CHAPTER - 1

INTRODUCTION
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Land and water are precious natural resources on which rely the sustainability of agriculture and the civilization of mankind. Unfortunately, they have been subjected to maximum exploitation and severely degraded or polluted due to anthropogenic activities. Human evolution has led to immense scientific and technological progress. Global development, however, raises new challenges, especially in the field of environmental protection and conservation. To date unprecedented, rapid change in environmental condition is observed due to urbanization. Both industrialization and natural resource extraction resulted in the release of large amounts of toxic and waste compounds into the biosphere. Industrial practices in the past century have led to discharge of a wide range of natural and synthetic compounds over great areas of land and water. The rate at which effluents are discharged into the environment have been on rapid increase. Today whole world is facing the problem of land and groundwater contamination from improper handling and disposal of hazardous materials and wastes. Though, the hazardous wastes generated by most of the industries, has different kind of toxic substances, which are of inorganic as well as organic in nature, almost all of them are differentially toxic to the various components of biosphere including the human being (Branzini et al., 2012). They often exist as co-contaminants at various source points and thus their mitigation appear to be more critical and complex as it has been realized earlier. About 40% of the hazardous waste sites on the US Environmental Protection Agency’s (USAP) National Priority List (NPL) are co-contaminated with toxic metals and organic pollutants simultaneously (Sandrin and Hoffman, 2007). The situation is not too different in other countries including that in India, which are observing a fast pace of ill regulated industrialization.

The major pollutants of the industrial origin which are of much concern to the environmental scientists are toxic heavy metals e.g. Arsenic, Barium, Cadmium, Chromium, Lead, Mercury, Nickel and Zinc etc. Heavy metals are natural components of the Earth's crust, but in many ecosystems the concentration of several heavy metals has reached toxic levels (Abii, 2012). Fifty three elements fall into the category of heavy metal till date and defined as the group of elements whose densities are higher than 5 g cm$^{-3}$ and recognized as
ubiquitous environmental contaminants (Massa et al., 2010) Many of these elements are essential to the body in very low concentrations like Iron, Copper, Cobalt, Zinc etc. but in high concentrations these can be toxic. Some heavy metals have no essential function in the body (e.g. mercury, lead, Ni etc.) and any concentrations can be harmful.

The presence of any metal may vary from site to site, depending upon the source of individual pollutant. The primary sources of heavy metals pollution in coastal lagoons are input from rivers, sediments and atmosphere, which can affect aquaculture profitability in certain areas. Many activities lead to pollution of soil and groundwater, such as: leaching from municipal and chemical landfills; abandoned dumpsites; accidental spills of chemical or waste materials; improper chemical application of fertilizers and pesticides for agricultural and domestic vegetative processes.

Several common pesticides used fairly extensively in agriculture and horticulture in the past contained substantial concentrations of metals (Tang et al., 2012). For instance in the recent past, about 10% of the chemicals have approved for use as insecticides and fungicides in UK were based on compounds which contain Cu, Hg, Mn, Pb or Zn (Wuana et al., 2011). Sources of heavy metal contaminants in soils also include metalliferous mining and smelting, metallurgical industries, sewage sludge treatment, warfare and military training, waste disposal sites, and electronic industries (Chen et al., 2010). For example, mine tailings rich in sulphide minerals may form acid mine drainage (AMD) through reaction with atmospheric oxygen and water, and AMD contains elevated levels of metals that could be harmful to animals and plants.

It is estimated that in the United States, more than half of approximately 5.6 million dry tonnes of sewage sludge used or disposed of annually is land applied, and agricultural utilization of biosolids occurs in every region of the country. In the European community, over 30% of the sewage sludge is used as fertilizer in agriculture (Weggler et al., 2004). In Australia over 175000 tonnes of dry biosolids are produced each year by the major metropolitan authorities, and currently most biosolids applied to agricultural land are used in arable cropping situations where they can be incorporated into the soil (Wuana et al., 2011). Extensive Pb and Zn ore mining and smelting have resulted in contamination of soil that poses risk to human and ecological health (WHO 2008). Ground-transportation also causes metal contamination.
Highway traffic, maintenance, and de-icing operations generate continuous surface and ground-tread ware, brake abrasion, and corrosion are well documented heavy metal sources associated with highway traffic. Heavy metal contaminants in roadside soils originate from engine and brake pad wear (e.g. Cd, Cu, and Ni), lubricants (e.g. Cd, Cu and Zn). Industrial and military activities have led to widespread contamination of the environment, including thousands of sites, termed Superfund sites, that are severely polluted. The concentrations of the contaminants can vary from highly toxic concentrations from an accidental spill to barely detectable concentrations that, after long-term exposure, can be detrimental to human health. Industrial and commercial activities as well as the treatment and disposal of waste are reported to be the most important contamination sources.

Soil is a crucial component of rural and urban environments, and in both places land management is the key to soil quality. Heavy metal concentration in soils persists for a long time after their introduction. Soils are the major sinks for heavy metals released into the environment by anthropogenic activities and unlike organic contaminants which are oxidized to carbon (IV) oxide by microbial action, most metals do not undergo microbial or chemical degradation, and their total concentration in soils persists for a long time after their introduction. Changes in their chemical forms (speciation) and bioavailability are, however, possible. The presence of toxic metals in soil can severely inhibit the biodegradation of organic contaminants.

According to the Commission of the European Communities, 2002 in the EU an estimated 52 million ha of soil, representing more than 16% of the total land area, are affected by some kind of deterioration. Vast number of pollutants and waste materials containing heavy metals are disposed into the environment per annum. Approximately $6 \times 10^6$ chemical compounds have been synthesized, with 1,000 new chemicals being synthesized annually. Almost 60,000 to 95,000 chemicals are in commercial use. According to Third World Network reports, more than one billion pounds (450 million kilograms) of toxins are released globally in air and water. Land and water pollution by heavy metals is a worldwide issue. All countries have been affected, though the area and severity of pollution vary enormously. In Western Europe, 1,400,000 sites were affected by heavy metals (Lone et al., 2008), of which, over 30,000 sites were contaminated, and the estimated total number in Europe could be much larger, as pollution problems increasingly occurred in Central and
Eastern European countries. In USA, there are 600,000 brown fields which are contaminated with heavy metals and need reclamation (McKeehan, 2000).

According to government statistics, coal mine has contaminated more than 19,000 km of US streams and rivers from heavy metals, acid mine drainage and polluted sediments. More than 100,000 ha of cropland, 55,000 ha of pasture and 50,000 ha of forest have been lost (Ragnarsdottir and Hawkins, 2005). The problem of land pollution is also a great challenge in China, where one-sixth of total arable land has been polluted by heavy metals, and more than 40% has been degraded to varying degree due to erosion and desertification (Yizong et al., 2009). Soil and water pollution is also severe in India, Pakistan and Bangladesh, where small industrial units are pouring their untreated effluents in the surface drains, which spread over near agricultural fields. In these countries raw sewage is often used for producing vegetables near big cities.

In India alone there are about 20,000 abandoned mine sites covering about 60 different kinds of minerals (Jadia and Fulekar, 2009). The world's most polluted places threaten the health of more than 10 million people in many countries, according to a report released by a U.S. environmental action group (ENS, 2006). According to report, the Chinese city of Linfen, located in the heart of the country's coal region is as an example of the severe pollution faced by many Chinese cities; Haina, Dominican Republic, is the site of a former automobile battery recycling smelter where residents suffer from widespread lead poisoning. The Indian city of Ranipet, where some 3.5 million people are affected by tannery waste, contains hexavalent chromium and azodyes. the Russian industrial city of Norilsk, which houses the world's largest heavy metals smelting complex is where more than 4 million tons of cadmium, copper, lead, nickel, arsenic, selenium and zinc emissions are released annually; the Russian Far East towns of Dalnegorsk and Rudnaya Pristan, residents suffer from serious lead poisoning from an old smelter and the unsafe transport of lead concentrate from the local lead mining site; and in the city of Kabwe, Zambia, mining and smelting operations have led to widespread lead and cadmium contamination. Tannery runoff in India is polluting the water supply of some 3.5 million people (ENS, 2006).

Metalloid(s) of environmental concern are As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn, Tl, Sb and others. Their anthropogenic application to soils is often related to the use of residuals, like biosolids, livestock manure and compost, adversely affecting human, crop and wildlife.
health. In plants, some metals play an important role as micronutrients, being essential for growth at low concentrations. Most of them are cofactors of enzymes and are involved in important processes such as photosynthesis (Mn, Cu), DNA transcription (Zn), hydrolysis of urea into carbon dioxide and ammonia (Ni), and legume nodulation and nitrogen fixation (Co, Zn, Co) (Lin et al. 2009). Some are involved in flowering, seed production and plant growth (Cu, Zn), especially when their availability is very low. Interactions for uptake and transport may occur between metals or with macronutrients, depending on their relative concentrations. For instance, Cu reduces the uptake of Cd and Ni, whereas its uptake is inhibited by Cr, Cd, Co and Ni in some plant species (Haiyan et al., 2003; An et al., 2004). Nickel can compete with Cu, Zn and Co and, to a greater extent, with iron uptake. Lead is also an antagonist in the uptake of Fe, more than Mn and Co and can inhibit enzymes such as ureases.

Interactions between metals for uptake across cellular membranes and vacuoles and transport depend on the expression and functionality of specific transporter families shared by various metals ((Israr et al., 2011). Phytotoxicity is mainly associated with non-essential metals like As, Cd, Cr and Pb, which generally have very low toxicity thresholds and lower values for hyperaccumulation (especially for Cd) than the other metals. The above-mentioned metals, except Cr, are also not essential for humans, and may enter the food chain through ingestion of contaminated edible products at various levels, depending on the metal in question. As, Cr and Pb are not easily transferred to above-ground plant biomass, mainly being stored in root cells (Tiwari et al. 2009; Mellem et al. 2009), whereas Zn is easily accumulated in green tissues like leaves (Probst et al. 2009). High concentrations of heavy metals in soil can negatively affect crop growth, as these metals interfere with metabolic functions in plants, including physiological and biochemical processes, inhibition of photosynthesis, and respiration and degeneration of main cell organelles, even leading to death of plants (Tripathi et al., 2007).

According to Allen (1988), soil remediation is the return of soil to a condition of ecological stability together with the establishment of plant communities it supports or supported to conditions prior to disturbance. Conventional technologies involve the removal of metals from polluted soils by transportation to laboratories, soil washing with chemicals to remove metals, and finally replacing the soil at its original location or disposing of it as
hazardous waste (Monferran et al., 2012). This decontamination strategy is an ex situ approach and can be very expensive and damaging to the soil structure and ecology.

Immobilization of heavy metals through the addition of lime, phosphate and calcium carbonate (CaCO$_3$) have been suggested as remediation techniques. These remediation technologies have the advantage of immediately reducing the risk factors arising from metal contamination, but may only be considered temporary alternatives because the metals have not been removed from the soil environment. In response to a growing need to address environmental contamination, many remediation technologies have been developed to treat soil, leachate, wastewater, and ground-water contaminated by various pollutants, including in situ and ex situ methods (Aboulroos et al., 2006).

A particular contaminated site may require a combination of procedures to allow the optimum remediation for the prevailing conditions. Biological, physical, and chemical technologies may be used in conjunction with one another to reduce the contamination to a safe and acceptable level. Conventional methods to remediate metal-contaminated soils (soil flushing, solidification/ stabilization, vitrification, thermal desorption, encapsulation) (Chen et al., 2010) can be used at highly contaminated sites but are not applicable to large areas. These remediation methods require high energy input and expensive machinery. At the same time they destroy soil structure and decrease soil productivity.

Some micro-organism-based remediation techniques, such as bioremediation, show potential for their ability to degrade and detoxify certain contaminants (Weyens et al., 2009). Although these biological systems are less amenable to environmental extremes than other traditional methods, they have the perceived advantage of being more cost effective. Bioremediation is most applicable for sites that have been contaminated with organic pollutants, and as such, this condition has been the focus of the majority of bioremediation research (Prasad et al., 2010). Because heavy metals are not subject to degradation, several researchers have suggested that bioremediation has limited potential to remediate metal-polluted environments. The use of natural materials to remediate contaminated waters and soils has been investigated for the past thirty years. Scientists and engineers have been investigating the ability of live plants and inactivated biomaterials as remediation alternatives.
Over the past decade there has been increasing interest for the development of plant-based remediation technologies which have the potential to be low-cost, low-impact, and environmentally sound, a concept called phytoremediation. In phytoremediation (uptake), the roots of established plants absorb metal elements from the soil and translocate them to the above-ground shoots where they accumulate. After sufficient plant growth and metal accumulation, the above-ground portions of the plant are harvested and removed, resulting in the permanent removal of metals from the site (Meighan et al., 2011). Some researchers suggest that the incineration of harvested plant tissue dramatically reduces the volume of the material requiring disposal. In some cases valuable metals can be extracted from the metal-rich ash and serve as a source of revenue, thereby offsetting the expense of remediation.

Phytoremediation is an integrated multidisciplinary approach to the cleanup of contaminated soils, which combines the disciplines of plant physiology, soil chemistry, and soil microbiology. Phytoremediation has been applied to a number of contaminants in small-scale field and/or laboratory studies. These contaminants include heavy metals, radionuclides, chlorinated solvents, petroleum hydrocarbons, PCBs, PAHs, organophosphate insecticides, explosives, and surfactants (Rahman and Hasegawa, 2011). Certain species of higher plants can accumulate very high concentrations of metals in their tissues without showing toxicity (Wei et al., 2009). Such plants can be used successfully to clean up heavy metal polluted soils if their biomass and metal content are large enough to complete remediation within a reasonable period.

Phytoremediation has gained popularity with government agencies and industry in the past ten years. This popularity is based in part on the relatively low cost of phytoremediation, combined with the limited funds available for environmental cleanup. The costs associated with environmental remediation are staggering. Currently, $6–8 billion per year is spent for environmental cleanup in the United States, and $25–50 billion per year worldwide (Pilon-Smits et al., 2009). Because biological processes are ultimately solar-driven, phytoremediation is on average tenfold cheaper than engineering-based remediation methods. The fact that phytoremediation is usually carried out in situ contributes to its cost-effectiveness and may reduce exposure of the polluted substrate to humans, wildlife, and the environment. Phytoremediation also enjoys popularity with the general public as a "green clean" alternative to chemical plants and bulldozers. Thus, government agencies like to
include phytoremediation in their cleanup strategies to stretch available funds, corporations (e.g., electric power, oil, chemical industry) like to advertise their involvement with this environment-friendly technology, and environmental consultancy companies increasingly include phytoremediation in their package of offered technologies.

The U.S. phytoremediation market now comprises $100-150 million per year, or 0.5% of the total remediation market. For comparison, bioremediation (use of bacteria for environmental cleanup) comprises about 2% (Tsao 2003). Commercial phytoremediation involves about 80% organic and 20% inorganic pollutants. The U.S. phytoremediation market has grown two- to three fold in the past 5 years, from $30-49 million in 1999 (Pilon-Smits et al., 2009). In Europe there is no significant commercial use of phytoremediation, but this may develop in the near future because interest and funding for phytoremediation research are increasing rapidly, and many polluted sites in new European Union countries (Eastern Europe) await remediation. Phytoremediation may also become a technology of choice for remediation projects in developing countries because it is cost-efficient and easy to implement.

In general, remediation technologies, whether in place or ex situ, do one of two things: they either remove the contaminants from the substratum (“site decontamination or clean-up techniques”) or reduce the risk posed by the contaminants by reducing exposure (“site stabilization techniques”). One “gentle” plant-based site stabilization approach, suitable for heavily contaminated sites, is phytostabilization aimed to decrease soil metal bioavailability using a combination of plants and soil amendments (Vangronsveld et al., 2009). Another approach directed towards real decontamination is trace element phytoextraction, representing use of plants for trace element removal from the soil by concentrating them in the harvestable parts (Xu et al., 2009). An opinion exists that trace element phytoextraction will be more economically feasible if, in addition to metal removal, plants produce biomass with an added economical value (Wei et al., 2009).

Understanding the processes affecting pollutant bioavailability can help optimize phytoremediation efficiency. Pollutant bioavailability depends on the chemical properties of the pollutant, soil properties, environmental conditions, and biological activity (Zhao et al., 2010). Soils with small particle size (clay) hold more water than sandy soils, and have more binding sites for ions, especially cations (CEC) (Pichtel and Bradway, 2008). The
concentration of organic matter (humus) in the soil is also positively correlated with CEC, as well as with the capacity to bind hydrophobic organic pollutants. This is because humus mainly consists of dead plant material, and plant cell walls have negatively charged groups that bind cations, as well as lignin that binds hydrophobic compounds. Heavy metals are usually present as charged cations or anions, and thus are hydrophilic. The bioavailability of cations is inversely correlated with soil CEC. At lower soil pH, the bioavailability of cations generally increases due to replacement of cations on soil CEC sites by H⁺ ions (Zeng et al., 2011). The bioavailability of ions is also affected by the redox conditions. Most terrestrial soils have oxidizing conditions, and elements that can exist in different oxidation states will be in their most oxidized form [e.g., as selenate, arsenate, Cr (VI), Fe(III)]. The oxidation state of an element may affect its bioavailability (e.g., its solubility), its ability to be taken up by plants, as well as its toxicity. Other physical conditions that affect pollutant migration and bioavailability are temperature and moisture. In general higher temperatures accelerate physical, chemical, and biological processes. Precipitation will stimulate general plant growth, and higher soil moisture will increase migration of water soluble pollutants. In polluted soils the more bioavailable (fraction of) pollutants tend to decrease in concentration over time due to physical, chemical, and biological processes, leaving the less or nonbioavailable (fraction of) pollutants (Usman et al., 2012). Consequently, pollutants in aged polluted soils tend to be less bioavailable and more recalcitrant than pollutants in soil that is newly contaminated, making aged soils more difficult to phytoremediate.

Plants are sessile organisms and have only limited mechanisms for stress avoidance; they need flexible means for acclimation to changing environmental condition. In order to improve a plant’s protection, it is important to understand the mechanisms contributing to stress tolerance. A common consequence of heavy metal stress is that they result, at some stage of stress exposure, in an increased production of reactive oxygen species. Consequences of reactive oxygen species formation depend on the intensity of stress and on the physico-chemical conditions in the cell (i.e. antioxidant status, redox state and pH). These reactive oxygen species react with lipids, proteins, pigments and nucleic acids and cause lipid peroxidation, membrane damage and inactivation of enzymes, thus affecting cell viability.

The deleterious effects resulting from the cellular oxidative state may be alleviated by enzymatic and non-enzymatic antioxidant machinery of the plant that vary at various cellular
and subcellular levels in different plants. The antioxidant protection in plant cells is complex and highly compartmentalized. For long time reactive oxygen species have been considered mainly as dangerous molecules, whose levels need to be kept as low as possible. Now this opinion is changing rapidly. It has been realized that reactive oxygen species play important roles in the plants defense system against pathogens, mark certain developmental stages such as tracheary element formation, lignifications and other cross linking processes in the cell wall and act as intermediate signaling molecules to regulate the expression of genes. Because of these multiple function of activated oxygen, it is necessary for cells to control the level of reactive oxygen molecules lightly, but not eliminate them completely. It is important to note that whether reactive oxygen species reacting with a cellular molecule will act as damaging, protective or signaling factors depends on the delicate equilibrium between reactive oxygen species production and scavenging at the proper site and time. Reactive oxygen species can damage cells as well as initiate responses such as new gene expression.

Plants use a diverse array of enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT) as well as low molecular weight antioxidants to scavenge different types of reactive oxygen species, thereby protecting potential cell injury against tissue dysfunction. Superoxide dismutases (SOD) are a family of metalloenzymes catalyzing the dismutation of $O_2^-$ to $H_2O_2$ (Sharma and Dietz, 2008). The bulk of $H_2O_2$ is removed by catalases (CAT), localized in peroxisomes, and peroxidases localized in vacuoles, the cell walls and the cytosol (Mittler, 2002). A fine regulation of the $H_2O_2$ level is achieved by the enzymes and metabolites of the ascorbate glutathione cycle. Ascorbate peroxidase (APX) allows the scavenging of small amounts of $H_2O_2$ in particular parts of the cell like chloroplasts and mitochondria (Dat et al., 2000). Ascorbate and glutathione are present in plant tissues in millimolar concentrations and in stress conditions their levels increase (Noctor and Foyer, 1998). Ascorbate is the major primary antioxidant reacting directly with ROS ($OH$, $O_2^-$, and $^1O_2$), it also acts as a secondary antioxidant by reducing the oxidized form of α-tocopherol and preventing membrane damage. Glutathione is the predominant non-protein thiol, redox-buffer, phytochelatin precursor, and substrate for keeping the ascorbate in reduced form in the ascorbate-glutathione pathway (Gill et al., 2010).
Plant stress tolerance may therefore be improved by the enhancement of in vivo levels of antioxidant enzymes. The antioxidant enzyme are found in almost all cellular compartments and hence demonstrates the importance of reactive oxygen species detoxification for cellular survival under heavy metal stress. Therefore, the present investigation was undertaken to develop efficient and cost effective phytoremediation system for remediation of heavy metals and hence present objectives are proposed in the light of above perspectives.

Thus the present study has been carried out with the following objectives:

1. Periodical Monitoring of toxic heavy metals and other co-pollutants at the source point.
2. Studies on natural populations of plants dominating the contaminated site.
3. Bioprospecting of plants at the industrial site and studies on phytoremediation efficiency of the selected plants.
4. Studies on interaction of various co-pollutants and their effect on phytoremediational potential of selected plants.
5. Developing a novel phytoremediation system of major toxicants using suitable plants.