Chapter 1 Contaminated Soil: Physical, Chemical and Biological Components

Aparajita Das, Irena Sherameti, and Ajit Varma

1.1 Introduction

Soil may be defined as a thin layer of earth's crust which serves as a natural medium for growth of plants. Soil contains organic and inorganic materials that serve as a reservoir of nutrients as well as water for crops and provides mechanical anchorage. The organic portion, which is derived from the decayed remains of plants and animals, is concentrated in the dark uppermost topsoil. The inorganic portion was formed over thousands of years by physical, chemical and microbiological weathering of underlying bedrock. Unpolluted and fertile soils are required for agriculture to supply the mankind with sufficient food and other necessary requirements. Due to extensive range of industrial and agricultural activities, a high number of chemical contaminants get released into the ecosystem. As a result of this, the soil gets contaminated with the build-up of persistent toxic compounds, chemicals, salts, radioactive materials or disease causing agents in soils, which have adverse effects on plant growth and animal health. Depending on soil type, such agents may be distributed with the water phase in soil and influence the functions adversely. Biological remediation using microorganisms and plants is generally considered a safe and a less expensive method for the removal of hazardous contaminants from the environment.

This chapter provides an overview of the contaminated soils, their physical, chemical and biological components and briefly discusses the importance of heavy

A. Das • A. Varma (🖂)

Amity Institute of Microbial Technology (AIMT), Amity University Uttar Pradesh (AUUP), Sec-125, Expressway, Noida, UP, India e-mail: adas@amity.edu; ajityarma@aihmr.amity.edu

I. Sherameti

Institute of General Botany and Plant Physiology, Friedrich-Schiller-University Jena, Dornburger Strasse 159, 07743 Jena, Germany e-mail: irenasherameti@yahoo.de

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metal tolerant AM fungi and heavy metal tolerant plants for reclamation of degraded soils.

1.2 Physical Properties of Soil

1.2.1 Soil Texture

Soil texture refers to the size distribution of soil particles and the relative percentage of sand, silt and clay particles present in a mass of soil with material sizes less than 2 mm. Materials of more than 2 mm are coarse fragments (gravel, stone, boulder, etc.). Soil texture includes only mineral particles that vary in size from those easily seen with the unaided eye to those below the range of a high-powered microscopy and it does not include any organic matter. Soils with the best structure for most crops are some types of loams, namely sandy loam, silty loam, sandy clay and clay loams which best retain water and supply dissolved minerals for nutrition. The available water holding capacity of soil is related to soil texture. Clayey soils show high water holding capacity, high plasticity and stickiness as well as swelling capacity whereas sandy soils are conspicuous by the absence of these properties. The most important way in which soil texture affects plant growth is the provision of water and, with it, nutrient supply (Pani 2007).

1.2.2 Soil Structure and Composition

Structure of soil refers to the arrangement of soil particles into groups that help in water and nutrient supplying ability of the soil, and air supply to plants' roots. Soil structure is the product of processes that aggregate cement and compact or unconsolidated soil materials. In essence, soil structure is a physical condition that is distinct from that of the initial material from which it has been formed, and can be related to processes of soil formation. The pads (natural aggregates) are separated from adjoining pads by surfaces of weakness. Soil structure influences plant growth rather indirectly. The pores sizes are the controlling factors governing water, air and temperature in soil, which in turn govern plant growth. One of the best examples of the effect of soil structure on plant growth is the emergence of seedlings in the seedbed. The seedlings are very sensitive to soil physical condition so that there should not be any hindrance to the emergence of tender seedlings and there should be optimum soil water and soil aeration. The soil in the seedbed should have a crumb structure so that the pads are soft and porous and roots of the seedling can penetrate it easily. A hard compact layer in contrast would hamper root growth. Soil structure can be divided into three groups as depicted in Table 1.1 (cf. Pani 2007). Most soils consist of four major components, namely mineral materials, organic

Types (form)	Class (size)	Grade (degree of aggregation)
Platy	Very fine	Structure less
Prism	Fine	Weak
Blocky and nuciform	Medium	Strong
Spheroid	Coarse or thick	Moderate
	Very coarse or very weak	

Table 1.1 Classification of soil structure (cf. Pani 2007)

matter, water and air. Of the total volume, about half is covered by mineral materials (45%) and organic matter (5%). The rest is contributed by air and water together.

1.2.3 Soil Water

Water is crucial for plant growth. Soil is capable of being a storehouse of water and becoming the main source of water for land plants. Mineral soil consists of 25% water and only half of it is available to plants (Pani 2007). If adequate water supply is unavailable in soil then normal plant functions are disturbed, and the plant gradually wilts, stops growing and dies. Thus, water is necessary in maintaining the turgidity of plants. Plants are most prone to damage from water deficiency during the vegetative and reproductive stages of growth. In addition, many plants are most sensitive to salinity during the germination and seedling growth stages.

If a cubic foot of typical silt loam topsoil was separated into its component parts, about 45% of the volume would be mineral matter (soil particles), organic residue would occupy about 5% of the volume, and the rest would be pore space. The pore space is the voids between soil particles and is occupied by either air or water. The quantity and size of the pore spaces are determined by the soil's texture, bulk density and structure. When rain or irrigation water is supplied to a field, it seeps into the soil. This process is called infiltration. Water is held in soil in two ways: as a thin coating on the outside of soil particles and in the pore spaces. Soil water in the pore spaces can be divided into two different forms: gravitational water and capillary water. As water infiltrates into a soil, the pore spaces fill with water. As the pores are filled, water moves through the soil by gravity and capillary forces. Water movement continues downward until a balance is reached between the capillary forces and the force of gravity. Water is pulled around soil particles and through small pore spaces in any direction by capillary forces. When capillary forces move water from shallow water table upward, salts may precipitate and concentrate in the soil as water is removed by plants and evaporation (according to http://www.ag.ndsu.edu, dated 16 May 2011). Most of the water that enters the plant roots does not stay in the plant. Less than 1% of the water withdrawn by the plant is actually used in photosynthesis (i.e., assimilated by the plant). The rest of the water moves to the leaf surfaces where it transpires (evaporates) to the atmosphere. The rate at which a plant takes up water is controlled by its physical

characteristics, the atmosphere and soil environment. Plants can extract only the soil water that is in contact with their roots (according to http://www.ag.ndsu.edu, dated 16 May 2011). Soil is a medium that stores and moves water. Thus, the amount of water present in the soil plays a significant role in several natural processes such as evaporation, infiltration and drainage of water, diffusion of gases, conduction of heat, and movement of salts and nutrients. Plants meet their water requirement from water stored in soil.

1.2.4 Soil Aeration and Plant Growth

Oxygen is required by microbes and plants for respiration (oxybiont). As the plant's roots depend on beneficial mycorrhizospheric microbes for optimal growth and development, both partners of this symbiotic/associative relationship need to be supported by sustaining soil structure and composition. Oxygen taken up and carbon dioxide evolved are stoichiometric. Under anaerobic conditions, gaseous carbon compounds in addition to carbon dioxide are released. Root elongation is particularly sensitive to aeration. Oxygen deficiency disturbs metabolic processes in plants, resulting in the accumulation of toxic substances in plants and low uptake of nutrients. Certain plants such as rice are adapted to grow under submerged conditions. These have large internal air spaces, which facilitate oxygen transport to the roots, under water saturated conditions.

1.3 Soil Contamination

Industrial revolution in the twentieth century has resulted in the generation of vast array of chemical products which has resulted in contamination of the environment. Since soil and ground water are preferred sinks for complex contamination, various chemical and biological soil properties are profoundly altered, which affects biodiversity and soil function (Singh et al. 2009).

1.3.1 Common Chemicals Involved in Soil Contamination

Soil pollution is caused by chemicals of both organic and inorganic contaminants. The most prominent chemical groups of organic contaminants are fuel hydrocarbons, polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorinated aromatic compounds, detergents and pesticides. Inorganic species include nitrates, phosphates and heavy metals such as cadmium, chromium and lead; inorganic acids; and radionuclides (radioactive substances). Among the sources of these contaminants are agricultural runoffs, acidic precipitates, industrial waste materials and radioactive fallout. Some of the prominent chemicals involved in soil contamination are discussed below:

(a) Petroleum hydrocarbons

In petrol polluted soil, benzene, toluene, ethylbenzene and xylene (BTEX) isomers are present in the water soluble fraction, causing pollution The most infamous class of hazardous compounds found in petrol, diesel, hydrocarbon-based oil as well as coal tar and its derivatives are the polycyclic aromatic hydrocarbons (PAHs). PAHs are hydrophobic, chemical compounds consisting of fused aromatic rings, not containing heteroatoms or carrying substituents, e.g., naphthalene, anthracene, coronene, pyrene, etc. (Surridge et al. 2009). They are detected in air, soil and sediment, surface water, groundwater, and road runoff and are dispersed from the atmosphere to vegetation and contaminate foods. As a result of their natural and anthropogenic sources, in combination with global transport phenomena, PAHs can be found in all regions of the world, including extreme environments with minimal human impact, such as Antarctica. Depending on the source of contamination, soils can contain PAH concentrations ranging between 1 µg/kg and 300 g/kg total PAHs. Further, due to incomplete combustion of materials such as coal and wood, atmospheric levels of PAHs ranging from 60 to 3 mg/m^3 air have been reported. Therefore, the development of practical bioremediation strategies for heavily impacted sites is urgently needed (Loick et al. 2009).

(b) Heavy metals

Heavy metals such as lead, arsenic and many more represent a highly abundant group of toxic compounds in the soil environment. Although their natural presence is limited to a few soil habitats, they are locally present as a consequence of human activities including mining, processing or the extensive use of the metal. Thus heavy metal pollution in soils constitutes a highly complex disruption of ecological equilibrium. Several heavy metals such as copper, zinc and iron are essential for the physiological functioning of living organisms, but they all become toxic at high concentrations. The toxicity of a metal depends on the metal itself, its total concentration, the availability of the metal to the organism and the organism itself (Kamal et al. 2010).

(c) Pesticides

A pesticide may be a chemical substance, biological agent (such as a virus or bacterium), antimicrobial, disinfectant used against any pest. Prominent chemical pesticide families include organochlorines, organophosphates and carbamates. Organochlorine hydrocarbons (e.g., DDT) could be separated into dichlorodiphe-nylethanes, cyclodiene compounds and other related compounds. Insecticides DDT and gammaxene were widely used at the end of World War II. Insects soon became resistant to DDT and as the chemical did not decompose readily, it persisted in the environment. Since it was soluble in fat rather than water, it biomagnified up the food chain and disrupted calcium metabolism in birds, causing eggshells to be thin and fragile. As a result, large birds of prey such as the brown pelican, ospreys, falcons and eagles became endangered (according to http://www.pollutionissues. com, dated 26.5.11).

(d) Solvents that are used in industries

Toluene is an aromatic hydrocarbon with a methyl side chain, widely used as an industrial feedstock, octane booster in fuel, solvent in paints, rubber, printing, adhesives, lacquers, disinfectants and in production of phenol (Surridge et al. 2009).

1.3.2 Sources of Soil Contamination

Ecosystems have been polluted with diverse kinds of chemicals which are released by various human and natural activities. Excessive levels of inorganic fertilizer related chemicals introduced into soil, such as ammonia, nitrates, phosphates which accumulate there or lead to contamination of water courses and air, have resulted in noteworthy environmental damage. 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. All these sources cause accumulation of metals and metalloids in our agricultural soils and pose serious threat for biota and human health (Forstner 1995). Sites contaminated by heavy metal include battery disposal areas, burn pits, chemical disposal areas, contaminated marine sediments, electroplating/metal finishing shops and fire fighting training areas as well as landfills and burial pits. Metals such as lead, arsenic, etc. are commonly called heavy metals, although this term strictly refers to metallic elements with a specific mass higher than 5 g/cm³ which are able to form sulfides (Gaur and Adholeya 2004; Singh et al. 2009). The other sources of metals in the soil are diverse, including burning of fossil fuels, mining and smelting of metalliferous ores, municipal wastes, sewage sludge amendments and the use of pigments. All these can also cause a considerable detrimental effect on soil ecosystems, environment and human health due to their motilities and solubility which determine their speciation (Khan 2005). At low concentrations, some metals such as copper, chromium, molybdenum, nickel, selenium and zinc are vital to healthy functioning and reproduction of microorganism, plants and animals (Marschner and Romheld 1995; Gaur and Adholeya 2004). However, at high concentrations, these essential elements may cause direct toxicity or reproductive effects. Some elements such as arsenic, cadmium, lead and mercury are not required by living components of ecosystem as these elements have no beneficial effect. These elements even at low concentrations can cause toxicity to plants and animals when they are present in the environment and their accumulation over time in the bodies of animals can cause serious illness (Alloway 1995; Gaur and Adholeya 2004; Adamo and Zampella 2008; Mohapatra 2008). 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; Khan 2005). Migration of these contaminants into noncontaminated areas as dust or leachates through the soil and spreading of heavy metal containing sewage sludge are examples of events that contribute towards contamination of our ecosystems. Soil contamination with heavy metal mixtures is receiving increasing attention from the public as well as governmental bodies, particularly in developing countries (Khan 2005). The remediation of such soils is important because these usually cover large areas that are rendered unsuitable for agricultural and other human use.

Trace metal contamination of soils can occur naturally from geological sources, for example Cu and Ni contamination of basaltic soils from the basalt parent material (Pal et al. 2010), or as a result of a wide range of industrial and agricultural activities. Metals and metalloids enter soils and waters because of many processes including atmospheric deposition from industrial activities or power generation; disposal of wastes such as sewage sludge, animal manures, ash, domestic and industrial wastes or byproducts; irrigation and flood or seepage waters and the utilization of fertilizers, lime or agrochemicals. Radionuclides are building up in some areas due to deliberate or accidental releases related to their use in energy production or for military purposes. It has been found that sewage sludge contains maximum amount of metals among different sources (Singh et al. 2009; Pal et al. 2010).

Heavy metals are deposited in soils by atmospheric input and the use of mineral fertilizers or compost, and sewage sludge disposal. It is well known that heavy metals are not biodegraded in contrast to organic pollutants and thus need to be physically removed or be immobilized (Gaur and Adholeya 2004; Mohapatra 2008). To some extent, microorganisms can modify the toxicity of metals by altering the bioavailability through oxidation and reduction. Traditionally, remediation of heavy metals contaminated soils involves either on-site management or excavation, and subsequent disposal to a landfill site. However, this method of disposal merely shifts the contamination problem elsewhere along with the hazards associated with transportation of contaminated soil and migration of contaminants from landfill into adjacent environments. Soil washing for removing contaminants from soil is an alternative to disposal to landfill. This method is, however, costly and produces a residue rich in heavy metals, which will require further treatment or burial. Conventional remediation methods usually involve excavation and removal of contaminated soil layer, physical stabilization (mixing of soil with cement, lime, apatite, etc.) and washing of contaminated soils with strong acids or heavy metal chelators (Pal et al. 2010). To prevent decrease in available arable land for cultivation from heavy metal contamination, remediation action is required.

1.4 Chemical Fate and Mobility of Heavy Metals

The fate and transport of a metal in soil and groundwater depend significantly on the chemical form and speciation of the metal. The mobility of metals in ground-water systems is hindered by reactions that cause metals to adsorb or precipitate, or chemistry that tends to keep metals associated with the solid phase and prevents them from dissolving. These mechanisms can retard the movement of metals and also provide a long-term source of metal contaminants (Evanko and Dzombak 1997).

1.4.1 Lead

The primary industrial sources of lead (Pb) contamination include metal smelter and processing, secondary metals production, lead battery manufacturing, pigment and chemical manufacturing, and lead-contaminated wastes. Widespread contamination due to the former use of lead in gasoline is also of concern. Lead released to groundwater, surface water and land is usually in the form of elemental lead, lead oxides and hydroxides, and lead metal oxyanion complexes. Most lead released to the environment is retained in the soil. The primary processes influencing the fate of lead in soil include adsorption, ion exchange, precipitation and complexation with absorbed organic matter. These processes limit the amount of lead that can be transported into the surface water or groundwater (Evanko and Dzombak 1997).

1.4.2 Chromium

Chromium (Cr) is one of the less common elements and does not occur naturally in elemental form, but usually as chromates. Chromium is mined as a primary ore product in the form of the mineral chromite, FeCr₂O₄. Hexavalent chromium is widely used in many industrial processes such as electroplating and wood preservation. Major sources of Cr contamination include releases from electroplating processes and the disposal of chromium containing wastes. Most of the chromium released into natural waters is particle associated, however, and is ultimately deposited into the sediment (Evanko and Dzombak 1997). Commercially available forms of hexavalent chromium (Cr (VI)) are potassium chromate and potassium dichromate. The chromium manufacturing industry produces a large quantity of solid and liquid waste containing hexavalent chromium. The treatment of these wastes is essential before discharging them to the environment. Cr (VI) compounds are highly water soluble, toxic and carcinogenic in mammals (Jeyasingh and Philip 2005). As chromium is widely used in many industries of which leather industries are the biggest consumers, wastes from tanneries pose a serious threat to the environment.

1.4.3 Arsenic

Arsenic (As) is a semimetallic element that occurs in a wide variety of minerals, mainly as As_2O_3 , and can be recovered from processing of ores containing mostly copper, lead, zinc, silver and gold. It is also present in ashes from coal combustion. Many arsenic compounds absorb strongly to soils and are therefore transported only over short distances in groundwater and surface water (Evanko and Dzombak 1997).

1.4.4 Zinc

Zinc (Zn) does not occur naturally in elemental form. It is usually extracted from mineral ores to form zinc oxide (ZnO). The primary industrial use for Zinc is as a corrosion-resistant coating for iron or steel. Zinc is one of the most mobile heavy metals in surface waters and groundwater because it is present as soluble compounds at neutral and acidic pH values. At higher pH values, zinc can form carbonate and hydroxide complexes which control zinc solubility. Zinc readily precipitates under reducing conditions and in highly polluted systems when it is present at very high concentrations, and may co-precipitate with hydrous oxides of iron or manganese (Evanko and Dzombak 1997).

1.4.5 Cadmium

Cadmium (Cd) occurs naturally in the form of CdS or CdCO₃. Cadmium is recovered as a byproduct from the mining of sulfide ores of lead, zinc and copper. Sources of cadmium contamination include plating operations and the disposal of cadmium-containing wastes.

Cadmium is relatively mobile in surface water and ground-water systems and exists primarily as hydrated ions or as complexes with humic acids and other organic acids. Under acidic conditions, cadmium may also form complexes with chloride and sulfate. Cadmium is removed from natural waters by precipitation and sorption to mineral surfaces, especially oxide minerals, at higher pH values (pH > 6). Removal by these mechanisms increases as pH increases (Evanko and Dzombak 1997).

1.4.6 Copper

Copper (Cu) is mined as a primary ore product from copper sulfide and oxide ores. Mining activities are the major source of copper contamination in groundwater and surface waters. Other sources of copper include algicides, chromated copper arsenate (CCA) pressure treated lumber and copper pipes. Solution and soil chemistry strongly influence the speciation of copper in ground-water systems (Evanko and Dzombak 1997).

1.4.7 Mercury

The primary source of mercury is the sulfide ore cinnabar. Mercury (Hg) is usually recovered as a byproduct of ore processing. Release of mercury from coal combustion is a major source of mercury contamination. Releases from manometers at

pressure measuring stations along gas/oil pipelines also contribute to mercury contamination (Evanko and Dzombak 1997). In contrast to the other metals and metalloids, microbial reduction of mercury can lead to volatilization of elemental mercury which has a high vapor pressure.

1.5 Soil Health and Need for Biological Remediation

Contaminated soils around the world have limited value for farming purposes. Affected soil that are excessively polluted become relatively sterile to maximum life forms. Many technologies are currently used to clean up heavy metal contaminated soils. The most commonly used ones are soil removal and land filling stabilization/solidification, physico-chemical extraction, soil washing, flushing, bioremediation and phytoremediation. None of above mentioned techniques are completely accepted as best treatment option because either they offer a temporary solution, or simply immobilize the contaminant or are costly when applied to large areas (Jeyasingh and Philips 2005). Productivity of conventional agricultural systems largely depends on the functional process of soil microbial communities. The structure and diversity of microbial communities are influenced by the soil structure and spatial distribution, as well as by the relationship between abiotic and biotic factors of the microbial communities (Jeyasingh and Philips 2005; Surridge et al. 2009). Biological remediation using microorganisms and plants is generally considered a safe and a less expensive method for the removal of hazardous contaminants from the environment. Certain indigenous microorganisms including bacteria and fungi are able to degrade PAHs in soil, leading to in situ rehabilitation of contaminated soils. Specific plants are also adapted to grow on contaminated sites. Arbuscular mycorrhizal fungi (AM fungi) can colonize the plants growing in contaminated soil and thus AM fungi can be exploited for phytoremediation (Bothe et al. 2010). Bioremediation, i.e., the use of living organisms to manage or remediate pollute soils, is an emerging technology. It is defined as the elimination, attenuation or transformation of polluting or contaminating substances by the use of biological processes. Phytoremediation, the use of plants to remediate or cleanup contaminated soils, is another promising method to remove and/or stabilize soils contaminated with heavy metals (Gaur and Adholeya 2004). Therefore, sustainable on-site techniques for remediation of heavy metal contaminated sites need to be developed.

1.6 Possible Role of AM Fungi in Remediation

Plants growing on contaminated soil are poor in health. Not all plants can grow on metal containing soil. The introduction of an AM fungal inoculum into such areas might be one of the strategies for establishing of mycorrhizal herbaceous plant

species. AM fungal isolates differ in their effect on heavy metal uptake by plants. Some reports indicate higher concentrations of heavy metals in plants due to AM, whereas others have found a reduced plant concentration, e.g., for Zn and Cu in mycorrhizal plants. Thus, selection of appropriate isolates could be of importance for a given phytoremediation strategy. AM fungal species can be isolated from areas which are either naturally enriched by heavy metals or old mine/industry waste sites in origin. In this context, AM fungi constitute an important functional component of the soil–plant system that is critical for sustainable productivity in degraded soils. Thus, AM fungi may also play a role in the protection of roots from heavy metal toxicity by mediating interactions between metals and plant roots (Khan 2005).

AM fungi are of importance as they play a vital role in metal tolerance and accumulation. External fungal mycelium of AM fungi provides a wider exploration of soil volumes by spreading beyond the root exploration zone which otherwise is unavailable for uptake by roots alone and thus provide access to a greater volume of heavy metals present in the root zone. A greater volume of metals is also stored in the mycorrhizal structures in the root and spores. For example, concentrations of over 1,200 mg/kg of Zn have been reported in fungal tissues of Glomus mosseae and over 600 mg/kg in *Glomus versiforme*. Another important feature of this symbiosis is that AM fungi can increase plant establishment and growth despite high levels of soil heavy metals, due to better nutrition water availability and soil aggregation properties associated with this symbiosis. AM fungus is significant in the ecological improvement of rhizosphere (Gaur and Adholeya 2004). Several of the heavy metal tolerant AM fungi isolated from polluted soils can be useful for reclamation of degraded soils as they are found to be associated with a large number of plant species in heavy metal polluted soil. A detailed review on role and prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils is available in Gaur and Adholeya (2004).

Gildon and Tinker (1981, 1983) isolated a mycorrhizal strain which tolerated 100 mg/kg of Zn in the soil. Considerable amount of AM fungal colonization was also reported in an extremely polluted metal mining area with an HCl-extractable Cd soil concentration of more than 300 mg/kg. Similarly, Weissenhorn et al. (1993) isolated mycorrhizal fungi from two heavy metal-polluted soils, which were found to be more resistant to Cd than a reference strain. Sambandan et al. (1992) reported 15AM fungal species from heavy metal contaminated soils from India. Of the 15AM species isolated, *Glomus geosporum* was encountered at all the sites studied. The percentage colonization ranged from 22 to 71% and spore count was as high as 622 per 100 g of soil. Weissenhorn et al. (1995) suggested a high tolerance of indigenous AM fungal population to elevated metal concentrations in soil and inside the roots (Gaur and Adholeya 2004).

Turnau et al. (2001) analyzed the community of AM fungi in the roots of *Fragaria vesca* growing in Zn-contaminated soil. Seventy percent of the root samples containing positively stained fungal hyphae were found to be colonized by *G. mosseae*. Another unique AM fungal species, *Scutellospora dipurpurascens* has been reported by Griffioen et al. (1994) from the rhizosphere of *Agrostis*

Table 1.2 AM fungi in metal polluted habitat (Mathur et al. 2007)	Glomus claroideum Glomus mosseae Gigaspora sp. Glomus tenue Glomus ambisporum Glomus fasiculatum Glomus intraradices Glomus macrocarpum
	Glomus etunicatum

capillaris growing in contaminated surroundings of a zinc refinery in the Netherlands. This indicates that these fungi have evolved Zn and Cd tolerance and that they might play an important role in conferring Zn or Cd tolerance in plants. Mycorrhizal fungi have also been shown to be associated with metallophyte plants on highly polluted soils, where only adapted plants such as *Viola calaminaria* (violet) can grow (Gaur and Adholeya 2004). A *Glomus* sp. isolated from the roots of the violet plant improved maize growth in a polluted soil (Hildebrandt et al. 1999; Gaur and Adholeya 2004; Bothe et al. 2010) and reduced root and shoot heavy metal concentrations in comparison to a common *Glomus* isolate or non-colonized controls. A list of AMF fungi present in contaminated habitat is given in Table 1.2.

1.7 Possible Role of Heavy Metal Tolerant Plants in Remediation

A metallophyte is a plant that can tolerate high levels of heavy metals such as lead, zinc and others. Plants that do well on heavy metal enriched soils can be used in the phytoremediation of polluted areas. The ability of metallophytes to tolerate extreme metal concentrations commends them as the perfect option for ecological restoration of metal-contaminated sites. Metallophyte can be used to stabilize soils against the erosion of the surface soil by wind and rainfall, and they can colonize disturbed areas. Metallophytes can be used to extract heavy metals (Bothe et al. 2010). As discussed above AM Fungi might contribute to metal tolerance. Plant species belonging to plant families Chenopodiaceae, Cruciferaceae, Plumbaginaceae, Juncaceae, Juncaginaceae, Amaranthaceae and few members of Fabaceae are believed not to form a symbiosis with AM fungi (Khan 2005). A detailed review on the role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation was done by Khan (2005).

Many hyperaccumulators belong to the family nonmycorrhized family Brassicaceae but there are conflicting reports regarding their mycotrophic status. Hirrel et al. (1978) reported them to be nonmycorrhizal but 1-5% AM fungal root colonization occurred in seven species of crucifers when grown in the presence of a mycorrhizal

companion plant. DeMars and Boerner (1996) made an extensive literature survey of crucifers and revealed that roots of 18.9% of the 946 members investigated were found to be colonized with internal hyphae, occasional vesicles but no arbuscules. Since arbuscules are the major site of nutrient exchange, including metals, between the plant and the AM fungi; these associations are expected to be non-functional (Bago 2000). However, a function as such for metals, e.g., in cell walls of dead hyphae, is still feasible. Pawlowska et al. (1996) found roots of *Biscutella laevigata*, a Brassicaceous plant colonizing the calamine mounds in Poland, to be mycorrhizal but without arbuscules. However, a latter study by Orlowska et al. (2002), who re-examined the mycorrhizal status of *B. laevigata* and the role of restoration of zinc-wastes on mycorrhization of this cruciferous plant species, observed AM hyphae, vesicles, as well as arbuscules in roots collected prior to seed maturity. Minuarita verna can endure the highest concentrations of heavy metals on any Central European metallophyte. Armeria maritima ssp. halleri occurs in coastal salt marshes and contains 20-fold and 88-fold greater concentrations of lead and copper, respectively, in its roots than in its leaves, indicating that the metals are immobilized in its roots. The genus *Thlaspi* comprises several closely related species

Table 1.3 Metal toleran	t plants	
Alyssum argenteum	Alyssum constellatum	Alyssum euboeum
Alyssum obovatum	Ariadne shaferi	Armeria denticulate
Austromyrtus bidwilliia	Berkheya coddii	Berkheya zeyheri
Blepharis acuminate	Clerodendrum infortunatum	Cnidoscolus bahianus
Cochlearia aucheri	Cochlearia sempervivum	Crotalaria trifoliastrum
Croton bonplandianus	Croton campestris	Dichapetalum gelonioides
Dicoma niccolifera	Dodonaea microzyga	Elsholtzia haichowensis
Elsholtzia patrini	Eremophila weldii	Eriachne mucronata
Euphorbia selloi	Evovulvus alsinoides	Garcinia polyneura
Garcinia reäoluta	Geniosporum tenuiflorum	Gochnatia recuräa
Grevillea acuaria	Heliotropium salicoides	Hybanthus epacroides sp. bilobus
Hybanthus floribundus	Merremia xanthophylla	Mosiera araneosa
Myristica laurifolia	Ouratea striata	Pearsonia metallifera
Pentacalia cristalensis	Pentacalia moaensis	Pentacalia trichotoma
Pentacalia trineura	Phyllanthus balgooyi	Phyllanthus chryseus
Phyllanthus cinctus	Phyllanthus comptus	Phyllanthus cristalensis
Phyllanthus discolour	Phyllanthus formosus	Phyllanthus incrustatus
Phyllanthus microdictyus	Phyllanthus mirificus	Phyllanthus myrtilloides
Planchonella oxyhedra	Polygonum posumbu	Psidium araneosum
Ptilotus obovatum	Rhus wildii	Richardia grandiflora
Rinorea bengalensis	Rinorea javanica	Senecio lydenburgensis
Senecio pauperculus	Shorea tenuiramulosa	Sida linifolia
Solidago hispida	Streptanthus polygaloides	Tephrosia polyzyga
Tephrosia villosa	Thlaspi montanum var siskiyouense	Trichospermum kjelbergii
Trymalium myrtillus	Turnera subnuda	Turnera trigona
Walsura monophylla	Waltheria indica	Westringia rigida

According to http://www.metallophytes.com, dated 06.05.2011

that grow well on heavy metal containing soils, e.g., *Thlaspi praecox* and *T. caerulescens* growing in heavy metal soils. *Thlaspi* species are generally short living annual plants and they have developed means to keep their seeds free of toxic heavy metal concentrations. *Viola lutea* ssp. *calaminaria* (yellow zinc violet) and *Viola lutea* ssp. *westfalica* (blue zinc violet) occupy heavy metal heaps with endemic occurrences (Tonin et al. 2001; Bothe et al. 2010). A list of metal tolerant plants is given in Table 1.3.

1.8 Conclusions

The present chapter discusses soil which consists of a complex mixture of particulate materials derived from abiotic parent minerals, living biota, organic detritus and humic substances. Soil can have naturally high concentrations of heavy metals as a result of the weathering of parental material with high amounts of heavy metal minerals or due to contamination associated with several human activities such as mining. Heavy metal contaminations of soils affect the population of living components present in it. Microorganisms have the capability to interact in a variety of specialized ways with metals. Specifically mycorrhizal associates may contribute to metal tolerance of plants. Such metalliferous plants that flourish on heavy metal rich soils can be used in phytoremediation of polluted areas.

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