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Heavy Metals: Biological Importance and Detoxification Strategies

Oves M[1]*, Saghir Khan M[2], Huda Qari A[1,3], Nadeen Felemban M[1] and Almeelbi T[1,4]

[1]Center of Excellence in Environmental Studies, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia
[2]Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh 202002, India
[3]Biological Department, Faculty of Science, King Abdulaziz University, Jeddah Kingdom of Saudi Arabia
[4]Department of Environmental Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

Abstract

Global modernization is responsible for industrialization, urbanization and several other anthropogenic activities, which involves the huge application of heavy metals. Heavy metals containing products during process and after disposal, release various heavy metals and ions in surrounding atmosphere which severely affect the soil and water quality. Heavy metals contaminated soils in general are nutrient deficient and are likely to become barren in future. If heavy metal polluted soil is used for crop cultivation then the heavy metals deposited in soil enter into food chain and at higher concentration create severe human health problems. On the contrary, at permissible limit, metals are important for enzymatic activity and genetic material integrity in biological system. To understand the importance and risk associated with heavy metals, a genuine attempt is made to present different aspects of metal contamination in soils. Furthermore, nutritive value of heavy metals and toxicity to bacteria and plants is discussed. Finally, different strategies adopted by biological systems to detoxify heavy metals are critically highlighted. This review is likely to help to better understand the over does risk of heavy metals and its biological detoxification strategies.

Keywords: Heavy metals; Toxicity; Detoxification; Pollution; Biological importance

Introduction

Due to consistently increasing human populations, the current agricultural systems are under tremendous pressure basically for two reasons: (i) cultivable land is declining very rapidly and (ii) the human food demand is on the rise. Therefore, well-directed and concerted efforts are needed in order to use the full potential of agro-ecosystems efficiently and to overcome these problems. However, the plant nutrients like nitrogen (N), phosphorus (P), potassium (K), and some other minor nutrients play important roles in crop improvement in conventional agriculture practices. In contrast, the deficiency in even micronutrients, which are typically present at <100 mgkg^{-1} dry weight, may significantly limit the crop yields in many production systems [1]. Some of micronutrients such as Cu, Fe, Mn, and Zn, are essentially required for various physiological functions of plants and animals. Although the majority of plants require these elements in minimal quantities, agricultural soils are often deficient in one or more of these micronutrients. In general, the concentration of such nutritional contents in plant tissues falls below the optimum levels. There are also minor elements, known as trace elements, or other metalloids which play important roles in functioning of living organisms including those of microbioma. In addition, these elements could also participate in other activities: (i) forming the structure of proteins and pigment (ii) redox processes (iii) regulation of the osmotic pressure (iv) maintaining the ionic balance and (v) acting as enzyme component of the cells [2,3]. Among these elements, Al, Co, Se, and Si play a role in promoting plant growth and may be essential for particular taxa [4]. Likewise, Zn plays a significant role in cellular division and amplification, protein synthesis, and contributes in carbohydrate, lipid, and nucleic acid metabolism [5]. On the other hand, structure and composition of microbiota [6] and plant growth [7-10] are reported to be significantly affected when the concentrations of such trace elements exceed the normal level. Moreover, the concentration of these trace elements also varies from soil to soil and/or region to region. For instance, multiple surveys conducted to determine the status of nutrient in agricultural soils in China and India revealed that Zn is commonly the most deficient micronutrient in soil. While the nutritional deficiency levels in Chinese soils were (%): Zn 51, Mo 47, B 35, Mn 21, Cu 7, and Fe 5 [11], the deficiency levels in Indian soils were: 49 Zn, 33 B, 12 Fe, 11 Mo, 5 Mn and 3 Cu [12]. Thereby, identifying the elements of soil nutrient pools and their consequential effect on both microbes and plants are necessary for enhancing crop production and plant nutritional value.

Source of heavy metal in soils

Heavy metals are defined as metals and metalloids having densities greater than > 5 g cm^{-3}. Heavy metals may be found in soils naturally [13-15] or can be added to soils through anthropogenic activities (Figure 1). Natural sources of heavy metals (HM) such as volcanoes emissions transport of continental dusts, and weathering of metal-enriched rocks due to long exposure to air, greatly adds higher amounts of HM to soils [16]. In addition, HM can also contaminate the soil through other human activities, such as: (i) exploitation of mines and smelters (ii) application of metal-based pesticides and metal-enriched sewage sludge in agriculture [17,18] (iii) combustion of fossil fuel, metallurgical industries, and electronics, and (iv) military training and weapons, etc. [19]. The anthropogenic activities have been categorized into five groups: (i) metalliferous mining and smelting (e.g., As, Cd, Pb and Hg), (ii) industry (e.g., As, Cd, Cr, Co, Cu, Hg, Ni and Zn), (iii) atmospheric deposition (As, Cd, Cr, Cu, Pb, Hg and U), (iv) agriculture (e.g., As, Cd, Cu, Pb, Se, U and Zn), and (v) waste disposal (e.g., As, Cd, Cr, Cu, Pb, Hg and Zn). Agricultural practices such as excessive application of phosphatic fertilizers for optimum crop production [20], extensive and injudicious usage of toxic pesticides, and use of sewage sludge can result in soil pollution [21].

***Corresponding author:** Oves M, Center of Excellence in Environmental Studies, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia, Tel: +966509399857E-mail: owais.micro@gmail.com

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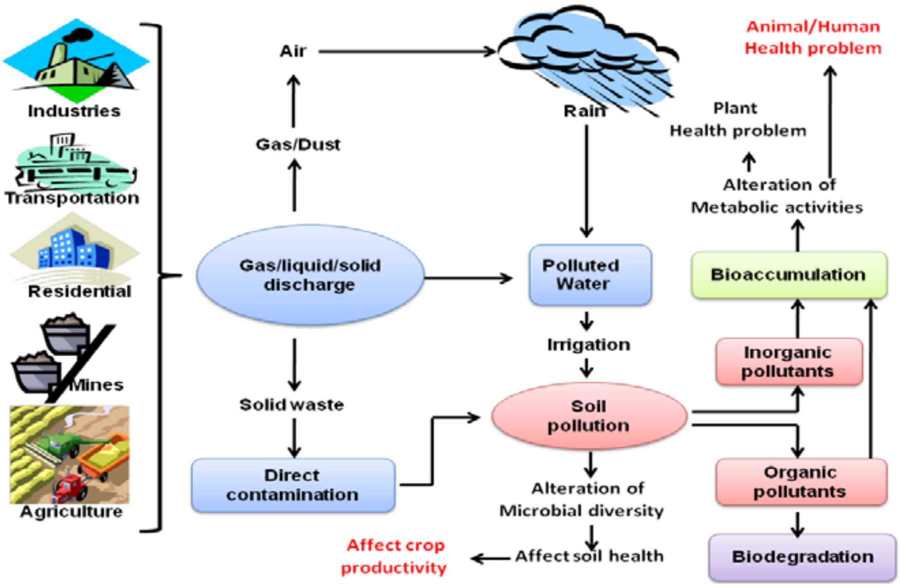

Figure 1: Source of metal contamination and toxic effects, adapted from Oves et al. [15].

The annual estimate of heavy metals release from all sources in worldwide is around (in metric tons) 22,000 of Cd, 939,000 of Cu, 783,000 of Pb, and 1,350,000 of Zn [22]. In 2009, the worldwide estimation of total production of ferrochromium and chromites was 7,000,000 and 19,300,000 metric tons, respectively [23]. Moreover, Pb, Cd, Zn and Ni are notable metals that originate from heavy traffic on roads and may also cause soil pollution [24,25]. Previous studies have reported that high concentrations of metals like Fe, Zn, Pb, and Cr can also be found in road dust, which may originate from the electric arc and furnace dust [26,27]. Another studies have shown that high concentrations of Fe, Zn, Cu, Cr and Ni can also be produced by serious wear and tear of tires and brake linings [28,29]. Other sources that may result in HM pollution includes: fly ash discharged from coal dependent power plants [30,31], polyvinyl chloride (PVC) products, colour pigments, several alloys, and chargeable Ni-Cd batteries [32]. Another major source of soil contamination is the unprocessed industrial wastewater [33,34]. Currently, the environmentalists around the world are paying more attention to the contamination of agronomic soils with heavy metals and their consequent adverse effect on the agro-ecosystems. This is because many chemical, physical, and biological activities are going on inside the soil constantly, which makes it an active and dynamic system. Moreover, the fertility of soil (nutrient pool) is mainly determined by the massive interactions between living and non-living components of soil. In addition, maintaining the soil in good quality is very important in any sustainable agriculture system. However, the nutrient status of soil is changed according to the following factors: time, prevailing conditions of climate and plant cover, and microbial composition of soil. When some stressors such as HM, temperature, extreme pH, or chemical pollutions are imposed on a natural environment, soil biota can be negatively affected and subsequently the whole ecological processes mediated by them are disturbed [35-37]. The assessment of soils for metal levels has therefore become important in order to assess its impact on different forms of water (groundwater and surface water) [38], microbial communities [39,40], plant genotypes [41,42], and animals and human health [43,44].

Bioavailability of Heavy metals

Although the total concentrations of HM in soil does not provide any specific information about the metal mobility and availability, it remains important to help in identifying the specific bio-availability, reactivity, mobility and how plants or other soil micro flora take up heavy metals [45,46]. Metals within soil have been categorized based on the available data into five major geochemical forms: (i) exchangeable (ii) bound to carbonate phase (iii) bound to Fe and Mn oxides (iv) bound to organic matter and (v) residual metal. These soil metals can vary greatly in their mobility, biological availability, and chemical behaviour in soil probably because of their ability to react to form organic compounds such as low-molecular organic acids, carbohydrates, and enzymes secreted by microorganisms [47]. Furthermore, the soil-derived bacteria can interact firmly with metal ions in soil solution through their charged surfaces. Bacterial cells have a huge capacity to enable them to adsorb and immobilize toxic ions from soil solution [48]. For instance, Huang et al. [49] have reported that when symbiotic bacteria such as rhizobia are used as inoculant, the adsorption of Cu and Cd in soil is significantly increased. The mechanisms of bacterial effects on the speciation and distribution of metals in soils, however, remain poorly understood. Previous studies have used numerous methods to investigate the possible chemical association of metals in soils, and to assess the metals mobility and bioavailability. The researches in these studies have illustrated various methods such as sequential extraction, single extraction, and soil column-leaching experiments [50,51]. In single extraction method, they use selective chemical extractant such as a chelating agent or a mild neutral salt [52,53], and it is frequently used to indicate the bioavailability or mobility of heavy metals. Moreover, this method provides valuable information that would help to predict the bioavailability of metals to plants, movement of metal in the soil profile, and metal transformation between different forms in soils [54]. There are some factors that affect the bioavailability and accumulation of heavy metals in soil. Such factors include- (a) type and texture of soil (b) physico-chemical properties of soils (c) plant genotypes and their photosynthates (d) soil–plant–microbes interaction and (e) agronomic practices such as fertilizer application, water managements and crop rotation system [55].

Heavy metal impact on human health-A concise view

Heavy metals usually enter the human body via different food chains, inhalation, and ingestion. In addition, heavy metals have been used for long time by humans for making metal alloys and pigments for paints, cement, paper, rubber, and other materials. Even today the applications of heavy metals in some countries are increasing despite their well-known toxic effects. However, once the heavy metals enters the human body by any means, it is likely to stimulate the immune system and may cause nausea, anorexia, vomiting, gastrointestinal abnormalities, and dermatitis [56,57]. The toxicity of heavy metals can also disrupt or damage the mental and central nervous systems [58], change blood composition, [59], damage lungs [60], kidneys [61], livers [62], and other important organs [63]. The long-term exposures of human population to HM have also shown physical, muscular, and neurological impairments. The degenerative processes are similar to Alzheimer's disease [60], Parkinson's disease [64], muscular dystrophy and multiple sclerosis [65]. Another disease such as obstructive lung disease has been linked to lung cancer, and damage to human's respiratory systems has also been found to develop following high rate exposure to metals. Apart from the toxic effects, certain metals like Cu, Se and Zn are reported to play some important and beneficial roles in human metabolism. As an example, Cu at lower concentration acts as co-factors for various enzymes of redox cycling [66,67]; however, at higher concentration disrupts the human metabolism leading to anaemia, liver and kidney damage, stomach and intestinal irritation.

Heavy metals importance for microorganisms

The uptake of HMs from soil has both direct and indirect effect on microbial composition [53,68], metabolism [69] and differentiation [70]. The interaction of metals and their compounds with soil microbes depends on several factors. These factors include metal species, interacting organisms and their habitat, structure and compositions, and microbial functions. Certain metals like Cu, Zn, Co, and Fe are essential for survival and growth of microbes. However, these metals can exhibit toxicity at higher concentrations and may inactivate protein molecules [71,72]. Although some metals such as Al, Cd, Hg and Pb remain with unknown biological functions, yet they accumulate within cells and may: (i) affect enzyme selectivity (ii) deactivate cellular functions (iii) damage the DNA structure and may result in cell death [73,74]. For instance, nickel among metals is one of the primary nutrients and plays essential roles in various microbial cellular processes. In addition, many microbes are able to locate and absorb Ni through permeases or ATP-binding cassette-type transport systems. Once the element gets into the cell, Ni is incorporated into several microbial enzymes like acetyl CoA decarbonylase/synthase, urease, methylenediurease, Ni-Fe hydrogenase, carbon monoxide dehydrogenase, methyl coenzyme reductase, certain superoxide dismutases, and some glyoxylases [75]. However, Ni is toxic to bacteria at higher concentrations. Therefore, bacteria have developed certain strategies to regulate the levels of intracellular Ni to overcome the toxicity problem as observed previously in two Gram-negative bacteria: *E. coli* and *H. pylori* [75,76]. In addition, *B. japonicum* HypB which was purified from an overproduction of *E. coli* strain, has shown its ability to bind up to 18 Ni ions per dimer as well as to contain GTPase activity [77]. In general, PGPR can produce siderophores under Fe-deficient environment [78-81]. Recent studies have reported that a ferric Fe-specific ligand increases the plant growth of cucumber (*Cucumis sativus* L.) by accelerating the access of Fe within rhizospheric environment [82]. In another study, certain strains of *Rhizobium ciceri* that form symbiotic association with chickpea, have the ability to produce phenolate type siderophores such as salicylic acid and 2,3-dihydroxybenzoic acid [83]. These compounds are produced in response to Fe deficiency; however, their production can be significantly affected by nutritive components of the culture medium. It sounds that Cu, Mo, and Mn ions bound competitively with Fe to siderophores, resulting in 34-100% increase in siderophores production [84,85]. For instance, Co is a biologically essential microelement with a broad range of physiological and biochemical functions [86,87]. For symbiotic association, Co is needed for N_2 fixation in legumes and root nodules of non-legumes. Interestingly, the requirement for Co is much greater for N_2 fixation than for ammonium nutrition, and in case of Co deficiency results in development of N_2 deficiency symptoms. Previous published results have observed a significant increase in leghaemoglobin formation when Co is applied, and play a role as an essential component of N_2 fixation. Therefore, it enhanced the nodule numbers per plant and ultimately increased the pod yield of soybean [88]. Many cobalamin-dependent enzymes of rhizobia are involved in nodulation and N_2 fixation such as methionine synthase, ribonucleotide reductase, and methylmalonyl coenzyme A mutase [89]. Preliminary studies have shown that the combined inoculations of *Rhizobium* and Co have significantly affected the total uptake of N, P, K, and Co by summer groundnut [90]. Similarly, molybdenum (Mo) acts as the catalytic centre of numerous enzymes. These enzymes have been categorized into two categories based on their cofactor composition and catalytic function that include: (i) bacterial nitrogenases containing a Fe-Mo-Co in the active site and (ii) pterin-based Mo enzymes. The second category of enzymes includes sulphite oxidase, xanthine oxidase, and dimethylsulfoxide reductase, each of which plays specific roles and has distinct activities. For instance, nitrate reductase has been reported in *D. desulfuricans* [91] while aldehyde dehydrogenase in *D. gigas* [92,93] and Oves et al. (def) has been also reported chromium reducing bacterial strain *Stenotrophomonas maltophilia and Pseudomonas aeruginosa* from soil samples [94-96].

Heavy metals importance for plant health

In general, plants usually need a continuous nutritional supply in order to remain healthy. Any shortage of a certain nutrient results in development of the nutrient deficient symptoms, and extreme condition of nutrient deficiency may lead to early mortality. In contrast, when the nutrient supply exceeds the required quantities, it may cause injury, and in some cases may lead even to death of plants at very high levels of nutrient enriched with heavy metals. However, plants require both macro-nutrients (C, H, N, O_2, P, S, etc) and micronutrients (B and Cl, Cu, Fe, Mn, Mo, Ni, and Zn) for their growth. The nutrients of both categories can be found in varied agro-ecological niches. A few plants living in symbiotic association with nitrogen-fixing microorganisms also require Co as a mandatory nutritional component. Metal can also be considered as a nutrient for plant health; however, there are two criteria used to define metals as essential nutrients for healthy plant which include: (i) it should be required by the plants to complete its life cycle, and (ii) it should be part of an essential plant constituent or metabolite. Plants are autotrophic organisms that use light energy during photosynthesis to convert CO_2 and H_2O into energy-rich carbohydrates and O_2. Therefore, the plant growth and development in general depends exclusively on photosynthesis, which in turn depends on sufficient supply of numerous chemical elements including metals like Cu, Fe, and Mn. Furthermore, heavy metals and metalloids can get into plants through uptake systems, which include different metal transporters [97,98]. However, in case of any metal defficiency, plants can regulate that defficiency and increase the metal availability in the root environment by either: i) lowering the pH through root exudates which may contain organic acids ii) or through the release of metalcomplexing agents. Subsequently, once the proper and sufficient supply is maintained, a signal from the shoot to the root is sent to shut down the exudation process. Several studies have reported that the presence of metals at low rates in plant systems can affect the plant growth by participating in the redox reaction and sometimes directly becoming an integral part of enzymes [99,100]. Furthermore, Zn plays important roles in maintaining the integrity of ribosome [101] in the formation of carbohydrates in catalysing the oxidation processes and in the synthesis of macromolecules [102]. Similarly, Mn plays an important role in certain enzymatic reactions such as malic dehydrogenase and oxalosuccinic decarboxylase. In addition, Mn is also needed for water splitting in photosystem II as well as for superoxide disumutase [103]. In microorganisms, Co cofactor is necessary to the formation of vitamin B12 , and Fe is an essential element for many metabolic processes and is indispensable for all organisms [104].

Heavy metal toxicity

Heavy metals significance in microbial activity

Altered structures of microbial community in response to metals is an important indicator of metal activity and its biological availability within soil ecosystem. In this context, the application of heavy metals individually such as Cd, Cr, Pb and mixture of both $CdSO_4$ and $Pb(NO_3)_2$ solutions at different rates have shown deleterious effects on soil microbes. Thereby, the polluted soil with heavy metals resulted in a significant decrease of the activities of various soil enzymes like acid phosphatase and urease of microbes [105]. Frostegard et al. [106] have also analyzed soil-derived microbes from metal-polluted soils and reported a gradual change in the microbial community structure based on alterations of the phospholipids and fatty acid profiles. However, the response of microbial communities to various metals depends on metal solubility, the bioavailability and toxicity in soil. The metal characteristics are greatly influenced by the physiochemical process of soil such as sorption, precipitation, and complexation ability [107]. Moreover, the interaction of metals with soil depends strongly on the physicochemical properties of soil, which may differ among various agro-climatic regions of the world. Barajas-Aceves [108] has suggested that when metal stress is applied, the total amount of microbial biomass decreased in parallel with decreasing the efficiency of substrate utilization of microbes [109]. The decline of the microbial biomass is an indicator of soil pollution with heavy metals, however its suitability in environmental monitoring is limited because of its high spatial variability [110] and failure to meet certain standard in its measurement. Previous study has also shown that the decline of the microbial biomass was also associated with changes in soil microbial community structure [111]. Another study has observed the structural changes of soil microbial community and the level of metal tolerance was increased in response to even slight metal contamination of the soil [112]. Taken together, the observed effects of metal toxicity on different soil microbial communities may be because of the metal-sensitive ability of populations or community has been changed. However, no specific threshold for metal toxicity was reported, but such threshold may be site specific as observed by Bunemann et al. [113].

Mechanism of heavy metal toxicity

Toxic metal species can bind to proteins and thereby affect the biological functions of the target molecule. For example, microbial biomass and enzyme activity of the soil have been found negatively affected by the elevated metal levels of Cu and Zn [114]. In addition, toxic metal may interact strongly with thiols and disulphides and subsequently cause disruption of the biological activity of certain proteins that contain sensitive S groups [115,116]. These reactions regularly produce reactive oxygen species (ROS), which are natural by-products of the normal metabolism. The destruction of sensitive thiol groups due to metal exposure may eventually impair the protein folding/ or binding of apoenzymes by cofactors, and thus the normal biological activity of the proteins is disrupted [72]. Moreover, certain metals in transition can participate in catalytic reactions, known as Fenton-type reactions that produce ROS. Collectively, these reactions can set the cell under an oxidative stress and thereby ROS levels significantly increased, which may result in DNA damage, destructions of lipids and proteins through a wide range of biochemical routes [117]. Toxic metal species may also get into cells through various transporters, or penetrate the cellular membrane and subsequently bind to lipophilic carrier. The transporter-mediated uptake of toxic metals interferes with the normal transport of essential substrates, and thus results in competitive inhibition of the transport process. Moreover, this transport process acquires energy from the proton motive force or ATP pool [118]. Some metal oxy-anions are reduced by the oxidoreductases that are able to draw electrons from the bacterial transport chain through the quinone pool [119]. Particular toxic metals can cause starving of microbial cells indirectly by siphoning electrons from respiratory chain [120]. The ROS are also produced during normal metabolic processes and results in DNA damage, destructions of proteins and lipids; however, this production is promoted during metal pollution and subsequently may lead to additional cellular damages [121]. Recently, Macomber and Hausinger [122] have suggested four mechanisms of Ni toxicity which involves- (a) replacement of essential metal by metalloprotein: a protein with a metal ion co-factor, (b) Ni binds to catalytic sites of non-metalloenzymes (c) Ni binds outside the catalytic site of an enzyme to inhibit its function and (d) Ni indirectly causes oxidative stress. In other example, the loss of cell viability after Cr(III) exposure was not because of the membrane damage or enzymatic inhibition, but was probably because of Cr(III) toxic effect on cellular morphology of the bacteria. After Cr(VI) was taken up into the cell, the toxic effect of Cr(III) appeared to be associated with extracellular interactions which ultimately resulted in deformed cell morphology [123].

Metal resistance strategies adopted by bacteria

Microscopic organisms have evolved some strategies to overcome the inhibitory effects of toxic heavy metals. Such metal detoxification strategies adopted by microbial communities are- (i) metal exclusion by permeable barriers (ii) active transport of metals far from the cell (iii) intracellular sequestration of the metal by protein binding (iv) extracellular sequestration (v) enzymatic detoxification of metal to a less toxic form and (vi) reducing the sensitivity of cellular targets to metal ions. Single or multiple detoxification mechanisms can be applied for any individual metal or a group of chemically related metals. However, the type of microorganisms can greatly affect the detoxification mechanisms [124]. For instance, a greater number of microbes have specific stress resistance genes that could regulate toxic metal resistance, and such regulatory genes are centrally located on plasmids or chromosomes [125,126]. In general, the regulatory system of metal resistance is chromosomal genes-based which is more complex than the plasmid system. On the other hand, plasmid-encoded systems are usually interacting with toxic-ion efflux mechanism. The determinants of plasmid-encoded resistance systems for toxic metal ions (such as Cu) have been reported to be inducible [127]. As an example, Pb-resistant *E. faecalis* was isolated and identified by employing biochemical approaches and 16S rRNA gene sequencing [128]. Lead-resistant *E. faecalis* also demonstrated resistance to other heavy metals and antibiotics. Moreover, *E. faecalis* had four plasmids with different molecular sizes 1.58, 3.06, 22.76 and 28.95 kb. Plasmid profile of the cured derivatives have shown that the ability of Pb resistance *E. faecalis* continues even after elimination of all the plasmids [128]. Furthermore, features of heavy metal resistances have been found on bacterial chromosomes. For example, Hg^{2+} resistance in *Bacillus*, Cd^{2+} efflux in *Bacillus* and arsenic efflux in *E. coli* have been reported in previous study [129]. Efflux pumps that were determined by plasmid and chromosomal systems are either ATPases or chemiosmotic systems. The mechanisms of efflux pumps are showing similarity among different types of bacteria. Cadmium resistance may involve: (i) an efflux ATPase in Gram-positive bacteria, (ii) cation-H^+ antiport in Gram-negative bacteria, and (iii) intracellular metallothionein in cyanobacteria [130]. Arsenic-resistant Gram-negative bacteria have an arsenite efflux ATPase and an arsenate reductase [which reduces arsenate (As V) to arsenite (As III)] which comprise the underlying biochemical mechanism [131]. Similar system for Hg^{2+} resistance was observed on plasmids of Gram-positive and Gram- negative bacteria with component genes being involved in transport of Hg^{2+} to the detoxifying enzyme, in which mercuric reductase plays a role in reduction of Hg^{2+} to elemental Hg^0 [132]. The enzyme organomercurial lyase can break the C-Hg bond in organomercurials [133-135]. In another study, plasmid-encoded resistance to chromate appeared to be unconnected with the reduction of chromate [Cr(VI)] to Cr(III) [135], but the mechanism of bacterial resistance depends on reducing the CrO_2^{-4} uptake [136].

Heavy metal–plant interactions

Exposure to heavy metals at higher concentrations results in severe damage to various metabolic activities leading consequently to the death of plants. The exposure of excess levels of metals to plants inhibits physiologically active enzymes [137], inactivates photosystems [138], and destruct mineral metabolism [139]. Sandmann and Boger [140] in a study have revealed the importance of lipid peroxidation under metal stress. Janas et al. [141] in other experiment have observed the impact of Cu on growth, lipid peroxidation, phenolic compound accumulation and localization in lentil (*Lens culinaris* Medic.) seedlings. Previous studies have reported that dissolved soil organic substances have significant effects on heavy metal transformations by increasing the solubility of heavy metal, root growth, and plant uptake [142,143]. Recently published work demonstrated that maize and soybean grown in Cu and Pb contaminated soil had reduced photosynthetic pigments poor stomatal conductance, and significantly lower biomass [144]. On the other hand, Cd application resulted in a remarkable decline in the net rate of photosynthesis, stomatal conductance, and biomass in pakchoi and mustard plant [145]. In another study, Cd contamination resulted in increased total chlorophyll content in tomato and decreased total biomass [146]. Qiu et al. [147] have shown that as a subsequent result of adding Zn and Cd individually into a hyperaccumulator plant (*P. griffithii*), accumulation of both metals in roots, petioles, and leaves was significantly increased. However, Zn supplement influenced the Cd accumulation, in which it decreased Cd concentration in roots and increased the accumulation of Cd in petioles and leaves [147]. The protective effect of Mg against Cd toxicity could partially be due to the maintenance of Fe status, the increase in anti-oxidative capacity, and protection of the photosynthetic apparatus [148].

Heavy metal translocation in plants

The first interaction of heavy metals with plants takes place during its uptake process. The maximum capacity of each plant to take up soil metals depends on metal concentration in soil and its availability to plants. The process of metal uptake by the plant roots depends on: (i) diffusion of elements along the concentration gradient (ii) root interception where soil volume is displaced by root volume due to root growth, and (iii) mass flow, transport from bulk soil solution along the water potential gradient.

Some metals can be absorbed by the apical region of the plants while others can be taken up by the entire root surface. Metals are transported further into the cells, some of which are transferred to the apoplast, and the other part bound to cell wall substances. At the apoplast side, metals further migrate through the plasma membrane into the cytoplasm where metals may affect the nutrient status of the plants. For instance, the toxic effect of Cr depends on its speciation, which determines its uptake, translocation and accumulation. Uptake and accumulation of Cr (Figure 2a), Cd (Figure 2b), Cu (Figure 2c) and Ni (Figure 2d) by various crops are well documented [149,150]. When the demand for element uptake by plant root is high and the nutrient concentration in the soil is low, the elements will move to the plant root by diffusion. Since there are some metals essential for plants, the uptake of such metals therefore, must be regulated by certain mechanisms. Previous study has reported that Zinc is transported with Zn transporters in a higher abundance with Zn accumulator plant species than in non-accumulator species [151]. Zinc was also reported to be actively transported as a free ion across the tonoplast. Other metals such as Cd, can easily enter the plant root through cortical tissue and translocated to the ground plant tissues [152]. As soon as Cd enters the roots, it can reach the xylem through apoplastic or symplastic pathway [153] and consequently forms complexes with some ligands, such as organic acids and/or phytochelatins. Normally, Cd ions are retained in the root system and only very small amounts are translocated to the shoot system. Metal ions are probably taken up by cells through membrane transport proteins that are designed for nutrient metal acquisition. Another study has found that Cd and Zn co-exist in aerial parts of *Arabidopsis halleri* plants [154]. The researchers have suggested that the uptake of Cd and Zn is genetically correlated and these metals are taken up by the same transporters, or their transporters are different and can be controlled by common regulators.

Heavy metals toxicity in plants: ***Effect on cell wall and plasma membrane:*** The root system of various plants including legumes is the primary target site for any pollutant in the soil. Ernst et al. [155] have reported that metal interacts well with cell wall but their findings about its binding properties and its role in providing resistance to metal were inconsistant. Most of heavy metals bind to polygalacturonic acids, to which the affinity of metal ions varies considerably [156-158].

The allocation of metals within plant varies with the age of plant organs. For instance, Nabais et al. [159] conducted a study to show the impact of root age on the allocation of metals, amino acid and sugars in different cellular fractions of perennial grass *Paspalum notatum* (Bahiagrass). In a recent study, the researchers compared the allocation of metals (Al, Fe, Cu and Ni), nutrient amino acids and sugars in different fractions of 21 and 120 days old root cells of *P. notatum* grown in quartz sand [159]. The researchers have found that the younger roots had a higher content of Al, Fe, Cu Ni, amino acids and sugars, compared to the older ones. This variation in metal distribution in roots could be probably due to the differences in metabolic activities and architecture of roots. However, the metal concentration in older roots was significantly lower than the concentration in the younger roots, and this is probably due to the active metabolism of young roots. For example, Al and Fe were mainly allocated to plant cell wall with pectin, hemicellulose and cellulose, in both younger and older roots. However, the older roots

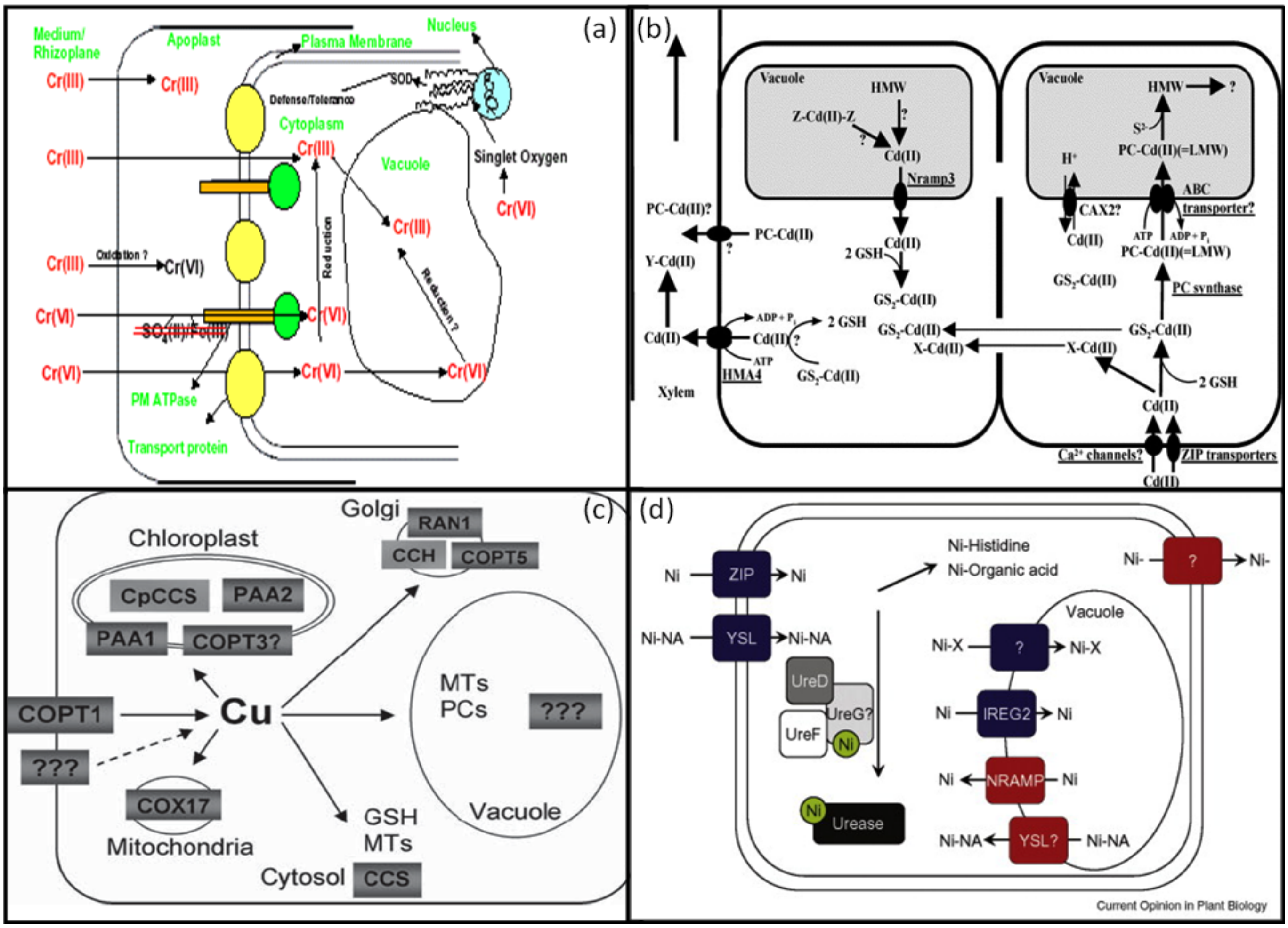

Figure 2: Hypothetical model of metal transport and accumulation in plant cells- (a) Cr (b) Cd (c) Cu and (d) Ni (Adapted and combined from Ref. [149,150,157,158]).

have shown a significant portion of Al which was also allocated to the intracellular fraction, suggesting that the older roots were less able to prevent the entry of Al into the cytoplasm. The proportion of Cu was higher in intracellular components in both younger and older roots, as expected from an essential nutrient. Based on this finding, it was concluded that the root cells of *P. notatum* suffered from severe changes in the composition of the cell wall with ageing. In a similar study, the *in vitro* Cd application increased the wall thickness and caused a destruction of the internal organization of chloroplast [160].

Another target site for metal toxicity is the plasma membrane, in which metal can bind and disrupt membrane functions [161]. Upon metal interaction, metal can induce qualitative and quantitative changes to the lipid composition of membranes, which in turn may alter the structure and functions of membrane, and other important cellular processes (Figure 3). Metal toxic consequences like oxidation and cross-linking of protein thiols, inhibition of key membrane proteins such as H^+-ATPase, or changes in the composition and fluidity of membrane lipids are reported [162]. Another study has reported the effect of Cr on the transport activities of plasma membrane [163]. In addition, the inhibition of ATPase activity was suggested to be due to membrane disruption by free radicals generated under metal stress [164]. Thereby, the reduction of ATPase activity results in proton extrusion, reduces the transport activities of the root plasma membrane, and ultimately limits nutrient uptake by the roots. Moreover, it was also reported that Cr interferes with the mechanism of controlling the intracellular pH [165]. Moreover, Cr may alter the metabolic activities of plants and it may (i) modify the production of photosynthetic pigments like, chlorophyll [166], and (ii) increase the production of metabolites like glutathione [83] and ascorbic acid [167] in response to metal stress which may cause damage to the plants. Among other metals, Cd and Cu have also been found to adversely affect the lipid composition of membranes [168]. Moreover, Cd treatment also reduces the ATPase activity of plasma membrane fraction of roots [169].

Membrane lipid peroxidation by heavy metals: Exceeding heavy metals induce changes in fatty acid composition of membranes, and injury to membrane may also occur when the per-oxidation of membrane lipid increases due to the action of highly toxic free radicals. In this perspective, numbers of metal ions have been reported to cause peroxidation of lipids of both the plasma membrane and chloroplast membrane [170,171]. For instance, lipid peroxidation was increased when plants were grown with Cd, Al, Cu and Zn [172] and Ni, Cr and As [173,174]. The application of Cu in the incubation medium and its uptake by the plant tissues resulted in (i) reduction of the content of photosynthetic pigments (ii) stimulation of lipid peroxidation, and (iii) enhanced permeability of the membrane.

Specific changes in the lipid composition were associated with

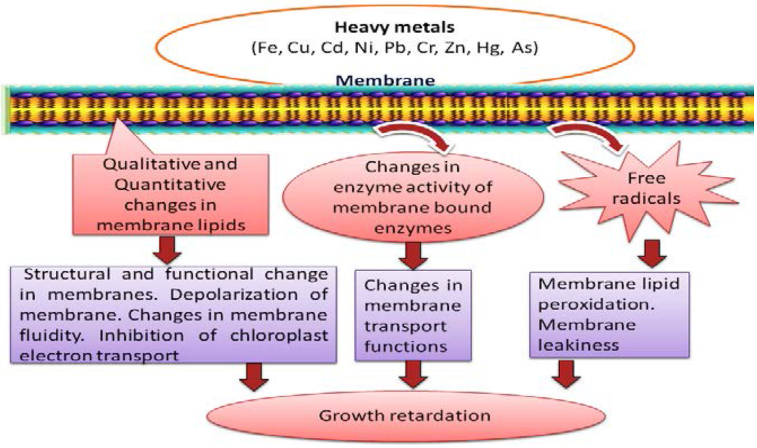

Figure 3: Heavy metal toxicity to cell membrane causing alterations in membrane lipids, enzyme activity and free radicals generation.

the gradual accumulation of Cu in the plant tissues. For instance, the decline in the content of sulfolipid was observed in chloroplasts; the increase in the content of monogalactosyl diacylglycerols, digalactosyl diacylglycerols, and phosphatidyl glycerols in chloroplasts and mitochondria was observed after one hour exposure to Cu. However, after 3 h of Cu exposure the content of all the lipids except phosphatidic acids decreased [171]. In general, the Fe and Cu compounds produced more free radicals and increased the peroxidation [175]. Thus, the alteration in membrane functions caused by metal exposure could be due to changes in both the structure and peroxidation of membrane lipids [176,177]. As an example, it has been reported that Al causes lipid peroxidation by disorganizing the membrane structure through generating free radicals [178]. The increased lipid peroxidation also alters the membrane properties, such as fluidity and permeability, and modulates the activities of membrane-bound ATPases [179]. Indeed, the process of peroxidation is a chain reaction, in which unsaturated fatty acids are converted in a stepwise to various small fragments of hydrocarbon such as malondialdehyde [180]. The resulting substances from lipid peroxidation processes have shown a deleterious effect on the plasma membrane functions leading to cell death. In a follow up study the researchers have demonstrated that the application of different levels of multiple heavy metal stress (such as Pb^{2+}, Cd^{2+} and Hg^{2+}) accelerated the lipid peroxidation of *Bruguiera gymnorrhiza* plants [181].

Heavy metals effect on photosynthesis: Photosynthesis is one of the most important physiological features of plant system. When plants are grown in metal enriched soils, the process of photosynthesis is adversely affected by heavy metals [182,183]. The interaction between toxic metals and the photosynthetic apparatus may result in (i) accumulation of metals in leaves and roots [182] (ii) alteration of the functional activities of chloroplasts which are abundant in different leaf tissues like stomata, mesophyll and bundle sheath [184], (iii) metal interaction with cytosolic enzymes and organics [185], (iv) effects at the molecular level on growth response and photosynthetic activity particularly on photosystem I, photosystem II, membrane acyl liquids and carrier proteins in vascular tissues [186], and (v) distortion of enzymes involved in photosynthetic carbon reduction (PCR) cycle and xanthophylls cycle. In this context, it has been reported that the elevated concentrations of metals such as Cu, have a deleterious effect on the formation of photosynthetic pigments [187]. In another study, the photosynthetic process like the one involved in the reduction of carbon when legume (greengram) was grown in heavy metal contaminated soils, was also affected [186]. Similarly, previous study has reported that the excess concentrations of Cu modified the ultrastructural responses of chloroplast in runner beans (*Phaseolus coccineus* L.) [188]. The chlorophyll was reduced when plants were grown in metal treated soils as reported by others [39,40]. Shanker [189] has suggested that the decrease in the chlorophyll a/b ratio following the Cr(VI) toxicity in plants was due to the destabilization and degradation of the proteins of the peripheral part [189]. When heavy metal stress applied, enzymes that mediate the chain reactions of chlorophyll biosynthetic pathways are inactivated, which could also contribute to the general reduction in the chlorophyll content in most of the plants including legumes.

Furthermore, the impact of metal stress of Cd^{2+} and Cu^{2+} on photosystem II activity has been reported in many previous studies. The Cd^{2+} affects both the PS II reaction centre and the light harvesting complex (LHC), which may result in inefficient energy transfer from the LHC to the reaction centre. In general, intensive studies have been conducted in order to study Cd-mediated inhibition of the reactions of photosynthesis [190]. Recent studies have reported that when Cd^{2+} is applied on protoplasts it showed a significant effect on the Calvin cycle, but it has no effect on the Rubisco [182]. In another study, Sheoran et al. [191] have induced a significant reduction in the Rubisco activity of pigeonpea plants when treated with Cd^{2+} at an early growth stage. However, Rubisco activity was not affected in older plants.

Genotoxic effects of heavy metals

When biologically non-degradable heavy metals persist in soil, it contaminates the urban and agricultural soils. In addition, heavy metals can also enter the human bodies via different food chains, subsequently accumulate in selected human body tissues, and eventually disrupt their metabolic functions [192,193]. However, some metals such as Cu, Fe, and Zn, are essential elements and play an important role in providing the required nourishment for healthy life. On the contrary, a few metals are even though essential, but may cause toxicity when their concentration exceeds the normal threshold level. In general, it has been reported that the soil with low pH is more genotoxic [194]. It has been known that the heavy metal pollutants have a high bio-accumulation rate. Thereby, when metal pollutant becomes above the optimal concentrations, it affects the human health [195,196], microbial composition and their function, soil enzyme activity, and plants [39,40,83,197,198]. The genotoxicant are reported to disrupt normal cellular processes, alters DNA structure, and consequently influence the cell survival. For instance, the excess concentrations of Cd and Cr have been found to be carcinogenic, mutagenic, and teratogenic for a large number of animal species [199,200]. Another effect of Cd and Cd-containing compounds include lung cancer (possibly prostate cancer) or tumours at multiple tissue sites [201,202]. In other reports, the genotoxic effect of Cd was assessed in human blood lymphocytes, and the researchers have shown the expression of DNA single strand breaks (SSBs) at even non cytotoxic concentrations [203]. Moreover, other metals such as Pb, Bi, In, Ag, and Sb can also act as a genotoxicant [204], and when such metal combines with phosphate, deoxyribose and heterocyclic nitrogenous bases of DNA, alters the genetic constitution as well as the integrity of the entire cell. Taken together, understanding the actions and reactions of chemical pollutants is important in order to preserve the gene pool and to manage a healthy ecosystem.

Some examples of effects of genotoxicants on plants

Indeed, heavy metals as genotoxicants have shown both direct and/or indirect effects on the synthesis and duplication of DNA and chromosom [205]. Also, metal genotoxicant can cause chromosomal aberrations in plant cells [206]. The genotoxic effects can be greatly influenced by the metal species and dosage of contamination. As an example, when barley (*Hordeum vulgare*) was grown in nutrient solution under controlled environmental conditions and then exposed to increasing concentrations (0, 15, 30, 60, and 120 $mmoll^{-1}$) of Cd at different time intervals, large genotypic variations among barley cultivars were observed. It has been suggested that the differences in the ability of barley cultivars to tolerate Cd pollution might be due to the involvement of internal antioxidative mechanisms. In addition, it has been suggested that the high sensitivity in Cd-sensitive barley cultivar was related to the oxidative damage, which involves the enhanced production of reactive oxygen species (ROS) [207]. Liu and Kottke [208] have reported that the exposure to Cd, Pb and Hg results in polyploidy, C-Karyokinesis, chromosome fragmentation, chromosome fusion, micronuclei formation, and nuclear decomposition in beans, garlic (*Allium sativum*) and onions (*Allium cepa*). Furthermore, Cd has also been known to cause cell death through accumulation of superoxide anions ($O_2^{\cdot-}$) of mitochondrial origin, and membrane peroxidation [209] in tobacco (*Nicotiana tabacum*). Previous studies have observed some Cd-induced genotoxic and cytotoxic effects in garlic and bean, and the excess levels of Cd can cause oxidative stress that involves the induction of lipid peroxidation in plants [210]. When cadmium chloride was applied on tobacco roots, greater damage of the DNA was detected by the cellular comet assay. In addition, it has been reported that the DNA damage induced by Cd^{2+} in roots of a transgenic catalase-deficient tobacco line (CAT1AS) is higher than the damage induced in wild-type tobacco (SR1) roots. By comparing the effects of ethyl methane sulfonate (positive control) and Cd^{2+}, it has been found that Cd^{2+} has no significant DNA damage in leaf nuclei. In addition, the somatic mutations or homologous recombination in leaves was assessed by the GUS gene reactivation assay, and it showed no significant results. Furthermore, Inductively Coupled Plasma (ICP) optical emission spectrometry has revealed that the Cd accumulation in the roots is 50 times greater than its accumulation in the above-ground parts of the tobacco seedlings [205]. The obtained data demonstrated that when tobacco and potato plants were grown in metal-stressed soil, the average leaf area (tobacco) and plant height (potato) were significantly reduced. In addition, a small but significant increase in DNA damage was also observed in nuclei of leaves of both plant species. In a similar study, Liu and Kottke [208] have reported that when excess concentration of heavy metals are added to medium (in which plants cannot grow normally) [209,210], it affects the frequency of sister chromatids exchange (SCE) in root tip cells of *Hordeum vulgare*. Likewise, Yi et al. [211] have used *Vicia* cytogenetic tests that are the most common tests used to assess the genotoxicity of environmental pollutants such as aluminium ($AlCl_3$). The observed results demonstrated that when *Vicia faba* root tips was exposed to $AlCl_3$ in a dose-dependent manner ranging from 0.01 to10 mM for 12 h, Al induced remarkable increases in their micronuclei formation and anaphase chromosome aberrations. In addition, Al-treated plants at pH 4.5 had greater number of micronucleated cells than those observed at pH 5.8. Following $AlCl_3$ treatment in a dose and pH dependent manner, the number of mitotic cells was significantly reduced, and in each mitotic phase the number of cells changed in response to Al-treatment. Moreover, the mitotic indices were reduced in response to the increases in pycnotic cells. Based on the deleterious effects of $AlCl_3$, it has been classified as clastogenic, genotoxic, and cytotoxic agent for *Vicia* root cells.

Futhermore, genotoxicants in some plants can also disrupt the sequential events of DNA processing such as replication [212], methylation [213], and repair system [214], which may eventually result in mutations [215]. For instance, Aina et al. [216] have evaluated the genotoxic effect of heavy metal stress on the DNA methylation of a metal-sensitive clover plant, *Trifolium repens* (L.), and a metal-tolerant hemp plant, *Cannabis sativa* (L.). The researchers used the immune-labelling monoclonal antibody technique in order to compare the variations in the level of 5-methylcytosine accumulation in the root DNA of plants grown in soils contaminated with different concentrations of Ni^{2+}, Cd^{2+} and Cr^{6+}. Their experimental findings demonstrated that the DNA of hemp control plants was methylated about three times more than the clover DNA. In response to heavy metal exposures, a considerable decline in DNA and RNA content of *Phaseolus vulgaris* [217] and submerged aquatic plant [218] has also been observed. Similarly, the exposure of plants to Cd, Cu, Cr, Ni, Pb, Hg, and Zn results in reduced efficiency of DNA synthesis, weaker DNA protection from declined chromatin proteins (histones) and increased deoxyribonuclease activity [215]. Heavy metals such as Cu, Ni, Cd, and Pb have also been reported to reduce RNA synthesis and to activate ribonuclease activity, which further leads to decrease in the RNA content [219].

The ability of plant to cope with the environmental stressors and to maintain its structural integrity provides the opportunity to assess the genotoxic effects of environmental pollutants. In this context, various methods dependent on chemical analysis have been developed to conduct risk assessments of the contaminated soils. And hence, to identify the presence of many mutagenic and carcinogenic compounds like heavy metals in soil. However, the standard chemical approaches employed to unravel soil metal concentrations have failed because majority of soil genotoxicants cannot be determined and most of the ecotoxic data are sometimes related to relatively less known compounds. Like bioremediation, there is a growing demand to develop new methods to assess soil genotoxicity. In this regard, bioassays provide a powerful tool to assess the toxicity of soil complex mixture without prior identification of its chemical composition. Considering the importance of bioassays, different genotoxic assessment tests such as Ames test [220-222], *Tradescantia* micronucleus test [223], *Tradescantia* stamen hair mutation [224], and *Vicia* root micronucleus assay [225] have been developed and applied in different countries as presented in Figure 4. Since these tests are simple to operate and more sensitive, they can be applied on regular basis for identifying genotoxic agents particularly in the detection of genotoxicants from contaminated environmental samples.

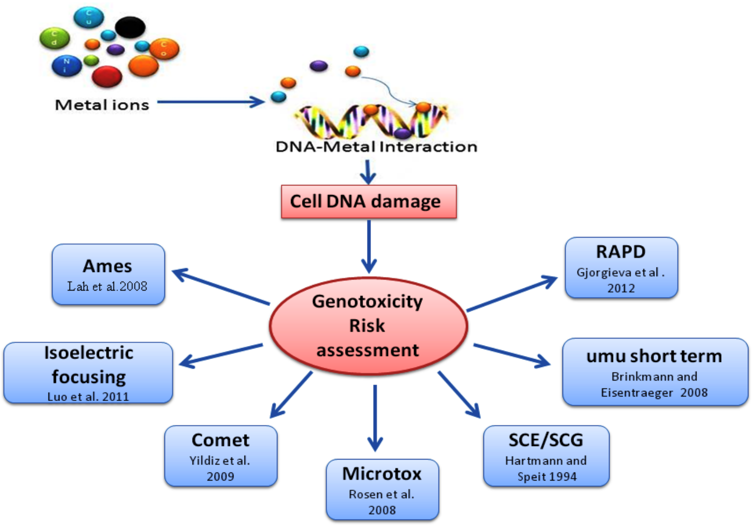

Figure 4: Types of genotoxicity assays commonly used for testing of heavy metal contaminated soils (Modified from Musarrat et al. [226]).

Heavy Metal Phytotoxicity: Challenges and remedies

Heavy metal contamination is a common factor restricting the plant growth in different agro-climatic regions. As an example, the accumulation of Cr(VI) in plant tissues has been found to induce some physiological changes, such as slowing down the plant growth rate, cause chlorosis in young plants, decreasing the pigment content, mutate enzymatic functions, impair root cells, and cause ultrastructural modifications of the chloroplast and cell membrane [226,227]. Chromium stress can also reduce seed germination and radicle growth in plants [228]. Excess concentrations of Cr can affect the roots of plants resulting in wilting of a plant and plasmolysis in root cells [229]. When Cr was applied at high concentration (1 mM), it completely distorted the chloroplastids membrane and caused severe disarrangement of thylakoids. Such destruction indicated that the Cr (vi) is more phytotoxic than other forms of chromium. The hexavalent chromium has been recognized as a genotoxicant and cytostaticant for plants. The cyto- and geno-toxicity of Cr were assessed in pea plants, which were grown in soil treated with Cr solutions in a dose-dependent manner with up to 2000 mgl^{-1}. Following incubation with soil metal pollutant for 28 days, there was no significant variations either in cell cycle dynamics or in ploidy level of the leaves; however, the flow cytometric histograms showed a significant damage to DNA. In parallel, comet assay was conducted as a confirmatory assay, which showed an increase in DNA damage particularly in treated samples with final concentration1000 and 2000 $mgCrl^{-1}$. When Cr was applied to the plant roots at 2000 mgl^{-1}, it affected the cell cycle progression and thus arrested the cycle at the checkpoint of G(2)/M phase. Moreover, the inhibition of plant growth involves the inhibition of cell division by induction of chromosomal aberrations [230]. According to Yoon et al. [231], metal accumulation in plants varies with plant species and genetic composition of plants. The genetic variation may be expressed as differences in morphological and physiological characteristics of genotypes [232]. Cadmium is yet another metal which is strongly phytotoxic and inhibits plant growth very severely leading to the death of plant. The main symptoms of Cd^{2+} toxicity to plants are stunting and chlorosis [233]. Treatment of cowpea for example with different concentrations of Cd decreased the germination percentage, growth parameters and biochemical contents [234]. In addition, Cd-induced toxicity impairs the mobilization of minerals and carbohydrates during seeds germination like those of bean [235]. Another Cd-induced effect, the absorption of nitrate and its transport from roots to shoots were reduced by inhibiting nitrate reductase activity in the shoots [236]. Moreover, Cd-induced oxidative damage involves accumulation of lipid peroxides and oxidized proteins as a result of the inhibition of antioxidant systems in plants [237,238]. In a recent study, Siddhu and Khan [239] have assessed the toxic effects of $CdCl_2$ on growth, morphology, yield and biochemical aspects of *Phaseolus mungo* L. At 10^{-2} M concentration, Cd has shown deleterious effects on seed germination, germination relative index, length and dry weight of root and shoot, shoot root ratio and seedling vigour index, plant height, phytomass, number of leaves and branches, leaf area and chlorophyll contents, nitrate and nitrite reductase activity. On the other hand, Pandey and Singh [240] have observed a significant increment in the SOD, APX and GR activity and a decrease in CAT following Cd treatment. The enhanced activity of SOD and inhibition of CAT and POD produced a high level of H_2O_2 which probably is the main cause of oxidative stress due to Cd regimes in plants. However, the NAD(P)H oxidase activities were strongly provoked in response to Cd exposure [241]. The plants have some mechanism for metal tolerance or removal which includes (i) cell wall binding, (ii) chelation with phytochelatins (PCs), (iii) compartmentation of metals in the vacuole, and (iv) Enrichment in leaf trichomes.

Antioxidant defence system

Generally, plants growing in the metal enriched environment suffers from oxidative damage and the increased concentrations of heavy metals decrease the activity of antioxidant enzymes [242]. Mechanistically, auto-oxidation and Fenton reaction may lead to oxidative loss of defence enzymes as shown in a previous study, in which the catalase activity was directly inhibited by O_2^- [243]. However, recently published studies have illustrated some important strategies to protect plants from metal induced toxicity like superoxide dismutase (SOD) induction and activation and synthesis of antioxidant catalase [244,245]. Gwozdz et al. [246] have reported that heavy metals increased the activity of antioxidant enzymes whereas the higher concentrations of such metals did not enhance the SOD activity instead it decreased the catalase activity. For example, when pea plants were exposed to 20 µM and 200 µM concentrations of Cr (VI) for seven days, the total SOD activity of root mitochondria was severely affected. Herein, the SOD activity was increased by 29% at 20 µM whereas the SOD activity was significantly inhibited at 200 µM concentration of Cr (VI) in comparison to 20 µM Cr (VI) treatments. Following an increase in Cr concentration from 20 to 80 ppm, a similar decline in the catalase activity was observed. The changes in enzyme activities in various organs of plants growing in soil intentionally treated with heavy metals or soils previously contaminated with such pollutants have also been used as a marker for identifying the metal tolerant plants. For example, Rout et al. have used peroxidase and catalase activities as enzymatic markers for identifying Cr-tolerant moongbean and rice cultivars. Likewise, Wani et al. [83] have also observed a reduction in the glutathione reductase activity in *R. leguminoserum* inoculated pea plants, grown in soil contaminated with Ni and Zn. In yet other study the excess levels of Cu applied against plants grown for five days in hydroponic condition inhibited the growth of shoots and roots of rice and resulted in an oxidative stress in response to ROS formation. However, the stimulated antioxidative system seems to be an adaptive response of rice plant against Cu-induced oxidative stress. Moreover, proline accumulation in Cu stressed plant plays a role in providing an additional protection against Cu-induced oxidative stress.

Conclusion

Soils contaminated with heavy metals pose serious threat to agro-ecosystems and consequently to human health. In order to circumvent metal toxicity and to recover soil fertility efforts have been directed to reduce/completely eliminate the risk of heavy metals. However, despite different approaches employed to clean up metal polluted sites, adequate success has not been achieved so far. In order to achieve greater success, efforts are needed to create public awareness about metal toxicity and help from government body to carefully monitor and regulate the discharge of properly treated by-products of different industries. Furthermore, the use of chemicals and fertilizers that contain heavy metals should be avoided in agricultural practices so that the accumulation of metals within soils can be stopped. Moreover, there is a growing demand to develop novel environmental monitoring methods to rapidly detect the presence of toxic substances in consumable items. Collectively, all these require intensive efforts to achieve safe and secure global environment.

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