Almas Zaidi · Parvaze Ahmad Wani
Mohammad Saghir Khan *Editors*

Toxicity of Heavy Metals to Legumes and Bioremediation

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Editors
Almas Zaidi
Aligarh Muslim University
Fac. Agricultural Sciences
Dept. Agricultural Microbiology
Aligarh
India

Parvaze Ahmad Wani
Crescent University
College of Natural and Applied Sciences
Department of Biological Sciences
Abeokuta, Ogun
Nigeria

Mohammad Saghir Khan
Aligarh Muslim University
Fac. Agricultural Sciences
Dept. Agricultural Microbiology
Aligarh
India

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Preface

Rapid industrial operations and constantly dwindling fresh irrigation water sources have resulted in the increased use of industrial or municipal wastewater in agricultural practices, which quite often adds considerable amounts of heavy metals to soil. And therefore, metal concentrations sometimes present in soils commonly go beyond the threshold level, which after uptake by soil microbes including nodule bacteria, rhizobia, and plants such as legumes cause severe toxicity to both microbes and plants. In addition, heavy metals via food chain may cause human health problems also. Maintaining good soil quality is therefore of major practical importance for sustainable agronomic production. Contamination of agronomic soils with heavy metals and their consequent deleterious effects on the production systems have, therefore, received greater attention globally by the environmentalists.

Among crops, legumes, which are grown largely in tropical and semiarid tropical regions, serve as a rich source of protein and provide a significant amount of nitrogen to soils. In addition, legumes are known to improve soil qualities, like organic matter, soil structure and porosity, fertility, microbial structure and composition, etc. In order to promote legume growth in varied ecosystems, microbes forming symbiosis with legumes and collectively called “rhizobia” are applied as inoculant to reduce dependence on chemical fertilizers frequently used in crop including legume production. Besides rhizobia, several other soil-inhabiting microbes possessing plant growth-promoting qualities, generally called as plant growth-promoting rhizobacteria (PGPR), have also been used and practiced as sole bioinoculant or as mixture with host-specific rhizobia for increasing the crop yields. These multipurpose organisms therefore broadly provide a practicable and low-cost substitute to compensate for alarmingly used synthetic chemical fertilizers in high-input agricultural practices in different production systems around the world for enhancing the quantity and seed quality of several crops including legumes. However, reports on the obvious toxicity of heavy metals to legumes and associated microflora and how such toxicities could be reduced/prevented employing inexpensive naturally abundant microbes are poorly documented. To circumvent the metal toxicity problems, several traditional physical and chemical methods have been applied, which, however, have not reached to optimum success level due to various socioeconomic or technical reasons. To overcome such barriers, there is therefore an urgent need to find an inexpensive and easily acceptable technology for metal cleanup from contaminated sites. In this context, both rhizobia and legumes have been found to play important roles in restoring the metal-contaminated soils and subsequently in enhancing legume production in polluted environment. Considering on the one hand the importance of *Rhizobium*–legume interactions in maintaining soil fertility and metal toxicity to symbiotic relationships and the role of PGPR in metal detoxification on the other, grave efforts have been made to compile such demanding research in a single volume.

Toxicity of heavy metals to legumes and bioremediation presents numerous aspects of metal toxicity to legumes and suggests quite a few bioremediation strategies that could be useful in restoring contaminated environments vis-a-vis legume production in metal-stressed soils. The mobility and availability of toxic metals, nutritive value of some metals, and the strategies to assess the human health risk by heavy metals are reviewed and highlighted. Heavy metal toxicity to symbiotic nitrogen fixing microorganism and host legumes is dealt separately. A focused insight into the possible effects of heavy metals on seed germination and important physiological functions of plants including popularly grown legumes around the world have been amply reviewed and discussed in this book. The interaction between chromium and plant growth-promoting rhizobacteria and how chromium toxicity could be managed are explored. The influence of glutathione on the tolerance of *Rhizobium leguminosarum* to cadmium is covered in detail. The book further describes in a separate chapter, “Bioremediation: A natural method for the management of polluted environment,” several bioremediation strategies commonly used in cleaning up the heavy metal-contaminated sites. “*Rhizobium*–legume symbiosis: A model system for the recovery of metal contaminated agricultural land” has been sufficiently discussed in this book. Microbially mediated transformations of heavy metals in rhizosphere are critically addressed. “Rhizoremediation: A pragmatic approach for remediation of heavy metal contaminated soil” is reviewed and highlighted. Plant growth-promoting rhizobacteria facilitate the growth and development of various plants in both conventional and stressed soils by one or combination of several mechanisms. This interesting aspect of PGPR in the management of cadmium-contaminated soil is dealt separately. The importance of mycorrhizal fungi in enhancing legume production in both conventional and derelict environment and site-specific optimization of arbuscular mycorrhizal fungi-mediated phytoremediation have been reviewed and discussed. Further in this book, heavy metal resistance in plants and putative role of endophytic bacteria are highlighted.

We indeed enjoy sharing especially with legume growers some of the most exciting developments in bioremediation and legume production in stressed environment and presenting this book as a key point of reference for everyone involved in research and development of legumes around the world. The data and methodologies described in this book are likely to underpin the development of sustainable legume production and serve as an important and rationalized source material. In addition, a broad perspective toward an issue of concern to researchers, students, professionals, policymakers, and practitioners in legume production in contaminated soil with minimum resources is highlighted. It would also serve as a valuable resource for agronomists, environmentalists, soil microbiologists, soil scientists, biologists, and biotechnologists involved in the management of contaminated lands.

We are very grateful to our expert colleagues for providing their vital, reliable, and progressive information to construct this book. Chapters in this book are well explained with suitable tables and pictures, and contain most recent literature. We are undeniably very thankful to our family members for their constant and unrelenting support during the whole period of book preparation. And most of all, we are extremely thankful to our lovely children Zainab and Butool for helping us to avoid some tense moment during book preparation by their joyful activities. We are also very pleased with the book publishing team at Springer-Verlag, Austria, who always provided us their unconditional support in replying to all our queries very quickly. Finally, there may be a few basic errors/inaccuracies or printing mistakes in this book, for which we feel sorry in anticipation. However, if such mistakes are brought to our notice at any stage, we will certainly try to correct and improve them in subsequent print/edition. Any suggestion or decisive analysis of the contents presented in this book by the readers is welcome.

Aligarh, India Almas Zaidi, Mohammad Saghir Khan
Abeokuta, Nigeria P. A. Wani

Contents

Contributors

Ees Ahmad Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India, eesahmad@gmail.com

Lima Ana Centre for Cell Biology, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

Rabindranath Bhattacharyya Department of Botany, Presidency College, Kolkata 700 073, West Bengal, India, rabindranathbpc@yahoo.co.in

Figueira Etelvina Centre for Cell Biology, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal, efigueira@ua.pt

Velmurugan Ganesan Biocentre, University of Cologne, Cologne 50674, Germany; Department of Molecular Biology, School of Biological Sciences, Madurai Kamaraj University, Madurai 625021, India, oomvel@gmail.com

Mohammad Saghir Khan Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India, khanms17@rediffmail.com

Natalia Kozyrovska Institute of Molecular Biology and Genetics of National Academy of Sciences, Kyiv, Ukraine

Ashok Kumar Department of Biotechnology, Himachal Institute of Life Sciences, Rampur Ghat Road, Paonta Sahib 173025, Himachal Pradesh, India, asokumr@gmail.com

Ewa Kurek Department of Environmental Microbiology, Institute of Microbiology and Biotechnology, University of Maria Curie-Skłodowska, 19 Akademicka St., 20-033 Lublin, Poland, e.kurek@poczta.umcs.lublin.pl

Małgorzata Majewska Department of Environmental Microbiology, Institute of Microbiology and Biotechnology, University of Maria Curie-Skłodowska, 19 Akademicka St., 20-033 Lublin, Poland

Santi M. Mandal Central Research Facility, Indian Institute of Technology, Kharagpur, West Bengal 721 302, India

Diriba Muleta College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia, dmuleta@gmail.com

Mohammad Oves Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India, owais.micro@gmail.com

Corticeiro Sofia Centre for Cell Biology, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

Pereira Sofia Centre for Cell Biology, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

Tünde Takács Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, 15. Herman O. Street, Budapest, Hungary, takacs@rissac.hu

Parvaze Ahmad Wani Department of Biological Sciences, College of Natural and Applied Sciences, Crescent University, Abeokuta, Ogun state, Nigeria, wani1889@rediffmail.com; waniparvaiz1889@gmail.com

Delelegn Woyessa Department of Biology, Jimma University, P.O.Box 307, Jimma, Ethiopia

Iryna Zaets Institute of Molecular Biology and Genetics of National Academy of Sciences, Kyiv, Ukraine, zkora@ukr.net

Almas Zaidi Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, UP, India, alma29@rediffmail.com

Soil Contamination, Nutritive Value, and Human Health Risk Assessment of Heavy Metals: An Overview

1

Mohammad Oves, Mohammad Saghir Khan, Almas Zaidi,
and Ees Ahmad

Abstract

Globally, rapidly increasing industrialization and urbanization have resulted in the accumulation of higher concentrations of heavy metals in soils. The highly contaminated soil has therefore become unsuitable for cultivation probably because of the deleterious metal effects on the fertility of soils among various other soil characteristics. In addition, the uptake of heavy metals by agronomic crops and later on consumption of contaminated agri-foods have caused a serious threat to vulnerable human health. Considering these, a genuine attempt is made to address various aspects of metal contamination of soils. In addition, the nutritive value of some metals for bacteria and plants is briefly discussed. Here, we have also tried to understand how heavy metals risk to human health could be identified. These pertinent and highly demanding discussions are likely help to strategize the management options by policy makers/public for metal toxicity caused to various agro-ecosystems and for human health program.

1.1 Introduction

The rapid industrial operations and consistently declining fresh irrigation water sources have led to the increase in use of industrial or municipal waste water in agricultural practices probably due to its (1) easy availability, (2) scarcity of fresh water, and (3) disposal problems. Even though sewage when applied provides water and valuable plant nutrients, it contributes sufficient amounts of heavy metals (HMs) to agricultural soils (Chen et al. 2005; Maldonado 2008; Zhang et al. 2008). In addition, heavy metals have been used over the years as building materials, in

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M. Oves (✉)
Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh 202002,
Uttar Pradesh, India
e-mail: owais.micro@gmail.com

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pigments for glazing ceramics, and pipes for transporting water, in batteries and other electronic products, and in painting (Horowitz 2009; Callender and Rice 2000). After discharge without proper care from various industrial sources or fertilizer application, HM accumulates in soils. Metal concentrations found in contaminated soils frequently exceed those required as nutrients or background levels, resulting in uptake by plants and deposition to unacceptable levels. When the level of HM goes beyond the permissible limits, they affect adversely the growth of beneficial soil microflora including nodule bacteria, rhizobia (Tyler 1993; McGrath et al. 1995; Paudyal et al. 2007). Furthermore, through food chain, HM cause problems to living organisms including microbes, plants, and humans/animals (Akoumianakis et al. 2009; Fu et al. 2009; Salvatore 2009; Zhang et al. 2008), as presented in Fig. 1.1. However, some of these metals which even may be there in foods such as iron and copper are essential as they affect many important biological systems. These elements can on the other hand be toxic for living organisms if their concentration is excessively high in the body. Other elements like mercury, arsenic, lead, and cadmium are not important; rather, they are toxic, even at fairly low concentration (Celik and Oehlenschlager 2007; Zarei et al. 2010). Despite these conflicting properties, metals in general have a unique ability to move and accumulate in various systems including precious but variable food chains over a period of time. The consistent and unchecked accumulations of

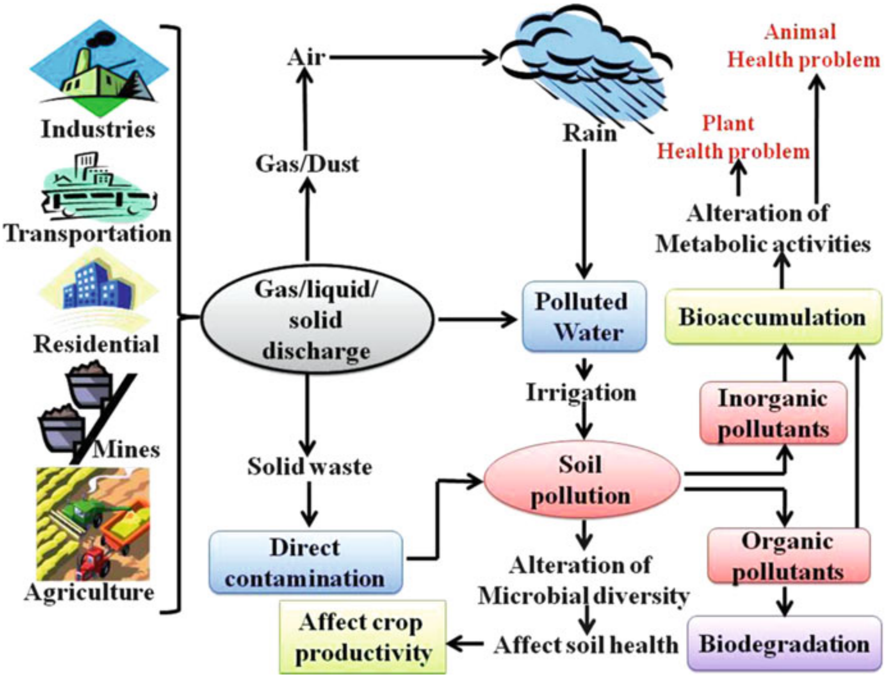

Fig. 1.1 Heavy metal contamination and its toxic effects on microbes, plants and animals

metals in the food chain damage different ecological niches and therefore pose a major threat to human health (Mishra et al. 2007; Efendioglu et al. 2007). For example, the consequence of certain metals has been reflected in the form of cancer, nervous system damage, and other diseases in humans (Zwieg et al. 1999).

1.2 Source of Heavy Metal in Soils

Heavy metal generally refers to metals and metalloids having densities greater than 5 g cm^{-3}. Heavy metals in soils may be found naturally or results from anthropogenic activities (Fig. 1.2). Natural sources include the atmospheric emissions from volcanoes, the transport of continental dusts, and the weathering of metal-enriched rocks (Ernst 1998). The other major source of contamination is anthropogenic origin: the exploitation of mines and smelters; the application of metal-based pesticides and metal-enriched sewage sludges in agriculture; the combustion of fossil fuel, metallurgical industries, and electronics (manufacture, use, and disposal); the military training, etc. (Alloway 1995).

According to Ross (1994), the anthropogenic sources of metal contamination can be divided into five major groups: (1) metalliferous mining and smelting (e.g., arsenic, cadmium, lead, mercury), (2) industry (e.g., arsenic, cadmium, chromium, cobalt, copper, mercury, nickel, zinc), (3) atmospheric deposition

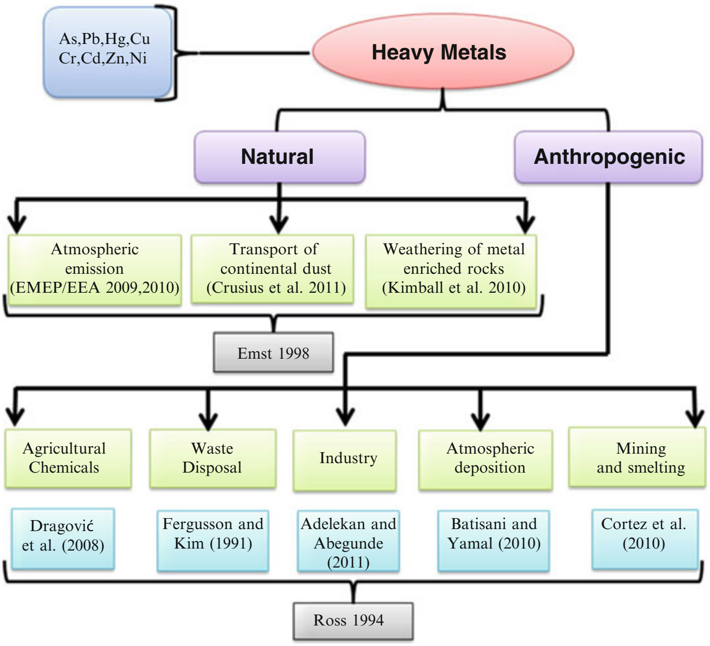

Fig. 1.2 Origin of soil contamination by heavy metals

(arsenic, cadmium, chromium, copper, lead, mercury, uranium), (4) agriculture (e.g., arsenic, cadmium, copper, lead, selenium, uranium, zinc), and (5) waste disposal (e.g., arsenic, cadmium, chromium, copper, lead, mercury, zinc). The use of intensive farm management practices, like application of phosphatic fertilizers (Mortvedt 1996; Nicholson et al. 2003), sewage sludge input, and pesticide treatments, are also the cause of soil pollution. Considering all source of origin, it is estimated that the annual worldwide release of heavy metals is about 22,000 tons (metric ton) for cadmium, 939,000 tons for copper, 783,000 tons for lead, and 1,350,000 tons for zinc (Singh et al. 2003). In 2009 alone, the total annual ferrochromium and chromite world production was 7,000,000 tons and 19,300,000 tons, respectively (USGS 2009).

Other source of soil pollution includes the emission of metals from heavy traffic on roads which may contain lead, cadmium, zinc, and nickel and are found in fuel as antiknock agents (Suzuki et al. 2008; Atayese et al. 2009). The deposition of vehicle-derived metal and the relocation of metals deposited on road surface by air and runoff water have led to contamination of soils (Nabuloa et al. 2006; Ogbonna and Okezie 2011). Road dust originating possibly from the emissions of electric arc furnace dust (EAFD) is reported to contain high concentrations of metals like Fe, Zn, Pb, and Cr (Sammut et al. 2006; Fernández-Olmo et al. 2007; Geagea et al. 2007). The serious wear and tear of tires and brake linings may also produce high concentrations of Fe, Zn, Cu, Cr, and Ni (Li et al. 2001; Adachi and Tainosho 2004; Iijima et al. 2007). The fly ash of coal-fired power plants contains metals like Fe, Ni, Cr, Cu, Zn, and Pb (Reddy et al. 2005; Gómez et al. 2007). Cadmium may be added to soil from sources like Cd-made compounds when used as stabilizers in PVC products, color pigment, several alloys, and also in rechargeable nickel–cadmium batteries. Industrial wastewater is yet other major metal contamination source of soils (Bergbäck et al. 2001; Sörme and Lagerkvist 2002). Contamination of soil may also result from dispersal and discharge of metals from various other sources. Such dispersal includes gas–dust release into the atmosphere from different technological processes requiring high temperature like power plants; metal smelting; burning of raw materials for cement, etc.; waste incineration; and fuel combustion. Another route of metal entry into soil is motor transport, which is widely connected with the use of lead as an additive to gasoline. Heavy metals in pristine river catchments originate from natural sources and processes as chemical weathering, soil erosion, fallout of natural aerosols from marine, and volcanic or arid soils sources (Avila et al. 1998; Gaillardet et al. 2003).

Contamination of agronomic soils with heavy metals and their adverse impact on the agro-ecosystems are therefore currently the focus of attention by the environmentalists around the world. This is because soil is an active and dynamic system where many chemical, physical, and biological activities are going on constantly. The massive interaction among living and nonliving components of soil determines the nutrient pool (fertility) of soil. Maintenance of good soil quality is therefore of prime importance for sustainable agriculture. However, the nutrient status of soil changes with time, prevailing conditions of climate and plant cover, and microbial composition of soil (Ademorati 1996). In addition, when some stressors such as HM, temperature, extreme pH, or chemical pollution are imposed on a natural environment, soil biota can be affected, and whole ecological processes mediated by them are disturbed (MacGrath 1994; McGrath et al. 2005). Moreover, every 1,000 kg of "normal soil" contains 200 g chromium, 80 g nickel, 16 g lead, 0.5 g mercury, and 0.2 g cadmium, theoretically (IOCC 1996). Assessment of metal status in soils corresponding to pollution level is therefore of great practical interest due to their variable impact on different forms of water (groundwater and surface water) (Clemente et al. 2008; Boukhalfa 2007), microbial communities (Wani and Khan 2011), plant genotypes (Pandey and Pandey 2008; Stobrawa and Lorenc-Plucińska 2008), and animals and humans (Lagisz and Laskowski 2008; Korashy and El-Kadi 2008).

1.3 Metal Bioavailability

The total contents of metal present in soil do not provide any information regarding the availability and mobility of metals, while the assessment of metal availability is more important because it helps to better understand the specific bioavailability, reactivity, mobility, and uptake by plants (McBride 1994; Luo and Christie 1998). Based on the data available, metals present within soil have been categorized into five major geochemical forms as (1) exchangeable, (2) bound to carbonate phase, (3) bound to iron and manganese oxides, (4) bound to organic matter, and (5) residual metal. Metals found in any of these forms vary greatly in mobility, biological availability, and chemical behavior in soil probably because they react differently to various organic compounds such as low-molecular organic acids, carbohydrates, and enzymes secreted by microorganisms inhabiting soil (Huang et al. 2002). Also, the soil bacteria have charged surfaces which interact very strongly with metal ions in soil solution. They could absorb a greater amount of heavy metals than inorganic soil components such as montmorillonite, kaolinite, or vermiculite (Ledin et al. 1996). Bacterial cells have an extremely high capacity of adsorbing and immobilizing toxic ions from soil solution (Beveridge et al. 1995). In this context, Huang et al. (2000), for example, reported that symbiotic bacteria such as rhizobia when used as inoculant significantly increased the adsorption of Cu and Cd in soil. The mechanisms and adsorption kinetics are still poorly understood, regarding how bacteria affect the speciation and distribution of heavy metals in soils, especially under field conditions. Numerous methods like sequential extraction, single extraction, and soil column leaching experiments have been used to determine the possible chemical associations of metals in soils and to assess mobility and bioavailability of metals (Li and Thornton 2001; Cukrowska et al. 2004). Of the various methods employed, single extraction method which involves the use of a selective chemical extractant such as a chelating agent or a mild neutral salt (Ure 1996) is frequently used to indicate the bioavailability or mobility of heavy metals in a short or moderate term. The consequential extraction could provide valuable information for predicting metal availability to plants, metal movement in the soil profile, and transformation between different forms in soils in a long term (McGrath and Cegarra 1992). Batch or column leaching experiment has also been used (Anderson et al. 2000) as a tool to assess the metal mobility in soil, sediment, and slag. This method can be applied to assess metal mobility and bioavailability that closely simulates the practical conditions (Cukrowska et al. 2004). Generally, the factors that affect the bioavailability and accumulation of heavy metals in soil/plants include (a) soil type, which includes soil pH, organic matter content, clay mineral, and other soil chemical and biochemical properties; (b) crop species or cultivars; (c) soil–plant–microbes interaction, which plays an important role in regulating heavy metal movement from soil to the edible parts of crops; and (d) agronomic practices such as fertilizer application, water managements, and crop rotation system. These factors together influence the thresholds for assessing dietary toxicity of heavy metals in the food chain, as reviewed by Islam et al. (2007).

1.4 Heavy Metal as Nutrient: An Overview

With ever increasing human populations, there is a continuous pressure on agricultural systems to produce more and more foods to fulfill the human food demands. To address these problems, well-directed and concerted efforts are required to efficiently use the full potential of agro-ecosystems. However, in agricultural practices, both major like nitrogen (N), phosphorus (P), and potassium (K) and minor nutrients play important roles in crop improvement. Apart from the major nutrients, the deficiency of micronutrients (which are typically present at <100 mg kg^{-1} dry weight) also limits the crop production severely in many production systems (Aghili et al. 2009). Some of the micronutrients essentially required for various metabolic activities of plants including legumes are copper, iron, manganese, and zinc. Even though these elements are required in smaller quantities by majority of plants, agricultural soils are usually deficient in one or more of these micronutrients. And hence, the concentration of these nutrient elements in plant tissues falls generally below the optimum levels. The minor elements, also called trace elements or other metalloids, play important roles in the functioning of living organisms and could participate in (1) forming structure of proteins and pigment, (2) redox processes, (3) regulation of the osmotic pressure, (4) maintaining the ionic balance, and (5) acting as enzyme component of the cells (Kosolapov et al. 2004). Among these elements, aluminum, cobalt, selenium, and silicon, for example, are known to promote plant growth and may be essential for particular taxa (Pilon-Smits et al. 2009). Also, some of these beneficial elements have been reported to enhance resistance to biotic stresses such as pathogens and herbivory and to abiotic stresses such as drought, salinity, and nutrient toxicity (Pilon-Smits et al. 2009). Similarly, zinc plays a vital role in the division and expansion of cells, protein synthesis, and also in carbohydrate, nucleic acid, and lipid metabolism (Collins 1981). On the other hand, when the concentrations of such trace elements rise above the normal threshold level, zinc, for example, inhibits the growth of both microbial communities (Wani and Khan 2011) and plants, for example, pea (Stoyanova and Doncheva 2002) and peanuts (Davis and Parker 1993; Davis et al. 1995).

The uptake of such elements differs from organisms to organisms (Beal et al. 2009) which, however, could be enhanced by increasing microbial biomass (Odokuma and Akponah 2010). The concentration of these trace elements also varies from soil to soil or region to region. For instance, the surveys conducted to determine the nutrient status of agricultural soils in China and India have revealed that zinc is the most common deficient micronutrient in soil. The levels of nutrient deficiencies in Chinese soils were (%) Zn 51, Mo 47, B 35, Mn 21, Cu 7, and Fe 5 (Zou et al. 2008), while in Indian soils, it were Zn 49, 33 B, 12 Fe, 11 Mo, 5 Mn, and 3 Cu (Singh 2008). Therefore, the understanding of the nutrient pool of soils and consequential impact of these elements both on microbes and plants are critical for improving the crop production and plant nutritional value for alarmingly increasing world populations.

1.4.1 Heavy Metals Importance in Microorganisms

Metals discharged from various sources followed by their deposition into soils and uptake by microbial communities affect directly and/or indirectly various stages of microbial growth, metabolism, and differentiation. The interaction of metals and their compounds with microbes, however, depends on the type of metal species, interacting organisms and their habitat, structural and biochemical compositions, and functional ability of the microbes (Khan et al. 2009a). These factors together influence the solubility, mobility, bioavailability, and toxicity of variously distributed metals in different locations (Gadd 2005, 2007). Some of the metals like copper, zinc, cobalt, and iron are essential for the sustenance but can exhibit toxicity when present above certain threshold concentrations probably because they form a complex with protein molecule which renders them inactive, for example, enzyme inactivation. On the other hand, some metals such as aluminum, cadmium, mercury, and lead, even though have no known important biological functions, could accumulate within cells and lead to variation in enzyme specificity, disrupt cellular functions, damage the DNA structure, and finally may result in cell death.

Nickel among metals, for example, is an essential nutrient and plays important roles in various cellular processes of microbes. Many microbes have the ability to locate nickel and absorb this element employing permeases or ATP-binding cassette-type transport systems. Once inside the cell, nickel is incorporated into several microbial enzymes like acetyl CoA decarbonylase/synthase, urease, aci-reductone dioxygenase, methylenediurease, NiFe hydrogenase, carbon monoxide dehydrogenase, methyl coenzyme M reductase, certain superoxide dismutases, and some glyoxylases (Hausinger 2003). At higher concentrations, nickel is, however, toxic to bacteria. To cope with such situation, bacteria have evolved certain strategies to regulate the levels of intracellular nickel as observed in two Gram-negative bacteria: *Escherichia coli* and *Helicobacter pylori* (Eitinger and Mandrand-Berthelot 2000; Mulrooney and Hausinger 2003). *Bradyrhizobium japonicum* HypB purified from an overproducing strain of *Escherichia coli* has been shown to bind up to 18 nickel ions per dimer and also to contain GTPase activity (Fu et al. 1995). Another metal such as copper (a modern bioelement) exists in Cu^{2+} and Cu^{+} forms and is considered one of the most important cofactor for various enzymes of higher organisms (Karlin 1993). In bacteria, washed cell suspensions of *Thiobacillus ferrooxidans* reduced Cu(II) to Cu(I) in the presence of S as a potential electron donor (Sugio et al. 1990); Cu(II) could be reduced under both aerobic and anaerobic conditions. However, only net reduction occurs under aerobic conditions when azide or cyanide is added to prevent the iron oxidase from oxidizing Cu(I). Copper reduction by *T. ferrooxidans* may play a role in copper leaching (Sugio et al. 1990). Similarly, under iron-deficient environment, plant growth-promoting rhizobacteria in general produce siderophores, a ferric iron-specific ligand, which are reported to increase plant growth by accelerating the access of iron within rhizospheric environment. For example, strains of *Rhizobium ciceri* able to form symbiosis specifically with chickpea (*Cicer arietinum* L.) produced phenolate-type siderophores such as salicylic acid and 2,3-dihydroxybenzoic acid. Although these compounds are produced in response to iron deficiency, nutritive components of the culture medium significantly affected their production. It seems that Cu(II), Mo (VI), and Mn(II) ions bound competitively with iron to siderophores, resulting in a 34–100% increase in production (Berraho et al. 1997).

There are certain metals which are also required during *Rhizobium*–legume symbiotic process. For example, cobalt is one such biologically essential microelement with a broad range of physiological and biochemical functions (Williams 2001; Balogh et al. 2003). Nevertheless, it becomes deleterious for many organisms when present at higher rates (Nies 1999). However, cobalt has been found associated with variable enzymatic activities in many organisms (Antonyuk et al. 2001; Kamnev et al. 2004) and can be located in magnetosomes (Vainshtein et al. 2002). Cobalt occurs mainly in the cofactor B12. Moreover, nitrile hydratase, a new class of cobalt-containing enzymes, has also been identified by Kobayashi and Shimizu (1998). For symbiotic association, cobalt is required for N_2 fixation in legumes and in root nodules of nonlegumes. Interestingly, the demand for cobalt is extremely greater for N_2 fixation than for ammonium nutrition. And if there is any deficiency, cobalt results in N deficiency symptoms. Therefore, whenever cobalt is applied, it has been observed to increase the formation of leghemoglobin, an essential component of N_2 fixation, and hence, it enhances the nodule numbers per plant and ultimately pod yield of legumes, for example, groundnut (Yadav and Khanna 1988). Among the various cobalamine-dependent enzyme systems of rhizobia involved in nodulation and N_2 fixation are methionine synthase, ribonucleotide reductase, and methylmalonyl coenzyme A mutase (Das 2000). The mixture of *Rhizobium* and cobalt has therefore been reported to significantly affect the total uptake of N, P, K, and Co by groundnut, when analyzed at harvest (Basu et al. 2006). Similarly, molybdenum forms the catalytic center of numerous enzymes which on the basis of cofactor composition and catalytic function have been grouped into two categories: (1) bacterial nitrogenases containing an FeMo-co in the active site and (2) pterin-based molybdenum enzymes. The second category enzyme includes sulfite oxidase, xanthine oxidase, and dimethyl sulfoxide reductase (DMSOR), each of which has distinct activities. Nitrate reductases, for example, have been reported in *Desulfovibrio desulfuricans* (Moura et al. 2007) while aldehyde dehydrogenase in *D. gigas* (Moura and Barata 1994; Rebelo et al. 2000; Moura et al. 2004).

1.4.2 Some Examples of Metals Important for Plant Health

Generally, plant remains healthy as long as there is continuous supply of nutrients to them. However, whenever there is shortage of a nutrient, it results in symptoms of deficiency and, at very low supply, in early mortality. In contrast, the excess of any nutrient may cause injury and, at high levels, even death of plants. Plants require on the one hand the excess amounts of certain elements called as macronutrients: C, H, N, O_2, P, S, etc.; in addition, they also require chemical elements which are necessary in small amounts and are called micronutrients. These include B and Cl, and the metals Cu, Fe, Mn, Mo, Ni, and Zn. The nutrients belonging to both categories are found in varied agro-ecological niche. A few plants living in symbiosis with nitrogen-fixing microorganisms also require Co as nutrient. However, so far, metal as nutrient is concerned; there are two criteria which are used to define a metal as essential for plant health: (1) it is required by the plants to complete its life cycle, and (2) it is part of a molecule of an essential plant constituent or metabolite. Since the plants are autotrophs and use light energy during photosynthesis to convert H_2O and CO_2 into energy-rich carbohydrates and O_2, the growth and development of plants in general depend exclusively on photosynthesis, which, in turn, is dependent on a sufficient supply of numerous chemical elements, including metals like Cu, Fe, and Mn. Heavy metals and metalloids can enter plants via uptake systems including different metal transporters (Eide 2004; Perfus-Barbeoch et al. 2002). However, if there is any deficiency of metal, plants increase the metal availability in the root environment by lowering the pH through root exudates which may contain organic acids, or through release of metal-complexing agents. After the proper and sufficient supply is maintained, a signal from the shoot to the root stops the exudation process. Once they enter the plant systems, some metals when present at lower rates have been found to affect plant growth by participating in redox reaction and sometimes directly becoming an integral part of enzymes (Baker and Walker 1989). For example, zinc is required to maintain the integrity of ribosome, is needed in the formation of carbohydrates, catalyzes the oxidation processes in plants, and plays important role in the synthesis of macromolecules (Alloway 2009; Pandey et al. 2006). Similarly, manganese plays an important role in reactions of enzymes like malic dehydrogenase and oxalosuccinic decarboxylase. It is also needed for water splitting at photosystem II and for superoxide disumutase. In plants, cobalt complex is found in the form of vitamin B_{12} while iron is an essential element in many metabolic processes and is indispensable for all organisms.

1.5 Heavy Metal Toxicity: A Brief Account

1.5.1 Effects of Heavy Metals on Microbial Diversity

Changes in microbial community structure in response to metals are considered an important indicator of the biological availability and activity of metals within soil ecosystem. In this regard, heavy metals such as Cd, Pb, and Cd/Pb mix using the $CdSO_4$ and $Pb(NO_3)_2$ solutions at different application rates have been found to exhibit toxicological effects on soil microbes which led to the decrease in their numbers, and enzyme activities like acid phosphatase (ACP) and urease (URE). Frostegard et al. (1993) also reported a gradual change in microbial community structure which was based on variation in phospholipids' fatty acid profiles, when organisms were analyzed from metal-contaminated soils. However, the response of microbial communities to various metals varies with solubility and consequently the bioavailability and toxicity of metals in soil which in effect are influenced greatly by sorption, precipitation, and complexation ability of soils (van Beelen and Doelman 1997; Oste et al. 2001). Moreover, the interaction of metals with soil depends strongly upon physicochemical properties of soil, which may differ among various agro-climatic regions of the world. One of the first observations of metal toxicity to soil microorganisms in the Woburn Market Garden experiment was a strong decrease in the amount of soil microbial biomass (Brookes and McGrath 1984). Later on, this type of study was confirmed by Barajas-Aceves (2005) who suggested that the decrease in the total amount of biomass was due to decrease in the substrate utilization efficiency of microbes when subjected to metal stress (Chander and Joergensen 2001; Chander et al. 2002). The reduction in microbial biomass is considered as an indicator of metal pollution, but its suitability in environmental monitoring as an indicator of soil pollution is restricted because of its high spatial variability (Broos et al. 2007) and shortcomings in its measurement (Dalal and Henry 1986). Decline in the amount of microbial biomass has also been found associated with changes in community structure (Abaye et al. 2005; Khan et al. 2010) and often to increased metal tolerance, even with small amounts of metal contamination (Witter et al. 2000). The resulting effects of metal toxicity on different microbial communities inhabiting varied agro-ecosystems may be due to changes in the metal-sensitive ability of populations or community. However, no distinct threshold for metal toxicity is reported, but such thresholds may be site specific as observed by Bunemann et al. (2006).

1.5.2 Heavy Metals–Plants Interactions

Heavy metals at higher concentrations cause severe damage to the various metabolic activities leading consequently to the death of plants including those of legumes, for example, green gram (Fig. 1.3A), pea (Fig. 1.3B), and chickpea (Fig. 1.3C). However, some plant species possess the ability to survive in soils even contaminated heavily with metals (Kneer and Zenk 1992). Metal at exceedingly

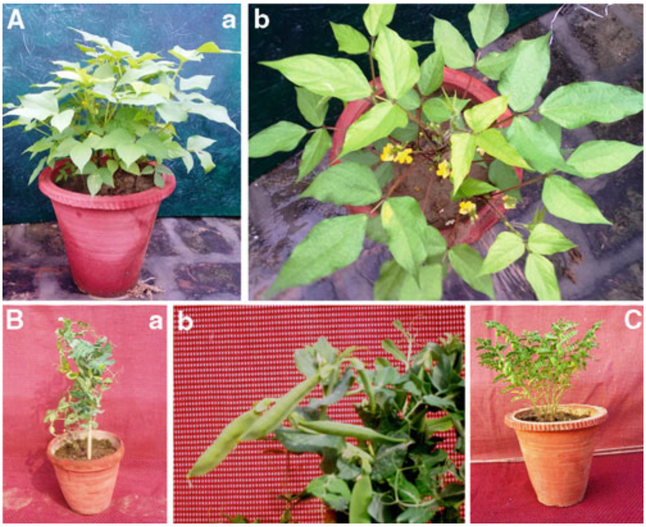

Fig. 1.3 Growth of greengram (**A** (a) vegetative growth and (b) with flowering), pea (**B** (a) vegetative growth and (b) with pods), and chickpea with seed pods (**c**) grown in conventional soils

higher concentrations is reported to damage plants by (1) inhibiting physiologically active enzymes (Stobart et al. 1985), (2) inactivating photosystems (Clijsters and Van Assche 1985; Somasundaram et al. 1994; Pandey and Tripathi 2011), and (3) disturbing mineral metabolism (Gadd 2007, 2010). In yet other study, Sandmann and Bflger (1980) have pointed out the importance of lipid peroxidation by metal (e.g., Cu) stress. Under nutrient deficient soil, the solubility of organic carbon and concomitantly the mobility of contaminants or pollutants such as heavy metals are increased. Dissolved soil organic matter has the significant effects on transformation of heavy metals through the increment of heavy metal solubility, root growth, and plant uptake (Quartacci et al. 2009; Kim et al. 2010). Copper and Pb accumulation in maize (*Zea mays* L.) and soybean (*Glycine max* L.) as affected by application of plant nutrients in soil such as N, P, and K (Xie et al. 2011) resulted in reduction in photosynthesis, stomatal conductance, and biomass while cadmium application caused a decline in the net rate of photosynthesis, stomatal conductance, and biomass in pak choi and mustard (Chen et al. 2011) but increased total chlorophyll content in tomato and decreased total biomass (Rehman et al. 2011). Accumulation of Zn and Cd in roots, petioles, and leaves of *Potentilla griffithii* was increased significantly with addition of these metals individually while Zn supplement decreased root Cd accumulation but increased the concentration of Cd in petioles and leaves (Qiu et al. 2010). The protective effect of Mg against Cd toxicity could in part be due to the maintenance of Fe status or to the increase in antioxidative capacity, detoxification, and/or protection of the photosynthetic apparatus (Hermans et al. 2011).

1.5.3 Metal Impact on Human Health

Heavy metals after release from various sources may enter into soil, vegetation, and water depending on their density. After their deposition in various systems, metals cannot be degraded and therefore persist in the environment causing human health problems through inhalation, ingestion, and skin absorption. On the other hand, heavy metals have willingly been used by humans for quite long times in metal alloys and pigments for paints, cement, paper, rubber, and other materials and are increasing even today in some parts of the world despite their well-known adverse effects. Acute exposure to metals may lead to nausea, anorexia, vomiting, gastrointestinal abnormalities, and dermatitis. Heavy metal toxicity can also damage or decrease mental and central nervous function (Gybina and Prohaska 2008), and damage blood composition (Cope et al. 2009), lungs (Kampa and Castanas 2008), kidneys (Reglero et al. 2009), livers (Sadik 2008), and other important organs (Lindemann et al. 2008; Lovell 2009). Furthermore, the long-term exposure of heavy metals may slowly impair physical, muscular, and neurological degenerative processes similar to Alzheimer’s disease (Kampa and Castanas 2008), Parkinson’s disease (Crawford and Bhattacharya 1987), and muscular dystrophy and multiple sclerosis (Turabelidze et al. 2008). High exposure can also lead to obstructive lung disease and has been linked to lung cancer, and damage to human’s respiratory systems. In contrast, some metals like copper, selenium, and zinc (trace elements) play an important role in maintaining the metabolism of the human body. Copper, for example, is an essential substance to human life, but in high doses, it can cause anemia, liver and kidney damage, and stomach and intestinal irritation.

1.6 Human Health Risk Assessment: A General Perspective

Contamination of soils by heavy metals followed by uptake of metals through various agencies like foods, feeds, water, etc. (Marshall et al. 2007; Sharma et al. 2007; Khan et al. 2008a, b; Sridhara Chary et al. 2008; Zhuang et al. 2009a, b), by humans has become one of the most serious environmental problems that has threatened the precious human health (Eriyamremu et al. 2005; Muchuweti et al. 2006; Moore et al. 2009). Therefore, there is indeed an urgent and collective effort required to clean up the contaminants from environment so that the risk of metal toxicities could completely or at least to some extent be minimized. The concern resulting from the potential exposure of populations vulnerable to toxicants has, however, forced workers of different disciplines to act together in order to develop methodologies so that the actual impact of heavy metals on both the varying environment and the human health could be assessed (Eriyamremu et al. 2005; Muchuweti et al. 2006).

1.6.1 What Is Human Health Risk Assessment?

A human health risk assessment is in fact the method of assessing the probability of harm caused to people resulting from exposure to contaminants at a site. And therefore, both the deleterious (toxic) effects of pollutants and the ways that people may be exposed to these substances are evaluated.

In this context, for evaluating the risk caused by heavy metals, different workers apply different approaches (Baes et al. 1984; Sauvé et al. 1998; Hough et al. 2003, 2004). However, the role of both scientists (risk assessors) and decision makers (risk managers) in the evaluation process is central to the understanding of the risk assessment. In general, two approaches can be applied for evaluating the risk of a specific pollutant to any individual population: direct approach (biological) and indirect (environmental monitoring). For example, different human biomonitors, like plasma and urine, human milk, hair, and adipose tissue, may be used in surveillance programs. Even though these sources may provide real and direct information about how population is exposed to pollution, they are variable and depend largely on personal characteristics, such as dietary habits, smoking, weight, etc., rather than on low-level environmental exposures (Paustenbach et al. 1997). On the other hand, the chemical analysis of the pollutant concentrations originating from different sources like air, soil, vegetation, sediment, etc., may be an interesting indirect methodology for human health risk assessment. However, in order to make chemical methods more viable and effective, it should be complemented with biological and toxicological methods (Vaajasaari et al. 2002; Tsui and Chu 2003; Robidoux et al. 2004; Gruiz 2005). Considering these, it is generally believed that health risk assessment may play an important role in protecting humans from the nuisance of heavy metals.

1.6.2 Why We Do Assessment and What Is Risk Assessment Process?

Risk assessment strategies often aimed at populations are a systematic and multistep process which is used to determine the magnitude, likelihood, and uncertainty of environmentally induced health effects (Sexton et al. 1995). Risk assessment has thus been suggested as a process which is generally used to collect scientific information regarding the toxicants and providing it to the policy/decision makers so that the human exposures to these substances could be regulated and managed. Broadly, risk assessment process includes four steps:

(a) Hazard Identification. In this step, site data relevant to human health are gathered and analyzed. And if there is any effect, that effect is again monitored to see whether it requires any further scientific investigations or not. For this, various tools, like quantitative structure–activity relationship (QSAR), short-term toxicity test, etc., are used in order to estimate the chemical damage of a single substance. However, this process also depends upon the origin of hazardous substances in question. For example, when establishing the hazard from