Chapter 1
Toxins and Their Phytoremediation

Muhammad Ashraf, Munir Ozturk, and Muhammad Sajid Aqeel Ahmad

Abstract The agricultural and industrial revolutions in the last few decades have resulted in increased concentration of toxins in our environment that are now-a-days a major cause of toxicity in plants and animals. Among different toxins, increasing levels of salts, heavy metal, pesticides and other chemicals are posing a threat to agricultural as well as natural ecosystems of the world. These contaminants result in soil, air and water pollution, and loss of arable lands as well as crop productivity. They also cause changes in species composition and loss of biodiversity by bringing about changes in the structure of natural communities and ecosystems. In this situation, different approaches are being adopted to reclaim polluted environments. Among these, *phytoremediation* has a potential in removing these toxins from the environment. This approach is based on the use of natural hyperaccumulator plant species that can tolerate relatively high levels of pollutants in the environment. Pollutants accumulated in stems and leaves of high biomass producing and tolerant plants can be harvested and removed from the site. Therefore, this approach has a potential to remove large amounts of toxins by harvesting the above-ground biomass. However, the effectiveness of *phytoremediation* approach can be increased if we have better knowledge of physiological, biochemical, molecular and genetic bases of plant resistance to natural and anthropogenic induced toxins. All these aspects of toxicity mechanisms and their removal techniques are comprehensively reviewed in this book.

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Contents

1 Introduction

With the increasing human population in the world, the issues related to environmental degradation are becoming more serious (Koptsik et al. 2003; Jarup 2003; Murch et al. 2003). Humans have accelerated the emission of organic and inorganic pollutants such as pesticides, salts, petroleum products, acids, heavy metals etc. Most of the pollutants cannot be easily degraded and hence they accumulate in the environment. Although, some pollutants such as salts and heavy metals naturally occur in soils, industry (Richards et al. 1997; Ortiz-Hernandez et al. 1999; Sharma 2005), and agriculture (Scancar et al. 2000; Yagdi et al. 2000; Delibacak et al. 2002; Suciu et al. 2008) are considered as the major sources of anthropogenic induced pollution in the environment. Accelerated accumulation of toxins in the environment results in soil degradation, deforestation, desertification, loss of species diversity, pollution, acid rain, greenhouse effect and other issues related to environmental degradation.

Toxins or toxic chemicals are the inorganic and organic compounds that have negative effects on plant growth and metabolism. These are emitted into the environment as a result of human activities. For example, salts and heavy metals are released from leakage during extraction by mining, smelting, combustion and industrial effluents (Nriagu and Pacyna 1988; Nriagu 1989). Similarly, extensive use of fertilizers and pesticides in agriculture has resulted in considerable soil contamination.

Other pollutants such as petroleum products, explosives, cyanides etc. also result in considerable toxicity to living organisms.

The toxicity of a particular pollutant is determined in terms of its (i) biological role, (ii) ability to bioaccumulate, (iii) poisonous nature, and, (iv) persistency in the environment (Wildhaber and Schmitt 1996; Barron 2002). However, all these aspects vary greatly for different pollutants depending upon their molecular structure and physical as well as chemical properties (Wildhaber and Schmitt 1996). Unlike organic pollutants which are eventually converted into CO_2 and H_2O, inorganic pollutants such as metals and salts tend to deposit in different environmental components, especially in lakes, and estuarine and marine sediments (Ingersoll et al. 1996; MacDonald et al. 1996). Therefore, their removal is much more difficult as compared to that of organic pollutants and requires a different strategy to adopt for their removal. In addition, metals can easily circulate from one environmental compartment to another. These features make them a highly environmental as well as health hazardous if they accumulate at higher concentrations in the environment (Philp 1995; Hu 2002).

There are various hyperaccumulator species from various groups of bacteria, fungi, lichens, and higher plants that have the ability to uptake, accumulate or detoxify various organic and inorganic pollutants (Verhaar et al. 2000; Gramatica et al. 2002). This process broadly known as *bioremediation* utilizes various mechanisms such as *phytoextraction*, *phytoimmobilization* or *phytostabilization*, *phytotransformation*, *phytodegradation*, *phytostimulation*, *phytovolatilization* and *rhizofiltration* to remove toxic materials from different environmental components especially from soil and water (Schwitzguebel 2000; Cummings 2009). All these strategies are based on different methods and are effective for the removal of specific pollutant. In addition, a particular strategy effective for removal of one pollutant could be entirely useless for the removal of others. For example, *phytoextraction* and *phytoimmobilization* could be remarkably effective for the removal of salts and heavy metals. However, it can be entirely useless for the removal of organic contaminants such as hydrocarbons and explosives where *phytotransformation* or *phytodegradation* could be more effective. Therefore, the selection of a particular plant species to recommend and grow in the contaminated areas depends on the nature of contaminant, mechanism used by that species to remove the Contaminant, tolerance of that plant species to the pollutant and other environmental constraints (Huang and Cunningham 1996; Meagher 2000; Memon et al. 2001).

2 Toxins and Their Types

Toxins are generally classified into biodegradable (organic) and non-biodegradable (inorganic) pollutants (Verhaar et al. 2000; Gramatica et al. 2002). Biodegradable toxins are easily broken down into simpler molecules (CO_2 and water) by the activity of living organisms when they enter in the biogeochemical cycles. Such toxins are generally not harmful as they occur in low quantities in our environment. However, at high concentrations they prove to be highly toxic to all living organisms. In addition, organic toxins such as petroleum products are toxic even at low concentrations. The examples of biodegradable pollutants include domestic and agricultural residues, petroleum products, urine and fecal matter and sewage water (Cunningham et al. 1996; Kazuya et al. 1999; Aboul-Kassim and Simoneit 2001). In contrast, non-biodegradable toxins cannot be broken down into simple and harmless products by living organisms even over long time period. These include inorganic fertilizers, pesticides and insecticides (DDT), heavy metals (nickel, mercury, copper, lead, aluminum, arsenic etc.), salts (NaCl), oxides of nitrogen and sulphur (NO_2 and SO_2) and cyanides (Van der Werf 1996; Misra and Mani 1991; Sigel et al. 2005). Unfortunately, these toxins persist in the environment for a long period of time and prove harmful to the organisms once they enter in the food chain. Therefore, the removal of these toxins from the environment is much more difficult as compared to bio-degradable one.

Another classification system is based on the environmental components (soil, air or water) in which these toxins accumulate. This classification system reflects the immediate environmental component which is exposed to the degradation by these toxins. Toxins that accumulate in soil include salts, heavy metals, inorganic and organic fertilizers, pesticides, and domestic, agricultural and industrial pollutants etc. Similarly, toxins that are released into air include primary (CO_2, CO, SO_2, NO_2, CH_4, ammonia, volatile organic compounds) and secondary (ozone, peroxyacetylene nitrate) air pollutants. Water pollution is mainly caused by sewage water, residues from food processing units, industrial wastes, petroleum products, fertilizers and pesticides from agricultural runoff etc. Most of the toxins can easily circulate from one environmental component to the other and finally accumulate in soil and water bodies. These pollutants can then be easily taken up by plants and aquatic fauna and flora and transfer to the human body where they cause serious illness and disorders (Philp 1995; Albering et al. 1999; Korte et al. 2000).

2.1 Salts

The excessive amounts of salts in different soil profiles are the largest source of pollutants in the environment causing the problem of salinity world-wide. It is estimated that about 7% of the total earth's land and 20% of the total arable area are affected by high salt contents. In addition, about half of the irrigated area is highly salinized and unfit for cultivation of agricultural crops (Szabolcs 1994; Zhu 2001). The most common salts that create soil salinity problem include NaCl and $MgSO_4$. On the basis of origin, soil salinity can be classified as Primary or natural and secondary or induced soil salinity. Primary or natural soil salinity arises by weathering of minerals derived from highly saline parent rocks (Ashraf 1994). In contrast, secondary salinization results from human interference with natural water regimes. It occurs when native perennial vegetation is replaced by shallow rooted seasonal crops. In addition, other activities such as overgrazing and deforestation greatly reduce plant cover (Ashraf 1994, 2004; Ashraf and Foolad 2007). This results in rise of underground water-table up to 2–3 m and then capillarity brings the salts dissolved in different soil profiles to the surface causing the problem of secondary soil salinity (Chhabra 1996; Datta and de Jong 2002). In addition, due to reduction in vegetative cover, the amount of water entering underground aquifers (recharge) is increased but water taken up by plants (discharge) is dramatically reduced. This results in rise of water-table bringing the salts stored deep in the soil to the earth surface (Dunin 2002). Sometimes, introduction of exotic crops as well as other plant species and extensive agronomic practices result in altered water-use requirements of the vegetation. If this results in greater recharge of underground aquifers than discharge, the groundwater level will rise, bringing up salts with it and thus causes secondary soil salinity (Srivastava and Jefferies 1996).

Although high level of salt in soil can have a variety of effects on crop plants at biochemical, molecular and physiological levels, the most common effects include inhibition in photosynthesis, nutrient imbalance, changes in metabolic activities, disturbance in solute accumulation, enzyme activities, and hormonal imbalance etc. (Ashraf 1994, 2004; Tester and Davenport 2003; Munns 2005; Munns et al. 2006). It is now widely accepted that salinity inhibits plant growth by four major ways, (i) salt-induced water stress, (ii) specific ion toxicity (ion imbalance or nutritional disorders), (iii) oxidative stress, i.e., production of reactive oxygen species, and (iv) hormonal imbalances (Greenway and Munns 1980; Munns 1993, 2002; Ashraf 2004; Flowers 2004; Munns and Tester 2008). In addition, the degree of growth inhibition due to salt stress depends on the duration of stress, plant growth stage, and type of plant species. However, early growth stages such as germination and seedling stages are contemplated as more susceptible to salt damage as compared to later adult stages (Hamdy et al. 1993).

The salt effects on plant growth and development have been discussed in detail in a number of reviews. Their main focus has been on physiology of salt toxicity and tolerance, intra- and inter-cellular ion transport as well as long distance transport in plants, identification and characterization of traits and/or genes responsible for ion homeostasis, osmotic adjustment, and antioxidants whose expression is regulated by salt stress (Ashraf 1994, 2004; Ingram and Bartels 1996; Tester and Davenport 2003; Flowers 2004; Munns 2005; Munns et al. 2006; Munns and Tester 2008). Of various plant responses to salt stress, accumulation of compatible solutes (organic compounds of low molecular weight) is one of the prominent responses of plants to salt stress, because this phenomenon helps the plant to become acclimated to different stressful environments (Bohnert and Jensen 1996; Ashraf and Harris 2004; Ashraf and Foolad 2007). Various compatible osmolytes such as proline and glycinebetaine are considered as extremely effective in regulating growth under stressful environments and are widely distributed in a wide variety of plants (Rhodes and Hanson 1993). These compatible solutes are of low molecular weight, high solubility, and non-toxic, even if they accumulate at high cellular concentrations. They protect cellular structures from abiotic stress-induced injuries. For example, they promote osmotic adjustment, scavenge reactive oxygen species, stabilize enzymes/proteins, and protect membrane integrity in plants subjected to stressful conditions (Hasegawa et al. 2000; Ashraf and Foolad 2007).

2.2 Heavy Metals

Heavy metals have gained considerable attention as a potential environmental pollutant in recent years (Misra and Mani 1991). This is the result of their excessive use in a number of industrial processes and therefore, their toxicity is more common as compared to deficiency in organisms (Lindberg and Greger 2002). Most metals are commonly used in a multitude of industrial processes, such as manufacture of batteries, alloys, electroplated metal parts, pesticides, textile dyes and steel etc. Consequently, they are emitted to the environment to supplement natural background geochemical sources (Barnes and Rudzinski 2006). The sources of metal pollution in the environment include leakage during extraction by mining and smelting, combustion (particularly during power generation, incineration, smelting and the internal combustion engines) and industrial effluents, (Duce et al. 1991; Galloway et al. 1982; Hutton and Symon 1986; Nriagu 1989; Nriagu and Pacyna 1988).

There are 35 metals that are of a concern to environmental health and 23 of them are called as heavy metals. These include arsenic (As), antimony (Sb), bismuth (Bi), cadmium (Cd), cerium (Ce), copper (Cu), chromium (Cr), cobalt (Co), gallium (Ga), iron (Fe), gold (Au), lead (Pb), nickel (Ni), manganese (Mn), mercury (Hg), platinum (Pt), silver (Ag), thallium (Tl), tellurium (Te), and zinc (Zn) (Philp 1995; Hu 2002). Among these, the most common heavy metals that cause toxicity in plants and animals are arsenic, lead, mercury, cadmium, nickel, iron and aluminum (Hutton and Symon 1986; Chaney and Ryan 1994). Most of the metals are easily absorbed by the plants and bioaccumulate in different organs (Wang et al. 2003). These metals may ultimately enter the human body through ingestion of food, use of metal contaminated water or breathing in air containing toxic metals (Philp 1995; Albering et al. 1999; Jarup 2003).

All metals are not toxic as some of them function as micro-nutrients in less concentration and hence are considered as essential nutrients (Taiz and Zeiger 2006; Timbrell 2005; Pechova and Pavlata 2007). Some of the metals are also called as trace elements (such as iron, copper, manganese, and zinc) due to their extremely low concentrations/requirement in biological systems (Nriagu 1989; Graham and Stangoulis 2003). Since they are found naturally in soil, their adequate amounts are naturally found in our foodstuffs, fruits and vegetables (Ghafoor et al. 1996; Islam et al. 2007). They are also a component of commercially available multivitamin products (Boullata and Armenti 2004). Most of the metals function as a cofactor of a number of metabolic reactions. For example, Fe, Zn, Cu, Ni and Mo are among the common metals that have known biological functions in plants (Westbroek and De Jong 1983; Seiler et al. 1994; Taiz and Zeiger 2006). These metals are mostly required as enzyme activator and some of them are even integral components of a number metaloenzymes. Hence, their deficiency may lead to suppression of growth and development of plants with visible deficiency symptoms reflected as chlorosis and subsequent necrosis of plant tissues (Dixon and Webb 1958; Ghani and Wahid 2007).

Despite the fact that some of the metals function as essential elements in low concentrations, they may become toxic if they accumulate at higher concentrations in the environment (Verkleij and Prast 1990). Other metals (biologically non-essential) may become toxic to organisms even at very low concentrations (Verkleij and Prast 1990; Islam et al. 2007). The general signs associated with metal toxicity in plants include reduced shoot and root growth, poor development of branching system, deformation of various plant parts and abnormal flower shape, decreased biomass production, leaf spotting, mitotic root tip disturbances, inhibition of germination, and chlorosis that can result in foliar necrosis (Ewais 1997; Madhava Rao and Sresty 2000; Pandey and Sharma 2002; Rahman et al. 2005; Gajewska et al. 2006). Ultimately, all these processes lead to reduction in yield of agricultural crops (Balaguer et al. 1998; Ahmad et al. 2007).

2.3 Herbicides and Pesticides

Herbicides and pesticides have long been used as the most effective means of crop protection by controlling or eliminating the pests and pathogens. They include fungicides, bactericides, insecticides, weedicides, herbicides, rodenticides and algicides (Ellenhorn et al. 1997). These chemical substances are applied to crops at different growth stages e.g., as pre-sowing seed treatments, during crop cultivation and after harvest to protect seeds, grains and cereals from the attack of pests and pathogens and to prolong their storage capacity (Morgan and Mandava 1988; Boesten 2000). These chemicals are applied as liquid sprays, powder and dusts, seed-treatments, oil-based solutions and aerosols. Different examples include dichlorodiphenyltrichloroethane (DDT), benzene hexochloride, lindane, malathion, and 2,4-dichlorophenoxy acetic acid etc. (Morgan and Mandava 1988; Laws and Hayes 1991). Most of the pesticides can effectively control pests and pathogens and therefore, they are the most popular, economical and effective technology for crop protection among farmers of different regions of the world (Mandava et al. 1985).

Although application of these chemical compounds is regarded as an effective mean to control pest and pathogens, their application can have adverse effects on plants and animals including invertebrate and vertebrate species (Schluz 2004). These pesticides and herbicides can enter the atmosphere and ecosystems during their preparation and processing procedures, application methods, post-application evaporation and volatilization and water runoff (Van der Werf 1996; Shreiver and Liess 2007). In addition, disposal of expired chemicals into soil and water bodies is also a major source of their pollution in the environment (Bacci 1994). Among different classes of these chemicals, insecticides are the most important in damaging environment and causing toxicity to living organisms. This is followed by fungicides and bactericides and herbicides (Marer 2000; Goel and Aggarwal 2007).

The toxic/damaging effects of pesticides and herbicides on organisms and environment are determined by a number of features. These include (i) their chemical nature (systemic or non-systemic), (ii) active ingredients (formulation), (iii) organism exposed, (iv) persistency in the environment, and (v) concentration used for application (Van der Werf 1996). Besides these facts, some other factors such as personnel skill of the applicator (farmer), time of application and weather condition also contribute significantly towards the pesticide’s actual toxicity and can make them extremely hazardous. These chemicals accumulate in soil and water bodies and prove extremely toxic to the non-target organisms including plants and animals as well as humans (Jeyaratnam 1990).

2.4 Cyanides

Cyanides are organic compounds that comprise the cyano group (C≡N) in their structure. Cyanide toxicity is also known as prussic acid poisoning (Vogel et al. 1987). Different forms of cyanides include hydrogen cyanide (HCN), potassium cyanide (KCN) and sodium cyanide (NaCN). Among these, HCN is a colorless gas with odor just like a bitter-almond while NaCN and KCN are white powders with a similar odor as that of HCN. Both NaCN and KCN are converted into HCN when they get mixed in water and cause toxicity to living organisms (Curry and LoVecchio 2001). Cyanogenic compounds occur naturally in certain bacteria, fungi, algae and higher plants. Therefore, they occur in a variety of food and plant products. Cyanogenic compounds naturally occur in a number of plant families including Poaceae, Papilionaceae, Sambucaceae, Euphorbiaceae and Rosaceae. They are found in small amounts in various plant fruits such as apple seeds, citrus seeds, plums, mango stones, peach stones and bitter almonds (Poulton 1990; Wong-Chong et al. 2006).

In plants, cyanides are generally found in bound forms as cyanogenic glycosides and play an important role in plant defense against herbivory. For example, cassava roots have been reported to contain excessive amount of cyanogenic glycosides (Emmanuel and Emmanuel 1981). Among different cyanogenic glycosides found in plants, amygdalin is the best characterized one, which is present in a number of plant species especially in the leaves and seeds of cherry, almond and peach, etc. (Santamour Jr 1998; Sánchez-Pérez et al. 2008). For example, cherry kernels may yield up to 170 mg while bitter almond pulps up to 250 mg 100 g^{-1} dry weight. Overall, cyanogenic glycosides have been reported to occur in more than 3000 plant species (ca. from 130 families) and thus these species have a potential to produce HCN toxicity if ingested by animals and humans. However, actual incidence of cyanide poisoning is low, because these plants are not frequently eaten up by animals or humans (Curry and LoVecchio 2001).

In addition to natural sources, cyanides are also released by various industrial sources. For example, thiocyanate is discharged in a variety of industrial wastewater discharges, while cyanogen halides are released upon chlorination or bromination of water containing free cyanides (Zheng et al. 2004). Cyanides are also used as a raw material during the production of chemicals (nylon and plastic), adhesives, cosmetics, dyes, computer electronics, pharmaceuticals, and road salts, pesticides, rodenticides, wine, anticaking agents, fire retardents, pharmaceuticals, painting inks, and other materials (Kjeldsen 1998). In addition, they are also directly used in a variety of processes, including electroplating and hydrometallurgical based gold and silver extraction (Kavanaugh 2004). Current industries that produce cyanide as a by-product include chemical manufacturing, iron and steel making, petroleum refining, and aluminum smelting (Wong-Chong et al. 2006). Overall, the approximate production of cyanides is 1.4 million tons per annum (Mudder and Botz 2001) which means over 10,000 tons of cyanide are being released into the environment each year (Mudder and Botz 2001; Korte et al. 2000).

Cyanogenic compounds, if accidentally ingested by animals or hydrolyzed by plants, prove extremely toxic (Schnepp 2006; Barillo 2009). This is mainly due to their ability to uncouple cytochrome C oxidase in mitochondria. HCN can readily bind to Fe in cytochrome in a stable and irreversible bonding (Cooper and Brown 2008). These result in disruption of electron transport chain thus blocking aerobic respiratory pathway that contributes to 95% of the energy produced in the cells in the form of ATP (Taiz and Zeiger 2006). In animals, tissues which are primarily dependent on aerobic respiration for source of energy, e.g., heart and central nervous system are markedly affected (Schnepp 2006; Barillo 2009). Thus, due to the blockage of ATP synthesis, plants or animals die quickly as no energy will be available to perform routine activities.

2.5 Toxic Explosives

Immense industrial and military activities are the main causes of substantial contamination of the environment with toxic explosives. Worldwide, a number of explosive-manufacturing, testing and storage facilities and military bases are contaminated with these chemicals. In addition, inappropriate disposal of explosive wastes and old and non-functioning weapons also contribute considerably towards environmental pollution (Pennington and Brannon 2002). The most common examples of explosives at hazardous waste sites are nitroglycerine (NG), 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (Royal Demolition Explosive - RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7- tetrazine (Rosenblatt 1980; Best et al. 1999). Among these, the most toxic materials used in military activities include TNT and RDX (Jenkins et al. 2006). Despite the threat of explosion upon exposure to large quantities of these explosives, exposure to these explosives such as TNT can cause severe health hazardous effects such as abnormal liver function, anemia, skin irritation, and cataracts. Similarly, RDX cause severe spasm when inhaled or eaten in large quantity. TNT and RDX also cause long-term health effects such as failure of nervous system and heart, which could lead to death of affected individuals (Lynch et al. 2002). In some cases, these toxic wastes may leach down to groundwater causing toxicity far away from the contaminated sites (Best et al. 1999).

There is only a little work on the effect of explosive materials on plants. However, the available literature suggests that these chemicals including nitroglycerine, TNT, RDX have a variety of effects on plants growing in contaminated areas (Harvey et al. 1991; Just and Schnoor 2004; Vila et al. 2007a; Rao et al. 2009). These effects include retardation of seed germination, growth (fresh and dry biomass) and development, and induction of leaf chlorosis and necrosis of plant tissues (Peterson et al. 1996; Robidoux et al. 1996; Vila et al. 2007b). Since the chemicals are mutagenic, they can also cause lethal mutations in animals as well as plants (French et al. 1999; Podlipna et al. 2008).

3 Plant Resistance to Toxins

3.1 *Salts*

The extent of the adverse effects of salt stress on crops or other naturally growing plants greatly differs and it depends on the type of species or cultivar, growth stage and interaction with other environmental constraints (Ashraf 1994; Ashraf et al. 2008; Munns and Tester 2008). Therefore, a variety of information is available in the literature depicting genetic variation for salt tolerance in crop plants. For example, while appraising the relative salinity tolerance in field pea, canola, dry bean, and durum wheat, Steppuhn et al. (2001) ranked these crops in an ascending order as dry bean < field pea < durum wheat < canola. Of different *Brassica* species, *B. napus* was found as the most salt tolerant, while *B. campestris* and *B. nigra* the most salt-susceptible (Kumar 1995). Some other studies entailing the exploration of mechanism of salt tolerance in canola have shown that cv. Dunkeld has high salt tolerance due to having higher photosynthetic, antioxidant, ion exclusion and osmotic adjustment capacities which make it highly salt tolerant (Ali et al. 2006; Ulfat et al. 2007; Ashraf and Ali 2008).

Plants use different mechanisms to overcome high salt concentration in soil. These include osmoregulation, compartmentalization of toxic ions, ion excretion, scavenging of reactive oxygen species and accumulation of compatible solutes etc. Salt tolerance in plants can be achieved by avoiding high ion concentration, i.e., delayed germination or maturity until favorable conditions, salt exclusion at root level or preferential root growth in non-saline areas, compartmentation of salts in vacuole or specialized cells such as salt glands and salt hairs or storage in older leaves, and selective discrimination of Na^+ against K^+ or Ca^{2+} (Marschner 1995; Hasegawa et al. 2000; Munns 2002, 2005; Tester and Davenport 2003; Flowers 2004). The antioxidant defense system includes antioxidant compounds (tocopherols and carotenoids) and enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and many others. Plants differ in their ability to scavenge ROS. For example, SOD in plants can catalyze the dismutation of superoxide to dioxgyen and hydrogen peroxide. Peroxidase or catalases can counteract H_2O_2 (Shalata and Tal 1998; Garratt et al. 2002).

Accumulation of compatible solutes such as polyols, sugars, glycinebetaine, proline, and other free amino acids is considered as one of the most vital components of salt tolerance in plants. Under saline conditions, these solutes not only allow the cells to adjust the osmotic potential to a level in the cytoplasm so as to maintain a sufficient amount of water content (Bohnert and Jensen 1996; Subbarao et al. 2001; Yokoi et al. 2002), but also safeguards proteins from the salt-induced dissociation of their respective subunits (Incharoensakdi et al. 1986). Moreover, in photosynthetic organisms, these organic solutes play a vital role in maintaining integrity of photosystem II at high levels of salt (Murata et al. 1992; Papageorgiou and Murata 1995), as well as the activity of enzymes involved in the mechanism of photosynthesis (Yokoi et al. 2002; Bohnert and Jensen 1996) such as ribulose 1,5- bisphosphate carboxylase/oxygenase (Nomura et al. 1998). Among the compatible solutes, accumulation of proline and glycinebetaine plays a crucial role in osmoregulation and osmotolerance in plants (Rhodes and Hanson 1993; Hasegawa et al. 2000). They also protect membranes and proteins against the destabilizing effects of abiotic stresses such as salt stress and water stress. In addition, their ability to scavenge free radicals generated under stress conditions renders them as an important marker of salt tolerance (Kavi Kishore et al. 2005; Ashraf and Foolad 2007).

3.2 Heavy Metals

Although some of the metals function as essential elements such as copper and zinc in low concentrations, they may become toxic if they accumulate at higher concentrations in the environment (Verkleij and Prast 1990). Other metals (non-essential) may become toxic to organisms even at very low concentrations (Verkleij and Prast 1990; Loska et al. 2000; Islam et al. 2007). The concentration of essential elements in organisms is generally controlled homeostatically i.e., they are taken up from the environment according to the nutritional demand of a plant (Sigel et al. 2005; Mueller-Roeber and Dreyer 2007; Alloway 2008), except for some elements like selenium, iodine and technetium (Wolterbeek 2001; Windisch 2002). If this regulatory mechanism breaks down either due to insufficient supply (deficiency) or excess (toxicity) of metal, its effects on growth are manifest as deficiency or toxicity symptoms in organisms (Grusak et al. 1999; van Wuytswinkel et al. 1999; Grusak 2002; Welch 2002).

The differential variability of uptake of different metals depends on various aspects such as the metal itself, the absorbing organism, the physico-chemical properties of the soil environment and the levels of other important metals and complex chemicals present in waters from different sources (Cataldo and Wildung 1978; Battarbee et al. 1988; Antosiewicz 1992). For example, free ions are largely bioavailable forms of a metal, and the free ion concentration is usually a potential indicator of toxicity (Seiler et al. 1994). However, in some other cases the situation is different. For example, in case of mercury, the organic form (methylmercury) is more toxic than the inorganic mercury ion (Wright and Welbourn 2002). In addition, the valency of a particular metal ion also has great influence on its bioavailability and mobility in soil and plants (Deoraj 2003; Deoraj et al. 2003).

A great deal of controversy exists in the literature on the prospective mechanisms of metal tolerance. This is likely due to a lack of knowledge on issues related to metal toxicity or due to the complexity of plant responses to metal toxicity. Furthermore, a variety of mechanisms may have been evolved in different species to tolerate high amounts of metals and even within the same plant species more than one mechanism may be operational (Memon et al. 2001; Meharg 2005; Gao et al. 2007). In most studies, plant species are tested for tolerance ability by using only one or a combination of a few metals. However, under natural conditions, most of the sites are polluted with more than one type of pollutants (organics and in-organics) having varying degrees of toxicity. In addition, other environmental and geophysical features also contribute considerably for their availability and uptake. Therefore, it becomes extremely difficult to distinguish their toxicity and mechanism operative for their tolerance in pants (Cataldo and Wildung 1978; Antosiewicz 1992; Deoraj et al. 2003).

Plants can employ numerous strategies to counteract excess external metal levels. These can be categorized into two main types, i.e., limiting the uptake or transport of metals, and internal metal tolerance mechanisms (Taylor 1987; Clemens 2006). In the first strategy, the toxic effects of metals are reduced by preventing the entry of excess metals in the plant by reduced uptake. This is brought either by complexing or precipitating metals in the root zone. Plants have the ability to precipitate metals by elevating the pH of the rhizosphere or by excreting them in the form of anions (Taylor 1991). However, a great deal of work has been done with limited number of metals such as Al and extensive work for other metals is essential to appraise the extensive validity of this mechanism.

True metal tolerance in plants could be, however, realized if metals are sequestered/compartmentalized within the cell of different tissues so that metals are unable to react with metabolically active cellular substances (Volesky 1990; Barley et al. 2005; Rajamani et al. 2007). In many studies, a significant increase in the level of organic molecules and amino acids (such as histidine) has been reported to occur in roots of metal stressed plants (Hall 2002). These results suggest that the complexation of metals with these organic molecules and amino acids might be involved in reduced delivery of metals from roots to shoots and hence reduced toxicity in aerial parts. However, once metals are transported to the aerial parts, there must be an effective mechanism to reduce their toxicity. As a first strategy, compartmentation of metal ions in the vacuole is the most plausible method of cellular sequestration (Rajamani et al. 2007). In addition, most of the metals lead to the production of reactive oxygen species. Therefore, most of the plants have evolved an effective scavenging system consisting of enzymatic (superoxide dismutase, peroxidase, catalase, glutathione reductase and ascorbate reductase) and non-enzymatic (proline, ascorbic acid, tocopherols, glutathione, carotenoids and phenolics) antioxidants. These antioxidants scavenge reactive oxygen species and protect micro- and macro-molecules and other cellular structures from oxidative damage (Luna et al. 1994).

3.3 Herbicides and Pesticides

Herbicides and pesticides have different effects on animals and plants. A few of these chemicals are selective in nature while others are broad spectrum in action. Therefore, broad spectrum pesticides are more hazardous to environment and organisms as compared to selective one (Laws and Hayes 1991; Marer 2000). Most of these chemicals persist in the environment which ultimately proves extremely toxic to non-target plants and animals. In addition to the toxic effects of these chemicals to plants and animals, these chemicals also contribute to soil degradation and affect soil microorganisms (Arthur Coats 1998; Andreu and Pico 2004).

Pesticide pollution causes considerable threats to a wide variety of non-target organisms including useful soil microbes, crops, livestock and other aquatic species.

Avoiding or minimizing the use of toxic chemicals is essential to improve continued existence of these non-target organisms (Calderbank 1989; Goel and Aggarwal 2007). It is now well known that soils have diverse composition and mainly consist of mineral particles and organic matter. Different types of pesticides may interact with the soil and form toxic residues in soils with minerals and organic matter, which may not be recovered from the soil even through extensive extraction (Gevao et al. 2000). The bioavailability of these bound residues is of great significance that determines toxicity to microorganisms and plants (Khan 1982; Calderbank 1989). Although, it has been documented that the activities of soil microorganisms primarily depend on the release of bound residues from the soil, but other factors like agronomic practices and application of some other chemicals that may change the chemical nature of soil may cause the release of soil bound residues (Khan 1982; Calderbank 1989; Goel and Aggarwal 2007). This might result in recycling of the compounds into the soil solution that could be ultimately absorbed by the plants and causes sever toxicity in plants (Andreu and Pico 2004).

Excessive use of pesticides and herbicides has been shown to produce a variety of toxicity symptoms in plants. However, there is great variation in toxicity symptoms depending upon type of chemical, active ingredient and concentration in the growing environment (Morgan and Mandava 1988; Boesten 2000; Hendersona et al. 2006). The most common toxicity symptoms in non-target plants are inhibition of seed germination, growth retardation, loss of photosynthetic pigments, damages to the photosynthetic machinery, fruit drop, reduced yield and a variety of other symptoms. These defects could result in chlorosis and necrosis of plant tissues eventually leading to the death of whole plants (Nair et al. 1993; Hendersona et al. 2006; Shreiver and Liess 2007).

3.4 Cyanides

The concentrations of cyanogenic glycosides greatly vary with phenology, growth stage, infection by pathogens, herbivory and environmental conditions (Gebrehiwot and Beuselinck 2001; Dzombak et al. 2006; Ballhorn et al. 2007). In plants, cyanogenic glycosides are usually compartmentalized in cell vacuoles and thus cells are prevented from their toxicity (Gruhnert et al. 1994; White et al. 1994; Gleadow and Woodrow 2002). Therefore, cyanogenic glycosides in plant tissues are not toxic unless they are hydrolyzed by plant enzymes (or rumen microorganisms) to form free HCN (White et al. 1998). This hydrolysis is usually carried out by the enzyme β-glucosidase that is found in plant cytoplasm. This conversion is also enhanced when the plant cells are injured (crushing, insect attack, herbivory) or when the plants are subjected to sever environmental stresses such as wilting or freezing stress (Ballhorn et al. 2009).

Some plants species contain an enzyme system that is able to detoxify cyanide by converting certain amino acids such as alanine and asparagine to cyanogenic glycosides in which a simple sugar is bonded to a cyanide molecule (Miller and Conn 1980; Galoian et al. 1982). In some plant species, β-cyanoalanine synthase

(CAS) was found to be able to catalyze the conversion of cyanide plus cysteine to β-cyanoalanine and sulfide (Miller and Conn 1980; Maruyama et al. 2001). This enzyme occurs in a number of higher plants and plays a vital role in the metabolism of cyanides (Maruyama et al. 2001). Since mitochondria are potential sites of cyanide toxicity and this enzyme is exclusively localized in this organelle, its principal physiological role has been attributed to its detoxification capability of cyanides (Manning 1988). In another study conducted on both cyanogenic as well as non-cyanogenic plants, asparagine was the only metabolic product found when they were exposed to labeled ^{14}CN (Manning 1988). In an experiment by Yu et al. (2004) 28 plants belonging to 23 families were appraised for their performance for removal of cyanide. These authors found that most of the plant species were capable of readily metabolizing cyanide to non-toxic chemical. This evidence shows that the mechanism of cyanide detoxification in plants needs to be fully explored.

3.5 Toxic Explosives

The toxicity of explosives containing nitro groups is usually attributed to the number of nitro groups. It has been suggested that different plants can take up and degrade toxic explosives such as nitroglycerine into simpler non-toxic compounds. In this regard, Podlipna et al. (2008) showed that the toxicity of nitroglycerine decreased with the decreasing number of nitro groups during *phytodegradation* of these chemicals by mustard (*Sinapis alba*), *Juncus inflexus*, *Phragmites australis* and flax (*Linum usitatissimum*). Most recently, genetically engineered plants have been shown to have greater ability to detoxify these compounds. In these plants, toxic explosives such as TNT are converted to different compounds that are used by the plant enzymes for further processing (Rylott and Bruce 2008). In response to the explosive presence several genes are up-regulated, including transferases, which by transferring a particular residue to the acceptor molecule, alter its bioactivity, solubility and/or transport properties (Ekman et al. 2003; Mezzari et al. 2005). A full characterization of the activity of the most promising enzymes such as transferases should be performed so that new concepts are added to the biochemical scheme of transformation of toxic explosives.

4 Phytoremediation of Toxins

Phytoremediation, a subcategory of *bioremediation*, is generally defined as removal of toxins from the environment by the use of hyperaccumulator plants. This word has been derived from the Greek “Phyto” meaning plant, and Latin “Remedium” meaning refurbishing balance, removal, or remediation. Thus, in the process of *phytoremediation*, pollutant/toxins from contaminated soils, water or air are mitigated/removed by using plants which are able to hold, breakdown or remove metals, salts, insecticides, pesticides, organic solvents, toxic explosives, crude oil and its derivatives, and a variety of other contaminants from different environmental components. *Phytoremediation* is generally considered as efficient, inexpensive and environment-friendly technique, as compared to other mechanical or chemical methods of remediation that involves excavation of soil from contaminated site and ex-situ treatment for the removal of contaminants (Cunningham and Ow 1996).

Phytoremediation of contaminated soils can be achieved through various processes. These include *phytoextraction*, *phytoimmobilization* or *phytostabilization*, *phytotransformation*, *phytodegradation*, *phytostimulation*, *phytovolatilization* and *rhizofiltration* (Schwitzguebel 2000; Cummings 2009). Of these strategies, *phytoextraction* or *phytoaccumulation* consists of natural or induced (enhancement through use of chelating agents) potential of plants, algae and lichens to uptake and remove pollutants from soil, water environment by accumulating them into harvestable biomass. This method is traditionally used for the removal of heavy metals and salts from the contaminated soils. *Phytostabilization* is stabilization of the toxic pollutants over a long-term. Some plants have natural ability to immobilize pollutants by providing a region around the roots where these pollutants can be precipitated and stabilized. Unlike *phytoextraction*, *phytostabilization* involves sequestering of toxins into the *rhizosphere*, thereby preventing metal uptake by plant tissues. Therefore, pollutants turn out to be less mobile and bioavailable to plants, wildlife, livestock, and humans. *Phytotransformation* is the conversion of different types of organic pollutants by certain plant species to non-toxic substances. In addition, microorganisms living in soil and water and those associated with plant roots may metabolize these substances to non-toxic ones. However, it is imperative to note that these tenacious and complex compounds cannot be degraded to simple molecules such as water, carbon dioxide etc. by plant metabolism. However, in this process, a change in their chemical structure is brought about that reduces their toxicity to living organisms. *Phytostimulation* involves the enhancement of uptake of pollutants by increasing the activity of soil microorganisms to degrade the contaminants. This involves normally the activity of those organisms that live in association with the roots of higher plants. *Phytovolatilization* is the removal of substances from soil or water and hence, their release into the atmosphere. *Rhizofiltration* is the filtration of contaminated water through a mass of roots so as to remove toxic substances or surplus nutrients (Raskin and Ensley 2000).

The use of *phytoremediation* approach for the removal of environmental toxins has been greatly appreciated due to its environmental friendliness. In comparison to the conventional methods being used for cleaning up contaminated soil that damage soil structure and hamper soil fertility, *phytoextraction* can clean up the soil without causing any major change in soil quality and fertility. Another potential benefit of *phytoextraction* is that it is comparatively cost-effective as compared to any other traditional clean up method in vogue. In addition, the effectiveness of plants in the process of *phytoremediation* can be easily monitored by their growth potential under contaminated soils (Salt et al. 1995, 1997; McIntyre and Lewis 1997; Sadowsky 1999; Raskin and Ensley 2000; Schwitzguebel 2000). Despite all these advantages, the process of *phytoremediation* is criticized due to its certain limitations. For example, it can reclaim only surface soils as well as up to the depth occupied by the plant roots. As this process depends on the ability of plants to uptake and degrade/metabolize, so more time is required as compared to traditional but highly efficient methods used for cleaning of contaminated soils. In addition, with plant-based remediation systems, preventing leaching of pollutants to groundwater aquifers is not easy without the complete removal of the pollutants from the soil. The survival of the plants growing in the contaminated land is determined by the extent of toxicity of pollutants. Finally, there is always a risk of bio-accumulated contaminants in plants to enter into the food chain, from primary producers to primary consumers and upwards, and finally to humans (McIntyre and Lewis 1997; Chaudhry et al. 2002; Prasad 2004a, b; Lupino et al. 2005).

Remediation of saline soils by using highly salt tolerant plants (halophytes) has been suggested as an economical approach. Some halophytic species (e.g., those of *Atriplex*, *Suaeda, Salsola*, *Chenopodium* and *Portulaca*) could uptake salt ions through roots and metabolize or store them in the leaves through the process of *phytoextraction* (McKell 1994; Grieve and Suarez 1997). The salt uptake and accumulation by these halophytes can reduce the salt level at least at rhizospheric level, and make the soil suitable for growth of the agricultural crops with better yield (Zuccarini 2008). This approach seems to be effective because many halophytic and highly salt tolerant plant species naturally grow on highly saline soils and hence can be employed to reclaim saline soils. This approach appears to be less expensive when conventional soil reclamation and advanced biochemical and genetical modification approaches are costly. However, it should be clear that the salt tolerance ability varies greatly within species as well as within populations of the same species. In addition, it also depends on interaction of salinity stress with other environmental adversaries that limit plant growth under that set of environments (Ashraf 2004). Therefore, the successes of a particular halophyte may differ greatly under different environments that need to be explored by proper experimentation. In addition, if the *phytoremediation* potential of halophytes is aided by other conventional techniques, the amelioration processes would be more fast, effective, reliable and sustainable (Ashraf et al. 2008).

Heavy metals from contaminated soils can best be removed by *phytoextraction* or *phytoaccumulation* techniques without destroying the soil structure and fertility. In this approach, toxic metals are absorbed and accumulated into the biomass that can be easily harvested and removed from the contaminated areas (Huang and Cunningham 1996; Chaney et al. 2000; Lasat 2000). *Phytoextraction* can be achieved using natural or chelate assisted extraction of heavy metals from the contaminated soils. Continuous or natural *phytoextraction* involves the removal of metals depending on the natural ability of a particular plant species to accumulate metal contaminants without showing any significant symptoms of toxicity (Salt et al. 1995, 1997). In contrast, in chelate assisted or induced *phytoextraction*, the *phytoremediation* potential of different species is enhanced by synthetic chelates such as ethylenediaminetetraacetic acid (EDTA), *S,S*-ethylenediaminedisuccinic acid (EDDS), trisodium nitrilotriacetate (Na_3NTA), *N*-hydroxyethyl-ethylenediamine-triacetic acid (HEDTA), ethylenediamine di-(*o*-hyroxyphenylacetic acid) (EDDHA), trans-1,2-diaminocyclohexane-*N*,*N*,*N′*,*N′*-tetraacetic acid (CDTA), ethylene glycol-bis(β-aminoethyl ether), *N*,*N*,*N*′,*N*-tetraacetic acid (EGTA), and diethylenetriaminepentaacetic acid (DTPA) (Blaylock et al. 1997; Kulli et al. 1999; Kayser et al. 2000; Grcman et al. 2003; Kos and Lestan 2003). These chelates generally increase the mobility and uptake of metal contaminants by plants many-folds as compared to natural conditions. However, it must be understood that the success of *phytoextraction* technique mainly depends on the ability of a plant species to (i) extract large quantities of heavy metals into their roots, (ii) translocate the heavy metals to above-ground parts, and (ii) produce a large quantity of plant biomass (Grcman et al. 2003; Kos and Lestan 2003; Luo et al. 2004). Other factors such as growth rate, element selectivity, resistance to disease, methods of harvesting, are also important in determining the success of this technique (Baker et al. 1994; Cunningham and Ow 1996). Therefore, slow growth, shallow root system and small biomass production limit the potential of hyperaccumulator species (Brooks 1994). This technique has successfully been used for the removal of almost all known metal contaminants by various plant species.

Phytovolatilization involves the uptake of contaminants from polluted soil and their transformation into volatile compounds and their extraction into the atmosphere by transpiration. This technique is relatively less useful for removal of heavy metals as the pollutant must (i) be taken up by plants through roots, (ii) pass through the xylem to the leaves (iii) be converted into some volatilable compounds, and (iv) volatilize to the atmosphere (Mueller et al. 1999). Despite these limitations, this technique has been reported to be useful for the removal of mercury from the polluted soils by transgenic tobacco plants carrying bacterial mercury detoxification genes *merA* and *merB* (Rugh et al. 1996, 1998; Bizily et al. 1999, 2000). The genes (*merA*) encodes the enzyme mercuric ion reductase that reduces ionic mercury (Hg^{+}) to the less toxic volatile $Hg^{(0)}$ using NADPH reducing equivalents. In this process, the mercuric ion is transformed into methylmercury (CH_3Hg^{+}) and phenylmercuric acetate (PMA), that are fat-soluble and finally to metallic elemental mercury $Hg^{(0)}$ that is volatile at room temperature (Langford and Ferner 1999). In another study, plants growing on high selenium media have been shown to produce volatile selenium in the form of dimethylselenide and dimethyldiselenide (Chaney et al. 2000). However, this technique has the biggest disadvantage that most of the pollutants evaporated into the atmosphere are likely to return back to the ecosystems by precipitation (Hussein et al. 2007). Additionally, the success of this technique has a been test only for a limited scale under controlled conditions and a lot of work has to be done for determining its effectiveness for other metals as well as under field conditions.

Rhizofiltration i.e., removal of metals by passing through a mass of roots, can be used for the removal of lead, cadmium, copper, nickel, zinc and chromium, which are primarily retained with in the roots (Chaudhuri et al. 2002; United States Environmental Protection Agency Reports 2000). This technique has been tested using different crop plants such as sunflower, Indian mustard, tobacco, rye, spinach and corn, as well as tree plants such as poplar (Chaney et al. 1997; Eapen et al. 2003; Pulford and Watson 2003; Biró and Takács 2007; Lee and Yang 2009). Among these, sunflower and poplar have the greatest ability to remove metals from the contaminated environment (Prasad 2007; Zacchini et al. 2009). The greatest benefit of the *rhizofiltration* method is that it may be conducted in-situ, with plants being grown directly in the contaminated soil and water bodies. It does not involve removal and ex-situ treatment of contaminants. Therefore, it is considered as a relatively cheep procedure with low capital costs. Operational costs are also low but it depends on the type of contaminant as well as selection of plant species. Additionally, crop may be converted to biofuel, used as a substitute for fossil fuel or used in other domestic and agricultural purposes (Chaudhry et al. 2002; Rugh 2004). Despite this, the applicability of this method is very limited. First of all, the plants species selected may grow well in moderately contaminated areas but might show poor performance in highly contaminated sites. Secondly, contaminants that lie in deep soil below the rooting depth will not extracted by this method. Therefore, plants with shallow root system will not be much effective as the deep-rooted plants. Thirdly, it normally takes many years to reduce the concentration of the contaminant to regulatory levels. Fourthly, most sites are contaminated with a variety of contaminants including metals, inorganics and organics. In this case, the use of plants for removing the pollutant through *rhizofiltration* will not be sufficient and would require support of some other methods. Plants grown on polluted water and soils may become a threat to animal and human health. Therefore, a careful attention should be taken while harvesting and only non-fodder crops should be chosen for the remediation of soil and water through the *rhizofiltration* method (Cunningham and Ow 1996; Chaudhry et al. 2002).

In *bioremediation* of herbicides and pesticides, plant metabolism contributes to their removal by transformation, break down, stabilization or volatilization after uptake from soil and groundwater. Biodegradation of these chemicals is mainly carried out by both bacteria and plants. However, bacterial degradation of these chemicals is more efficient as compared to plants (Roberts et al. 1993; Allison et al. 1995; Hall et al. 2000; Hendersona et al. 2006; Liao and Xie 2008). *Bioremediation* by microbes is mostly active in the upper layer of the soil surface, where the organic matter is the source of nutrients for their activity (Navarro et al. 2004). The degradation process consists of formation of metabolites and their decomposition to inorganic and simple products that are generally harmless to living organisms (Sassman et al. 2004, Sparks 2003, Kale et al. 2001). Some fungal species such as *Phanerochaete chrysosporium* and *Phanerochaete sordida* have also been shown to actively degrade pesticides such as DDT from the contaminated soils. This extremely toxic chemical was transformed into comparatively less toxic products such as DDD and DDE (Bumpus and Aust 1987; Safferman et al. 1995). Although both these chemicals are less toxic to micro-organisms, which have the ability to metabolize and detoxify them into more simple products and their high concentration can prove extremely toxic to these organisms (Bumpus and Aust 1987; Safferman et al. 1995; Osano et al. 1999).

In addition to the role of bacteria in biodegradation of herbicides and pesticides, many plants contain certain enzymes that can break down and convert ammunition wastes, chlorinated solvents such as trichloroethylene and other herbicides to simpler and harmless molecules. The enzymes include oxygenases, dehalogenases and reductases (Black 1995). In some studies, it has bee reported that some grass species such as big bluestem, switchgrass, and yellow Indian-grass have a potential to remove pesticide residues from the contaminated soils. These species can develop a region around rhizosphere with microflora that can readily detoxify pesticide residues (Hoagland RE, Zablotowicz 1995; Marchand et al. 2002; Hendersona et al. 2006). Specific strains of atrazine-degrading bacteria have been shown to have atrazine chlorohydrolase that can enhance the rate of biotransformation of atrazine in soil. In addition, these prairie grasses were also found to reduce the rates of leaching of pesticides from soil to ground water (Hendersona et al. 2006). In another study by Coats and Anderson (1997) some members of *Kochia* sp. were found to be effective in degradation and detoxification of various chemicals such as atrazine and trifluralin. In this case, most of the degradation occurred in the rooting zone (rhizosphere), suggesting that micro-organisms residing in the rhizosphere of these plant were involved in enhanced degradation of these pesticides. Additional experimentation on members of *Kochia* sp. by the same authors have shown to be promising for the removal of pesticide from soils and groundwater (Arthur and Coats 1998). In laboratory experiments, poplar tree with fast growth potential and deep root system were found to be very successful in the removal of atrazine and arochlor from soil and groundwater. In this case, poplar plantations absorbed and metabolized these harmful compounds to less toxic chemicals (Burken and Schnoor 1996; Burken and Schnoor 1997; Nair et al. 1993).

Various plant species have the potential to remove cyanides from the polluted environments. These include hybrid willows (*Salix matssudana* Koidz x *Salix alba* L.), weeping willows (*Salix babylonica* L.), basket willows (*Salix viminalis*), poplar (*Populus deltoides*), upright hedge-parsley (*Torilis japonica*), Chinese elder (*Sambucus chinensis*), snow-pine tree (*Cedrus deodara* (Roxb.) Loud), water hyacinth (*Eichhornia crassipes*) and many other plant species (Ebbs et al. 2003; Yu et al. 2004 2005; Larsen et al. 2004; Taebi et al. 2008). However, their remediation ability varies greatly and differs with plant species, age and level of toxin in the environment. Hence, the decision whether to use a particular species for *phytoremediation* of cyanides should be carefully evaluated before any sound recommendation. In addition, it has also been shown that the removal of cyanide may also be carried out by certain species of micro-organisms through the process of biodegradation (Dubey and Holmes 1995).

As mentioned earlier, some plant species have the ability to uptake, transport and detoxify the cyanogenic compounds. The basic detoxification mechanism in tolerant species is *phytodegradation* in which the conversion of cyanides to cyanogenic glycosides is carried out by specific enzymes. This helps these plants to reduce the level of cyanide to non-toxic levels and maintain growth under cyanide polluted environment. In view of a report a small amount of cyanides can also be evaporated through *phytovolatilization* (Trapp and Christiansen 2003). This postulation was confirmed by the work of Yu et al. (2004) in which it was found that 1.5% of total cyanide fraction could be evaporated through leaves. However, they suggested that this small fraction is not sufficient enough to confirm whether the process of *phytovolatilization* is involved in the removal of cyanides from contaminated soils. Later, Larsen et al. (2004) did not find a significant relationship between evaporation and removal of cyanides by basket willows. However, they confirmed the involvement of two potential enzymes beta-cyanoalanine synthase and beta-cyanoalanine hydrolase in the ability of willow to detoxify cyanides. This evidence, although insufficient, shows that *bioremediation* of cyanides from the environments polluted can be carried out mainly by biodegradation and on a limited scale through *phytovolatilization*.

The primary solution for the remediation of soils affected with explosive chemicals is soil evacuation and ex-situ treatment by incineration or secured land-filling. However, this method is extremely cost-intensive, destructive to the environment, and not practicable by any means. In this situation, *bioremediation* is an affordable and environment-friendly method and has been evaluated using a number of bacterial strains and a few plant species. A number of fungi, yeast, bacteria and other microorganisms present in the root zone (rhizosphere) of higher plants have been shown to break down organics such as explosives, fuels and solvents (French et al. 1998; Bhadra et al. 1999; Burken et al. 2000; Hawari et al. 2000). Among plants, willow and poplar have been extensively used in the cleaning-up of soils contaminated with toxic explosives. It has been reported that hybrid poplar (*Populus deltoids* x *P. nigra*) is very effective in removal of TNT when it was grown in hydroponic solution, but it translocated only 10% of total TNT to the foliar parts (Thompson et al. 1998). In another study, clones of hybrid willow (*Salix clone* EW-20) and Norway Spruce (*Picea abies*), were found to be very effective in readily metabolizing TNT to non-toxic intermediates (Schoenmuth and Pestemer 2004).

A limiting factor for using *phytoremediation* approach of explosives is that it is a very slow and in most of the cases an incomplete process. This leads to accumulation of a variety of intermediate metabolites that can be further incorporated into the food chain and may ultimately reach humans (Dietz and Schnoor 2001; Aken 2009). Recently, a number of bacterial genes have been introduced into plants to enhance inherent limitations of plant detoxification capacities. For example, various bacterial genes encoding enzymes involved in the detoxification of explosives have been successfully introduced in plants. In this regard, the genes encoding nitroreductase and cytochrome P_{450}, have been successfully engineered in a number of plants. This has resulted in a considerable improvement in uptake, detoxification and tolerance to toxic explosives by these plant species (Cherian and Oliveira 2005; Park 2007; Aken 2009).

5 Conclusion

Although *phytoremediation* is very helpful in removing contaminants from polluted soil and water, it is absolutely not the complete answer to all contamination problems. It is a fact that once pollutants are added to the environment, they cannot be completely removed due to their ability to circulate among different environmental components and food chains. Therefore, as a first strategy, we must try to avoid or reduce the addition of pollutants to the environment. Secondly, if soil or water environment has been polluted, we must adopt in-situ and environment-friendly approach such as *bioremediation* to overcome this problem rather than ex-situ and destructive remediation methods.

The use of *phytoremediation* approach to remove contaminants has been greatly appreciated due to its environment friendliness. Perhaps, the greatest benefit of this approach is that plants are directly planted in the contaminated soils and it does not involve massive soil evacuation and ex-situ treatment for removal of contaminates. This feature greatly reduces the operational as well as capital costs incurred and renders this method less expensive than any other in-situ and ex-situ clean-up methods. In comparison to the traditional methods used for removing contaminants from contaminated soil that degrade structure of soil and reduce fertility, *phytoremediation* can clean-up the soil exclusive of bringing about any major change in soil quality and fertility. In addition, the effectiveness of plants in the process of *phytoremediation* can be easily monitored by examining their growth potential when grown in contaminated soils. Some crop products may be converted to biofuel, used as a substitute for fossil fuel or employed in other domestic and agricultural purposes.

Despite the attractiveness of *bioremediation* as environment-friendly, economical and feasible approach, it has certain limitations as its full potential is still being discovered. First of all, most plants have shallow root system and can generally grow and remediate in only top soil up to 3–4 feet. Even if we use deep-rooted plants, it can effectively remediate up to a depth of only 10 feet and thus may not be effective for the remediation of groundwater. Secondly, it requires a considerable time-period to effectively remediate a contaminated site and bring the level of contaminants to acceptable levels. It also requires a continuous monitoring of the effectiveness during this process that increases capital cost. Thirdly, in most of the *bioremediation* techniques such as *phytoextraction* and *phytostabilization*, plants uptake pollutants from soil and then transport and accumulate them to their above-ground parts such as stems or leaves. In this case, pollutants are not completely biodegraded to non-toxic compounds, but accumulate in plant tissues. This can be extremely harmful to primary (herbivores) and secondary (human) consumers. Fourthly, sometimes, it is impossible to predict the byproducts of transformation process and in this case degradation of some pollutants, such as DDT leads to accumulation of byproducts such as DDE and DDD that proved extremely toxic in most organisms. Although some microorganisms have the capacity to detoxify or metabolize them (DDE and DDD) to more simple and harmless products, their high concentrations can be toxic to them. Finally, some pollutants are extremely resistant to biodegradation and some are recalcitrant in nature. Therefore, the removal of these compounds requires superior and efficient organisms or alternative methods.

An extensive research work is required to fully understand the mechanism of bioremediation. It could be achieved through immense work in the fields of physiology, molecular biology, and biochemistry. Different species of plants and microorganisms need to be identified and carefully evaluated for their *bioremediation* potential. In addition, different genes found in micro-organisms with a potential of *bioremediation* can be identified and introduced into crop plants and trees. This would enhance the efficiency of natural hyperaccumulator species for the effective removal of environmental pollutants. Since most of the soils and water bodies are polluted with more than one type of pollutants, an integrated approach should be used to get the maximum benefits of *bioremediation*.

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