al*., 2009). The heavy metal polluted soils can be remediated by physical, chemical and biological techniques (Tangahu *et al*., 2011). But most of them are costly and far away from their optimum performance (Tangahu *et al*., 2011; Ochonogor and Harrison, 2014; Dixit *et al*., 2015). Physical remediation is a conventional technique mainly involving soil replacement and thermal desorption method. Contaminated soil is partially or completely replaced with clean soil (Yao *et al*., 2012). The thermal desorption is, however, used based on volatility of pollutants (Hg, As, etc.). Such soils are remediated by heating them using steam, microwave, infrared radiation to make the pollutants volatile followed by their collection (Li *et al*., 2010). An American company uses this technique for mercury collection and *in-situ* remediation (Yao *et al*., 2012). The chemical remediation of heavy metal contaminated soils includes chemical leaching, chemical fixation, electrokinetic remediation and vitrifying technology (Yao *et al*., 2012). In case of chemical leaching contaminated soil is washed with fresh water, reagents, and others fluids or gas for leaching the heavy metals from the soil through the mechanism of adsorption, ions exchange, precipitation, and chelation (Tampouris *et al*., 2001; Ou-Yang *et al*., 2010). Electrokinetic remediation is an almost new chemical remediation technology, which involves electric field gradient by applying voltage at the two sides of soil. The pollutants are carried via electromigration and electroosmotic flow to two poles of treatment room and then treated further (Yao *et al*., 2012). In vitrifying technology, soil
is heated at 1400~2000°C, the organic matters volatilize and the melt after cooling forms rock shape vitreous resulting in immobilization of heavy metals (Fu, 2008). All the physico-chemical techniques, however, are suitable only for small area, besides being expensive and labour intensive. They also cause extensive changes in physical, chemical and biological characteristics of the soil (Yao et al., 2012). The microorganisms cannot degrade the heavy metals rather help in remediation by affecting migration and transformation through changing their physical and chemical states. The mechanisms include extracellular complexation, precipitation, valance transformation and intracellular accumulation, volatilization etc. (Lone et al., 2008). These microbial remediation techniques are temporary because heavy metals are not removed from the soil only their physical and chemical states are transformed (Khan, 2005). There has been need for a cost effective biological soil remediation technique to remove heavy metal pollutants and improve soil quality and fertility (Padmavathiamma and Li, 2007; Tangahu et al., 2011). Over the time scientists have realized this and have tried for the plant based low cost, eco-friendly technique for heavy metal remediation (Jadia and Fulekar, 2009). Baumann (1885) for the first time reported the capability of some plants in accumulating high concentrations Zn, while Minguzzi and Vergananao (1948) reported Ni accumulating hyperaccumulator plants. The use of plants to remove, destroy or sequester hazardous contaminants from various media such as soil, water and air, therefore, is called as Phytoremediation. The term phytoremediation has been taken from Greek prefix “phyto” meaning plant and Latin suffix “remedium” meaning to clean or restore (Cunningham et al., 1997). The plants could subsequently be harvested, and processed by drying, ashing or composting for the recovery of heavy metals from the ash.

The plants that grow on metalliferous soils have evolved strategies to accumulate high concentrations of heavy metals in their tissues without showing any symptoms of toxicity (Padmavathiamma and Li, 2007). Several researchers have reviewed different aspects of this technology (Lasat, 2002; McGrath and Zhao, 2003; Singh et al., 2003; Prasad and Freitas, 2003; Alkorta et al., 2004; Ghosh and Singh, 2005; Yang et al., 2005; Khan, 2006; Padmavathiamma and Li, 2007; Lone et al., 2008; Jabeen et al., 2009; Hong-Bo et al., 2010; Tangahu et al., 2011; Eitim, 2012; Lee, 2013; Sharma and Pandey, 2014; Shafi et al., 2015; Ahemad, 2015).
These reviews have recommended phytoremediation as an emerging novel technology for improving the quality of heavy metal degraded soils. Majority of the plants that survive, grow and reproduce on heavy metal polluted soils behave as “excluders” relying on tolerance or hypertolerance strategies (Fig. 3).

This is accomplished by restricting the entry of heavy metals by retaining and detoxifying them in the roots with less translocation to the leaves which otherwise would show phytotoxic effects (Hall, 2002). Some hypertolerant plant species, nevertheless, can translocate heavy metals to shoot and accumulate them there at higher concentrations and are called as “hyperaccumulators”. These hyperaccumulators exhibit an opposite behaviour as far as heavy metal uptake and distribution in the plant is concerned (Fig. 3) (Rascio and Navari-Izzo, 2011). Conventionally, they can accumulate 100 fold greater concentrations than the non-accumulator plants. Lasat (2000) estimated that the hyperaccumulators accumulate more than 10 ppm Hg, 100 ppm Cd, 1000 ppm Cu, Pb, Cr and Co and 10000 ppm Ni and Zn.
More than 500 plant species, from distantly related families have been identified as hyperaccumulators (Goolsby and Mason, 2015) which indicated that the hyperaccumulation trait has evolved independently owing to selective ecological pressure (Rascioa and Navari-Izzo, 2011). The Phytoremediation process is categorized into four subgroups viz. phytoextraction, rhizofiltration, phytostabilization, phytovolatilization on the basis of nature of the heavy metal contaminant, underlying mechanism and applicability (Jabeen et al., 2009).

2.6.1 **Phytoextraction:** This includes uptake and translocation of heavy metals by plant roots into their above ground parts. These metals can subsequently be recovered by harvesting the plants, and burning them into ashes (Tangahu et al., 2011).

2.6.2 **Rhizofiltration:** It is defined as the use of plants to absorb, concentrate, precipitate and sequester contaminants from polluted aqueous sources in the roots (Ensley, 2000). The plants used in this process must produce significant amount of biomass, must be labour and cost effective and produce minimum secondary wastes (Dushenkov and Kupulnik, 2000). Plants with fibrous root systems with large surface areas are considered as effective rhizofiltrators (Jabeen et al., 2009). Indian mustard (*Brassica juncea*) removes Cd, Cu, Ni, Cr, Pb, Zn, whereas, sunflower (*Helianthus annus*) removes Pb and U from hydroponic solutions (Dushenkov et al., 1995; Dushenkov et al., 1997).

2.6.3 **Phytostabilization:** This is plant mediated transformation of toxic soil heavy metals into less toxic forms. It is a containment strategy and limits mobility and bioavailability of heavy metals in the soil rather than removing them from soil (Jadia and Fulekar, 2009). Plant roots grow into heavy metal polluted soils release exudates and organic acids that convert the contaminants to less toxic forms consequently changing the chemical, physical and biological properties of soil (Fig. 4) (Jabeen et al., 2009).

2.6.4 **Phytovolatilization:** Plants take up heavy metals from soil and transform them into volatile forms and subsequently release these volatiles into the atmosphere by transpiration (Jadia and Fulekar, 2009). Heavy metals/metalloids that exist in
gaseous forms in the environment *e.g.* Hg, As, Se utilize this technique. This technique though reduces heavy metals concentration in soil but causes air pollution. Genetically modified plants like *Chara canescens*, *Brassica juncea* and *Arabidopsis thaliana* have been reported to perform phytovolatilization (Fig. 5) (Jabeen *et al.*, 2009).

Phytoextraction is the most efficient mechanism (Fig. 6) (Padmavathiamma and Li, 2007). There are two basic mechanisms of phytoextraction i.e. chelate assisted induced phytoextraction (chelate assisted) and long term continuous phytoextraction. The chelate assisted strategy is employed in case of inadequate heavy metal availability and synthetic chelates and acidifying agents viz. EDTA (ethylene diamine tetra acetic acid), EGTA (ethylene glycol-O-O’-bis-[2-amino-ethyl]-N, N, N’, N’-tetra acetic acid), EDDHA (ethylene diamine di o-hydroxyphenylacetic acid), EDDS
Figure 5: Schematic mechanism of phytovolatilization (Adapted from Padmavathiamma and Li, 2007).

Figure 6: Schematic mechanism of phytoextraction (Adapted from Padmavathiamma and Li, 2007).
(ethylene diamine disuccinate), citric acid etc are added to soil to liberate heavy metals (Lasat et al., 1998; Tandy et al., 2006). The synthetic chelates, however, alter the characteristics of the soil besides increasing probability of leaching of metals to ground water (Padmavathiamma and Li, 2007). Luo et al. (2006) observed chelate mediated (EDTA, EDDS) phytoextraction of Pb in Zea mays. The continuous phytoextraction, however, is a long term process and does not require chelates (Padmavathiamma and Li, 2007).

Ebbs et al. (1997) reported hyperaccumulation of Zn and Cd by members of Brassicaceae family (Brassica juncea, B. juncea, B. napus and B. rapa) in hydroponics experiment. The genus Thlaspi has been extensively studied for hyperaccumulation of heavy metals e.g. Thlaspi caerulescens for Pb, Cd, Ni and Zn, T. goesingense Ni and Zn, T. rotundifolium for Ni, Pb and Zn (Prasad and Freitas, 2003). Frey et al. (2000) reported accumulation of Zn in the insoluble form within vacuoles of epidermal cells. Sarret et al. (2002), however, reported Zn accumulation in the mesophyll cells of Arabidopsis halleri. Excess Zn concentration (4~10-fold) does not affect shoot Cd accumulation in T. caerulescens (Uneo et al., 2004). The root Cd concentration, however, decreased with increasing Zn concentrations indicating the interaction between Zn and Cd. The results suggest different uptake systems for Cd and Zn e.g. Cd competed with Zn uptake while Zn did not compete with Cd uptake. Thlaspi caerulescens grown on the ZnS-enriched soil accumulated up to 6900 mg Zn/kg in the shoots. Whiting et al. (2000) observed that rhizosphere interaction affect T. caerulescens when grown with H. vulgare and L. heterophyllum in metal uptake under field conditions. Results prove that Cd concentration in H. vulgare increased by a factor of 2.4 when it was grown along the sides of T. caerulescens without any barrier. The uptake of Zn by H. vulgare, however, decreased significantly probably due to metal depletion within the rhizosphere zone of the Zn-hyperaccumulator. Gove et al. (2002) believed that T. caerulescens might have altered the conditions in the shared rhizosphere and thereby affected the availability of selected metals to neighboring plants. Wenzel et al. (2002) indicated that root exudates of organic ligands might contribute to Ni hyperaccumulation in T. goesingense. This was attributed to the ligand-induced dissolution of Ni bearing minerals in the rhizosphere of T. goesingense and appeared to be less effective in the
rhizosphere of excluder *Silene vulgaris* and *Rumex acetosella* growing on the same site. *Sedum alfredii* Hance was reported in China as hyperaccumulator and has been under extensive research (Li et al., 2005; Yang et al., 2006). Studies on mined and the non-mined ecotypes of *S. alfredii* indicated mined ecotypes (ME) have higher tolerance to Cd as reflected by dry matter yield than those of the non-mined one (NME) (Xiong et al., 2004). Kupper et al. (2001) studied the Ni uptake and its cellular compartmentation in three Ni hyperaccumulators i.e. *A. bertoloni* (Desv), *A. lesbiacum* (Candargy) and *T. goesingense* (Halacsy). It was observed that these species though were identical in hyperaccumulation of Ni, these three species showed similar hyperaccumulation of Ni, but *T. goesingense* appeared less tolerant to Ni than the other two species. Among different fern species, three accessions of *P. vitta*, two cultivars of *P. cretica*, *P. longifolia* and *P. umbrosa* were grown with 0~500 mg As/kg added to the substrate. The results show that in addition to *P. vitta*, *P. cretica*, *P. longifolia* and *P. umbrosa* also hyperaccumulate As to a similar extent. This study identified three new species of As hyperaccumulators in the *Pteris* genus (Zhao et al., 2002). The other hyperaccumulators discovered are viz. *Raphanus sativus* for Cd and Zn (Hamon et al., 1999), *Helianthus annus* for Cd, Ni, Pb, Cu, Fe, Mn and Zn (Liphadzi et al., 2003), *Festuca arundinacea* for Cu, Pb, Zn (Roy et al., 2005), *Scirpus littoralis* for Zn, Pb, Cu, Mn (Bhattacharya et al., 2006), *Leersia oryzoides* for As (Ampiah-Bonney et al., 2007), *Sorghum bicolor* for As, Cd, Mo, Pb, Zn (Muranyi and Kodobocz, 2008), *Pteris vittata* for As, Pb (Feng et al., 2009), *Triticum aestivum* and *Brassica* for Cu, Cd, Cr, Zn, Fe, Ni, Mn and Pb (Chandra et al., 2009), *Alternanthera phyloxeroides*, *Sanvitalia procumbens*, *Portulaca grandiflora* for Pb (Gupta et al., 2009), *Salix viminalis* for Cd and Zn (Hammer et al., 2003), etc.

The hyperaccumulators should ideally have rapid growth, produce high biomass have extensive root system, be easy to harvest and could accumulate a range of heavy metals in their harvestable parts (Yang et al., 2005; Tzvetkova et al., 2014). Though phytoremediation (phthoextraction) is an emerging technique, nevertheless, it cannot be applied universally for all polluted soils (Khan et al., 2000). This is because of meager understanding of biochemical, physiological and molecular mechanisms involved in hyperaccumulation. Many hyperaccumulators are yet to be discovered and most of known hyperaccumulators show slow growth and can produce limited amount
of biomass (Khan et al., 2000; Denton, 2007; Yadav and Srivastava, 2014). Jabeen et al. (2009) and Khan (2006) have advocated three main strategies for improving the phytoextraction of heavy metals viz. genetic strategy (use of genetically modified hyperaccumulator), chelate assisted strategy (use of synthetic/natural chelates) and rhizosphere manipulation (use of known indigenous microbes). Rhizosphere manipulation of mycotrophic hyperaccumulator with indigenous AMF, PGPR and biosurfactant producing bacteria has more prospects and can meet the present need as these organisms also help plants to grow better. This technique of using of mycorrhizal hyperaccumulator for phytoremediation is called as mycorrhizoremediation (Khan, 2006).

Chen et al. (2005) reported increased sequestration of U in roots compared to shoot of Medicago trunculata when inoculated with Glomus intraradices. Indigenous G. mosseae when inoculated to Pteris vittata improved biomass and phytoextraction efficiency of As (Leung et al., 2010). In contrast, improvement of nutrient and reduced uptake of Cd and Zn was observed when Thlaspi praecox was inoculated with indigenous Glomus sp. (Vogel-Mikus et al., 2006). Wang et al. (2012) reported enhanced biomass (1.7 fold compared to control) and Cd concentrations in root and shoot of Medicago sativa when inoculated with G. intraradices. The affect of AM fungi on the heavy metal uptake in hyperaccumulator is not uniform. Some AM fungi used in phytoremediation of heavy metals are Glomus mosseae in Trifolium repens for Cd (Vivas et al., 2003); Gigaspora sp. in Hordeum vulgare for Cd (Tullio et al., 2003); Gigaspora sp. and Glomus tenue in Berkheya coddii for Ni (Turnau and Mesjasz-Przybylowicz, 2003); G. constrictum, G. fasciculatum, G. ambisporum, Scutellospora pellucida, S. dipurpurescens were used as consortia in T. repens (Zhu et al., 2001), Agrostis cappilaris (Griffioen et al., 1994) for Zn; G. intraradices with Zea mays for Pb (Malcova et al., 2003) and G. caledonium in Z. mays for Cd and Cu (Liao et al., 2003).

Different bacterial strains like Achromobacter xylosidans strain Ax10 (Ma et al., 2008), Azotobacter chroococcum HKN-5, Bacillus megateriumm HKP-1, B. mucillaginosus HKK (Wu et al., 2006), B. subtilis SJ-101 (Zaidi et al., 2006), Sinorhizobium sp. Pb002 (Di Gregorio et al., 2006), Variovox paradoxus, Rhodoccus
sp. (Belimov et al., 2005) are used for rhizosphere manipulation. Wani and Khan (2010) reported reduced uptake of Cr in roots of *Cicer arietinum* by using *Bacillus* PSB10. Rajkumar et al. (2008) reported improved plant biomass and sequestration of Ni, Cu, Zn by *B. weihenstephanensis* SM3 inoculated *Helianthus annuus* in pots. Increased Pb and Cr were reported in *Zea mays* when treated with *Pseudomonas aeruginosa*, *P. fluorescens* and *Ralstonia metallidurans* (Braud et al., 2009). Wang et al. (2014) reported improved plant growth and Zn uptake in grains of *O. sativa* after inoculation with endophytes viz. *Sphingomonas* sp. SaMR12 and *Enterobacter* sp. SaCS20. Extractable Ni was increased when hyperaccumulator was used along with *Microbacterium arabinogalactanolyticum* (Abou-Shanab et al., 2006).

It is, therefore, clear that phytoextraction is an emerging green technology for the removal of heavy metals from contaminated soils and consequently to improve the soil quality. Nevertheless, it has also been understood that this technique is still not a cure all for contaminated soils as indicated by poor plant growth and less biomass production. Special attention is required to address these issues to make this novel technology a full flagged practical and field oriented technology. In the present research work, I have, therefore, tried to use indigenous mycorrhizal hyperaccumulator along with PGPR to manipulate the rhizosphere of hyperaccumulator so that the indigenous microbial resources could be used to develop an appropriate plant-microbe combination in order to tackle the soil pollution caused by open cast coal mining.