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Impact of Heavy Metals on Plants and Animals in Relation to Sewage Water – A Review

[1]Muhammad Mazhar Hussain, [3]Aiman Hina, [2]Asif Saeed, [4]Sanjeela Sabahat, [5]Fakiha tul Jannat and [3]Muhammad Aslam

[1]Directorate of Vegetable, Department of Horticultural Research and Development, National Agricultural Research Centre (NARC), Park Road, 45500, Islamabad, Pakistan

[2]Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan

[3]National Center for Soybean Improvement, National Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing, 210095, Jiangsu, Peoples Republic of China

[4]Pakistan Tobacco board, Ministry of Commerce, 45500 Islamabad, Pakistan

[5]Government College Women University, Faisalabad, Pakistan

Abstract: All plants are subjected to a multitude of stresses at some stage of their life cycle. This is a fact that, due to significant fiscal and agricultural importance, waste water is being extensively used for irrigation purpose. Heavy metal stress can be a major challenge to crop plants, particularly, in vegetable crops. The inhibition of plant growth and metabolic activities by heavy metals is one of the major reported impacts on plants. Lead (Pb) and cadmium (Cd) are well documented to show toxic influence on plants and human as well and they get entry of heavy metals via food chain. Due to toxic biological effects of heavy metals, different strategies may be used to overcome the problems caused by them. For using raw sewage water, detection of natural tolerance for heavy metals is very helpful for screening of crops, which are being grown on raw sewage water. Evolution is always present in all plants, so finding out the accessions which are tolerant to lead and cadmium, or finding out the genotypes which accumulate less metals in their fruit, could be achieved easily instead of finding out the exact gene for these metals. Plants irrigated by effluent have a greater tendency to accumulate cadmium and lead, which depends on plant genetic make-up, plant species and varieties, growth conditions, soil and environmental dynamics. Only, lead and cadmium are reviewed here for their possible impact on human and plants. Genetics of lead and cadmium tolerance is also reviewed, although lead has not been focused more due to its low solubility.

Key words: Cadmium, Heavy metal, Lead, Sewage water, Tomato.

INTRODUCTION

Heavy metal stress is an important a-biotic factor of crop yield reduction in metal contaminated soils. These soils mostly occur near factories and smelting units. Soils irrigated by raw sewage water also contain various types of heavy metals. It is very difficult to find out the impact of heavy metals on crop growth due to many factors, such as, soil pH, temperature, water availability, organic matter contents of soil and soil type. Moreover, if the cause of heavy metals is the use of sewage water, then it is very hard to assess the effect of these metals on crop growth as in most of the cases, sewage water has good economic impact on crop plants hiding the effect of heavy metals. Crop plants or wild types growing on metal effected soils are considered as a model for detecting the evolutionary effects of heavy metals on crop plants (Watkins and Macnair, 1991). Evolutionary mechanism vary among plants growing on natural metalliferourus or contaminated soils and these plants are categorized in three groups, viz. accumulators, metal excluders and metal indicators (Baker and Walker, 1990). Accumulator plants store more metals in their above ground part than that of present inside the soil while metal indicators also accumulate metals in above ground part but their concentration do not exceed from the heavy metal level in the soil. Metal excluders do not accumulate metal in above ground parts and prevent its entry into their system. Hyper accumulators contain more than 0.1% metal in their leaves on dry weight basis (Brooks *et al.,* 1998).

Heavy metals in Pakistan

The unplanned industrialization and untreated disposal of industrial effluents has led to increase the

Corresponding Author: Aiman Hina, National Center for Soybean Improvement, National Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing, 210095, Jiangsu, Peoples Republic of China
E-mail: aimanhina@yahoo.com

pollutants in the ecosystem (Diagomanolin *et al.*, 2004). In the province of Punjab only, there are about 46,000 industrial units of various categories, out of which 4,600 units are considered to be the major contributor of environmental pollution (Khalil *et al.*, 1996). The waste water from leather and steel industry has long been recognized as a main contributor to water pollution, as it contains highly toxic nature of water borne components, such as, Cr^{6+} and Cd^{2+}. Almost, all the industrial wastes are discharged directly in the environment without any treatment.

Most of the industrial effluents contain heavy metal ions; constantly add up the metal ion concentration in the environment that is highly toxic for terrestrial and aquatic organisms. Industrial wastewater pollution is also badly affecting human health as it gets into the food chain through drinking water (Singh *et al.*, 2010) and also through skin through absorption (Qu *et al.*, 2014). There is a lack of public awareness about environmental pollution, its adverse effects on life and need for remedy (Shentu *et al.*, 2008). In spite of the fact that fast expanding industrial areas are just flooding over the highly toxic metal ions loaded effluents, little is being done to save the human population from various health hazards, due to heavy metals. In addition to the natural metal crusts of the planet, heavy metals are also added into atmosphere in the dust particle form or vapors, the later may be controlled by some air filters. The presence of cadmium in soil poses a serious risk to all organisms. Despite the fact that cadmium has no known function in plants, due to high mobility and bioavailability, it enters into plant system and builds its level and become a cause of plant growth retardation, chlorosis and ultimately stunted growth. This raised level in plants acts as a reservoir for animals and human contamination source, yet, for the children, who ingest contaminated soil, an additional risk exists (Wagner, 1993). Elevated lead (Pb) levels in blood plasma of children from China have been reported by Huo *et al.*, (2007). Bone degradation, disorder of calcium and vitamin D metabolism and kidney damage are the result of cadmium toxicity in animals and humans (Wagner, 1993).

Heavy metals and plant growth

Metals with a density above $5g/cm^3$ are known as heavy metals. Out of total heavy metals which are found in nature, twenty one are non-metals, sixteen are light and the rest are heavy metals. Some of them, such as, zinc, iron, nickel and copper, are necessary for normal cellular growth, whereas, others, such as, Hg, Pb, and Ag, do not have any known cellular function (Nies, 1999). Transition metals are needed in trace concentrations and can become toxic for the cell when they exceed physiological levels. Similarly, metal ions, for which no physiological function has been shown, can be detrimental when they enter the cell in concentrations exceeding the tolerance limit (Inouhe *et al.*, 2000). Once heavy metal having positive charge particularly those with high atomic numbers, such as, Hg^{2+}, Cd^{2+} and Ag^{2+} enter inside the cell, tend to bind to SH group and stop the activity of sensitive enzymes. Other heavy metals having positive charge may interact with physiological ions, e.g., cadmium, may interact with zinc or calcium, Nickel and Cobalt interacts with iron, zinc with magnesium, thereby stopping the function of the respective physiological cation (Nies, 1999). High affinity of these metals with sulphur, nitrogen and oxygen, containing groups in biological molecules cause inactivation and damage to those biological molecules (Clemens, 2006). The apparent effect of heavy metals on plants is growth inhibition and chlorosis. Plants are reported to have various methods of detoxification for toxic metals, such as, uptake of selective metal, excretion by leaf falling, complex formation by specific ligands, and compartmentalization in the vacuole (Jiang and Liu, 2010; Gupta *et al.*, 2010; Singh *et al.*, 2010 and Maestri *et al.*, 2010).

Lead and cadmium uptake by plants

The source of lead uptake in plants is mainly through soil (Sharma and Dubey, 2005; Uzu *et al.*, 2009) although it is also from aerial parts (Uzu *et al.*, 2010) near roads and smelting industries (Srivastava *et al.*, 2015). According to Chang *et al.* (2014), fertilizers are main source of heavy metals in leafy vegetables. According to Seregin and Ivanov (2001), adsorption of lead takes place onto roots and then it binds to the rhizoderm cell surface by polysaccharides or in the carboxyl groups of mucilage uronic acid. Many plant species have been reported to have lead adsorption onto their roots such as *Lactuca sativa* (Uzu *et al.*, 2009), *Vigna unguiculata* (Kopittke *et al.*, 2007), *Brassica juncea* (Meyers *et al.*, 2008), *Brassica rapa* (Cenkci *et al.*, 2010), *Funaria hygrometrica* (Krzesłowska *et al.*, 2010) and *Festuca rubra* (Ginn *et al.*, 2008). On adsorption, lead follows passive pathway of water translocation to enter into plant. Observations of root apex for lead concentration gradient indicated that the absorption of lead is not uniform in plant roots (Seregin *et al.*, 2004). Lower pH of rhizodermic cells and thin cell walls increase solubility and absorption of lead in soil solution, that is why lead concentration is found more in root apical cells than others (Tung and Temple, 1996; Seregin *et al.*, 2004). From roots, lead reaches to endodermis by water streams of apoplast (Tanton and Crowdy, 1971; Lane and Martin, 1977). Here, it is choked by casparian strip and follows symplastic transport. Plant detoxification system acts here to get rid of lead.

Genetics and molecular mechanism of lead and cadmium tolerance

At present, no molecular mechanism has been reported for lead uptake into roots. Perhaps, it follows several pathways, particularly, ionic channels. As it is a non-selective phenomenon, the involvement of H+/ATPase pump is necessary for –ve membrane potential maintenance of rhizodermic cells (Wang *et al.*, 2007). Although several authors have reported the use of calcium channels for lead uptake (Pourrut *et al.*, 2008 and Wang *et al.*, 2007), but calcium inhibits lead absorption (Kim *et al.*, 2002), due to competition of lead cations for use of calcium channels (Huang and Cunningham, 1996). Despite the above reported selective pathways, lead also uses cation transporters which have less binding ability (Wojas *et al.*, 2007) and cyclic nucleotide-gated ion channels, which are non-selective pathways (Kohler *et al.*, 1999 and Arazi *et al.*, 1999).

The classification of plant species for lead tolerance, a transfer factor has been introduced, which indicates the lead concentration in plant and lead concentration in soil (Liu *et al.*, 2010; Arshad *et al.*, 2008; Bi *et al.*, 2010). But this feature is non-persistent for all the plant species depending on the physical and chemical properties of soil and also plant species used (Bi *et al.*, 2010; Liu *et al.*, 2010). Plants adopt following strategies in response to toxic heavy metals; Exclusion, in which plants evade unnecessary uptake of metals until and unless this phenomenon fails causing an unrestricted transport, 2) indication, whereby plants have no proper system to hinder metal uptake and internal accumulation represents the level of metal found in soils, and 3) accumulation and sequestration, whereby plants blindly accept large quantities of metals from soil, and transfer it to the aerial parts where it is stored (Baker, 1981). Another method of metal tolerance is hyper-accumulation (Adriano, 2001), which is being used as method of phytoremediation (gathering of expertise established on the use of plants to exclude or extract harmful organic and inorganic pollutants (Salt *et al.*, 1998; Vangronsveld and Cunningham, 1998; Wiszniewska *et al.*, 2015) Genetic variability in ninety nine pea genotypes was recorded, using five mg kg^{-1} cadmium, where its concentration in shoots varied by a factor of 2.8 (Belimov *et al.*, 2003).

The above review regarding lead and cadmium revels that plants irrigated by effluent have more tendency to accumulate cadmium and lead, which depends on plant genetic make-up, plant species and their varieties, growth conditions, soil and environmental dynamics. Legume crops tend to accumulate more cadmium than cereals. While investigating the response of different levels of cadmium on rice plant, Wu *et al.* (2006), established that the germination was stimulated to some extent in low cadmium application (0.01 to 1.50 mM Cd), while adversely affected under 2.0 mM Cd. Root and shoot Cd concentration increase by elevating cadmium level. Differences among genotypes were pronounced regarding cadmium contents in shoots rather than roots. Reduction in100 seed weight, seed yield and harvest index, number of pods per plant and seeds per pods in mung bean genotypes has also been reported (Wahid and Ghani, 2008). During the early growth period of wheat plants, cadmium uptake and accumulation was found (Shukla *et al.*, 2003). Cadmium has been reported to inhibit the transporters responsible in the translocation such as radial movement in the root, loading into the xylem vessels or absorption to the leaf (Sandalio *et al.*, 2001). Changes in conducting xylem tissue are also a clue that support restricted translocation of nutrients from the roots (Barcelo *et al.*, 1988). Genetic differences in heavy metals accumulation by cereal and legume crops have been reported (Kumar *et al.*, 1995; Cieslinski *et al.*, 1996) and this is due to the fact that cereal and legume crops differ in their root architecture, orientation and metal uptake capacity (Marschner, 1995). Varietal selection in cereal crops for contaminated soils may add to safer crop production on heavy metal contaminated sites (Chamon *et al.*, 2005).

A greater accumulation of metals has been reported in dicotyledonous than monocotyledonous crops (Kabata-Pendias *et al.*, 1993). Cereal crops, which are mostly monocots, are known as excluders of metal cations (Baker *et al.*, 1994). Cadmium accumulation behavior varies on the species level, cultivar level and even at individual plants level. Inbred lines of maize (Hinesly *et al.*, 1982), wheat (Grant and Bailey,1998; McLaughlin *et al.*, 1999; Bose and Bhattacharyya, 2008), rice and vegetables (Dong *et al.*, 2011; Zhao *et al.*, 2012), vetiver plants (Punamiya *et al.*, 2010), maize and beans (Guo and Marschner, 1996) accumulated different levels of cadmium and lead even at the same metal concentration. Among wheat cultivars, 2.5 times difference of cadmium in grain was reported (Wenzel *et al.*, 1996). Variation in heavy metal accumulation exists within plants and the elevated levels were found in roots followed by leaves and seeds (Machelett *et al.*, 1993).

Lead and cadmium accumulation in plants

On penetration into roots, lead is transported to aerial parts or it may be fixed in root zone rather than translocation to aerial parts. Above 95% fixation in root zone has been reported in *Vicia faba*, *Pisum sativum*, and *Phaseolus vulgaris* (Piechalak *et al.*, 2002; Shahid *et al.*, 2011), *Nicotianatabacum*, (Gichner *et al.*, 2008), *Avicennia marina* (Yan *et al.*, 2010), *V. unguiculata* (Kopittke *et al.*, 2007), *Lathyrus sativus* (Brunet *et al.*, 2009), non-accumulating *Sedum alfredii* (Gupta *et al.*, 2010), and *Allium sativum* (Jiang and Liu, 2010). The fixation of lead, more in root zone than aerial parts, may be due to immobilization by negatively charged pectins within the cell wall (Kopittke *et al.*, 2007; Arias *et al.*, 2010), lead salts precipitation in intercellular spaces (Kopittke *et al.*, 2007; Islam *et al.*, 2007; Meyers *et al.*, 2008), buildup level in plasma membranes (Jiang and Liu, 2010; Islam *et al.*, 2007), or sequestration in the vacuoles of rhizodermal and cortical cells (Seregin *et al.*, 2004; Kopittke *et al.*, 2007).

Hyper-accumulator plant species translocate most of the lead to their aerial parts, such as, brassica pekinensis and pelargonium (Liu *et al.,* 2008; Xiong *et al.*, 2006; Arshad *et al.*, 2008). Such plants tolerate higher concentrations of lead ions due to various detoxification mechanisms, such as, uptake of selective metals, excretion and compartmentalization. The presence of organic chelators like ethylene ediamine tetra acetate (EDTA) (Zaier *et al.*, 2010; Barrutia *et al.*, 2010) or micro-organisms (Arias *et al.*, 2010; Punamiya *et al.*, 2010) also help to increase translocation of lead to aerial parts. Transpiration mechanism (Liao *et al.*, 2006, Arias *et al.*, 2009) must be involved in translocation of lead through xylem (Verbruggen *et al.*, 2009). Lead reaches to leaves through vascular flow (Sharma and Dubey, 2005) and it also makes complexes with amino acids or organic acids (Vadas and Ahner, 2009; Roelfsema and Hedrich, 2005; Maestri *et al.*, 2010).

Arshad *et al.*(2008),Uzu *et al.*(2009) and Liu *et al.*(2010) suggested translocation factor for flow of lead to aerial parts from roots. The results of this translocation factor mostly indicate a lower value, which shows lower transfer rate of lead to aerial parts (Uzu *et al.*, 2009; Liu *et al.*, 2010). According to Kabata-Pendias *et al.* (1993), transport of metal to grain tissues is hindered and most of the quantity is dumped in roots. Thus, excluders may include cereal crops, such as, wheat, barley, oat, rye and corn (Kabata-Pendias *et al.*, 1993).Various vegetable species (Murtaza *et al.*, 2008); peanut (Su *et al.*, 2013), hot pepper (Xin *et al.*, 2013), rice varieties (Chamon *et al.*, 2005; Abbas *et al.*, 2006), mungbean (Wahid and Ghani, 2008), potato (Dunbar *et al.*, 2003); soybean and bean genotypes (Bell and Gonzalez, 2009; Metwally *et al.*, 2005), and wheat cultivars (Jalil *et al.*, 1994; Chamon *et al.*, 2005) differed in metal uptake and accumulation due to different levels in soil, metal characteristics, metal speciation, presence of other counter species of ions (Hernandez *et al.*, 1996); Obata and Umebayashi, 1997),environmental growth condition and crop genetic factors.

The lowest transfer factors of Cd were found for grains of maize, peas, oat and wheat whereas the highest values were reported for leaves of spinach and lettuce. The Cd accumulation by crop species decreases in the following order: leaf vegetable > root vegetable grain crops (Page *et al.*, 1987). The highest proportions of Cu and Cd taken up by rice and wheat varieties were retained in the root (Chamon *et al.*, 2005). In the grains of effluent irrigated wheat, the concentration of Cd was found above the permissible levels recommended by WHO for foodstuff sampled at Gandakhue, Mulkhanwala, Awanwala and Kanuwala along Satiana road drain, Faisalabad (Farid, 2003). The value of transfer factor (TF) from root to shoot was found larger than from shoot to grain. Gupta *et al.* (2006) observed that more quantities of Fe, Cu and Zn accumulated in seeds compared to Cd and Cr by chickpea varieties when grown under fly ash.

Effects on germination and growth

Inhibition of seed germination has been reported in *Hordeum vulgare*, *Elsholtzia argyi*, *Spartina alterniflora*, *Pinus halepensis*, *Oryza sativa*, and *Z. mays* (Tomulescu *et al.*, 2004; Islam *et al.*, 2007; Gautam *et al.*, 2010). At seedling stage, lead exposure in plants also strongly limits the development and sprouting of seedlings (Dey *et al.*, 2007; Gichner *et al.*, 2008; Gopal and Rizvi, 2008). Lead may speed up germination and simultaneously induce adverse effects on the length of radical and hypocotyl in *E. argyi* (Islam *et al.*, 2007) and this inhibition of germination may result from the interference of lead with protease and amylase enzymes (Gautam and Flora, 2010).

Inhibited growth of roots and aerial plant parts has been reported at low concentrations (Islam *et al.*, 2007; Kopittke *et al.*, 2007) specifically root growth, which may be correlated to its higher lead content (Liu *et al.*, 2008), swollen, bent, short and stubby roots that show an increased number of secondary roots per unit root length (Kopittke *et al.*, 2007), mitochondrial swelling, loss of cristae, vacuolization of endoplasmic reticulum and dictyosomes injured plasma membrane and deep colored nuclei as reported by Jiang and Liu (2010) are major toxicity symptoms of lead. Inhibited root growth has been reported by Arias *et al.*, 2010, Islam *et al.*, 2007; Kopittke *et al.*, 2007. Lead is a ubiquitous toxic metal, which has mutagenic, carcinogenic, genotoxic, anthropogenic and phytotoxic effects. Lead is the most toxic and frequently faced contaminants (Cecchi *et al.*, 2008; Grover *et al.*, 2010; Shahid *et al.*, 2011), which affects the plants.

Effect on plant biomass

The reduction in plant biomass has been reported (Gopal and Rizvi, 2008; Gichner *et al.*, 2008; Islam *et al.*, 2007; Piotrowska *et al.*, 2009;Wiszniewska *et al.*, 2015) by lead toxicity and this may be due to nutrient metabolic disturbances (Kopittke *et al.*, 2007; Gopal and Rizvi, 2008) and disturbed photosynthesis (Islam *et al.*, 2008). Inhibited growth may not necessarily be correlated to a reduction in biomass (Kosobrukhov *et al*., 2004; Yan *et al*., 2010). Serious retardation of plan growth could be observed when 50% fall in CK occurs for growth and physiological parameters (Li *et al*., 2014). Ultra-structural changes have also been observed in the cells of cyanobacteria after exposing to cadmium, disintegration, and the damage of thylakoid membranes in photosynthetic lamellae was also detected. The damage of thylakoid membranes resulted in reduced photosynthesis. Cadmium has also been reported to inhibit photosynthetic activity of nostoc. Many of the cyanobacterial cells possess pigment granules, which store arginine aspartate. These granules act as nitrogen storage device in the cell. It was proposed that these bodies may be the part of cells internal system damaging. The toxic limit, 10 or 15µg /ml produces great changes due to a greater accumulation of Cd in the cell. Mitochondria are the organelles for damaging and changing in the nucleus.

The nucleolar fusion bodies also changed in the presence of Cd, which is brought up by plants as Cd^{2+} and its amount in normal plants ranged from 0.1-2.4 milligram per kilogram (Alloway, 1995) and with great amounts, it severely lowered plant growth and dry biomass production. The low amount of Cd in the organism tissues, at which it has damaging effects, is five milligram per kilogram (Macnicol and Beckett, 1985). There are many radicals which interact with Cd uptake by plants. Therefore, tomato can be used as a model plant for genetic studies for other species with flesh berry fruits (Sun *et al.*, 2013; Ahsan *et al.*, 2007). Gratão *et al.*, (2008) reported increased peroxidation of lipids, catalase activity, GR activity and reduced GPOX enzyme activity in tomato plant under cadmium stress. Ammar *et al.*, (2008) and Delpérée and Lutts (2008) reported that these metals could move to the upper parts of plant such as fruit and their amount was found low in leaves compared to that fruits, which is edible portion of the plant. But the amount of cadmium was low in leaves of plants at the age of 204 days. The amount of cadmium reached to maximum when the plants were of seventy five days in the leaves, roots and fruits, which depicted that cadmium is transported to these organs after development.

When the plants were treated with cadmium chloride the catalase activity increased in fruits. Hence, there is a dire need of further research in the production of chemicals such as phytochelatins and even other antioxidant systems and metabolites, such as, GT (glutathion), AS (ascorbate), AA (amino acids), PA (polyamines) and OA (organic acids), which may change in the presence of cadmium in plant (Dong *et al.*, 2006). The activity of glutathion reductase increased suggesting that cadmium caused the fruit to be affected. It was necessary for the sampling to be taken shortly after the fruit development. In the experiment, it was found that as the enzyme activity was increased in the plant, so it responded as an antioxidant against the stress of heavy metals, such as cadmium chloride. As the activity of enzymes increased in all the organs of the plant indicating that the cadmium is increasing in the plant and the response of the plant against heavy metals also increased in all the organs especially three organs, namely leaves, roots and fruits. The peroxidation of lipids in the fruit was found and it displayed that there is enhanced enzyme activity and in leaves and roots the other systems may also be involved. All this was happening when cadmium concentration was increasing in these organs of the plant. This revealed that antioxidant system was playing an important role. It was also found that the activity was more prominent in fruits as compared to the roots and leaves. It was also an important finding that, as compared to roots and leaves, the fruit was exposed to cadmium for less time and activity of peroxidation of lipids was more than those organs for the treatment of cadmium and also for stepwise increase of level.

Effects on proteins and enzymes

Lead toxicity may reduce protein pool (Chatterjee *et al.*, 2004; Mishra *et al.*, 2006; Garcia *et al.*, 2006; Piotrowska *et al.*, 2009), which is the result of several lead effects: acute oxidative stress of reactive oxygen species (ROS) (Piotrowska *et al.*, 2009; Gupta *et al.*, 2009; Kumar *et al.*, 2015), modification in gene expression (Kovalchuk *et al.*, 2005), increased ribonuclease activity (Gopal and Rizvi, 2008), protein utilization by plants for the purposes of lead detoxification (Gupta *et al.*, 2009), activation of monoterpene indole alkaloids (Matsuura *et al.*, 2014) and diminution of free amino acid content (Gupta *et al.*, 2009) that causes disturbances in nitrogen metabolism (Chatterjee *et al.*, 2004). The increase in certain amino acid contents (Qureshi *et al.*, 2007) or in total protein content particularly redox maintaining proteins (Mishra *et al.*, 2006) is essential for plants to cope lead toxicity (Gupta *et al.*, 2010).

This behavior seems similar to that of ascorbate functions or similar to how metals are sequestered by glutathione (GSH) or phytochelatins (PCs) (Brunet *et al.*, 2009; Liu *et al.*, 2009; Yadav, 2010; Jiang and Liu, 2010). Changed protein profile of roots due to lead toxicity seems to be similar to that of transcriptome profile of several enzymes including isocitratelyase, cysteine proteinase SAG12, serine hydroxymethyl transferase, and arginine decarboxylase (Kovalchuk *et al.*, 2005). Metallothioneins (MTs) belong to a super family of intracellular metal-binding proteins, present in virtually all living organisms, with features common to the archetypal MTs. These unique biomolecules have captured the attention of biologists and chemists alike due to their remarkable chemical structure that confers a degree of specificity, stability and dynamic behavior almost impossible to predict from the properties of their organic and metallic ingredients. MTs form thiol bonds with metal ions to scavenge toxic heavy metals (cadmium, mercury, etc.), to store biologically essential metals (copper and zinc) and to regulate metal dependent processes fundamental to cellular pathways. MTs have been isolated from various organisms including plants, vertebrates, invertebrates, fungi, unicellular eukaryotes and some prokaryotes.

These proteins have a large number of cysteine residues which form thiolate bonds with transition metals (d10 metal ions) stabilize the protein 3D structure and result in high metal content. MTs have been isolated from different organisms in bound forms to Cd, Cu and Zn; on the other hand, they can also bind Hg, Pb, Bi, Ag, and Au in *in vitro* experiments. Their capacity to bind to both, the essential and nonessential metals, points to another function of these proteins in heavy-metal detoxification, in addition to regulation of the biological activities of essential trace elements.

Mineral and water status effects

Disruption of plant water status (Brunet *et al.*, 2009), decreased transpiration and moisture content, reduced leaf surface area for transpiration that is caused by decreased leaf growth, reduced cuticle layer and influence on turgor pressure, are the results of lead toxicity in plants. In order to cope with these problems, plants mostly produce high concentrations of osmolytes, under lead stress conditions (Qureshi *et al.*, 2007). Increased concentration of abscisic acid (ABA), a phytohormone (Roelfsema and Hedrich, 2005) in roots and aerial parts is correlated to the presence of Pb^{2+} ions (Parys *et al.*, 1998; Atici *et al.*, 2005). This increased concentration of ABA leads to stomatal closure (Mohan and Hosetti, 1997) limiting gas exchange and water losses by transpiration (Parys *et al.*, 1998).

Reduction in divalent cations, such as, Zn^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, and Fe^{2+} had been reported in leaves of *Z. mays* (Seregin *et al.*, 2004), *O. sativa* (Chatterjee *et al.*, 2004), *Brassica oleracea* (Sinha *et al.*, 2006), *Medicago sativa* (Lopez *et al.*, 2007), *V. unguiculata* (Kopittke *et al.*, 2007), and *Raphanus sativus* (Gopal and Rizvi, 2008). A changed physiological function of plant or competition of ions usually results in lower uptake of specific nutrient elements. Potassium and lead have similar radii (Pb^{2+}: 1.29 Å and K+: 1.33 Å): which results in a competition to enter into plant through the same potassium channels (Sharma and Dubey, 2005). Efflux of K^+ from roots is the result of lead interaction on K^+-ATPase and -SH groups of cell membrane proteins. The decreased shoot nitrate content, nitrate reductase activity and free amino acid content in *B. pekinensis* have been reported by Xiong *et al.* (2006).

Bioavailability of metals

The bioavailability of metals in soils depends on their solution concentration which in turn is dependent on the soil processes like cation exchange, specific adsorption, precipitation and complexation (Harter and Naidu, 1995; Basta *et al.*, 2001). Many factors like total concentration and speciation of metals, soil mineralogy, pH and CEC (Basta *et al.*, 2001), total soil organic content (Harter and Naidu, 1995), soil moisture percentage and biological influence (Harter and Naidu, 1995) govern the bioavailability of metals. Many of these factors vary seasonally and temporally. Besides being interrelated, they also inhibit prediction of metal bioavailability. So changing one factor may affect several others. The differential response of species and varieties to environmental changes also contributes to differences in the uptake of heavy metals from soil.

Effects of plant factors on metal bioavailability

Plant genetics, physiology and morphology can also control the bioavailability of heavy metals. However, these effects might be variable and require an intensive study. It is concluded that metals bioavailability is modified by soil processes, genetic and environmental factors (Xin *et al.*, 2013). These effects may be exerted either through the root or shoot or through the whole plant (Yan *et al.*, 2010). Soils irrigated with tube well, canal or effluent water differs in ionic composition and other properties; similarly plants grown on these soils may have variable metal composition (Islam *et al.*, 2007).

Effect of sewage water in plants

According to Lope *et al.*, (2007), in fact, tree growth was greater (P< 0.01) in the field irrigated using municipal waste water than in plots irrigated with well water, as indicated by different plant heights and diameter such as 17.95 ± 1.33 cm diameter at breast height, 10.04 ± 0.15 m height, 8 ± 0.27 m crown length, 2.53 ± 0.17 m crown average diameter, 264.20 ± 30.02 cm^2 basal area and 0.139 ± 0.013 m^3 standing volume of the trees in waste water irrigated field. Similarly, an increase in the growth of olive (*Olea europaea*) was observed in trees, due to irrigation with municipal waste water. Addition of municipal waste water on Eucalyptus has resulted in a doubling of growth rate when compared to *Eucalyptus grand* is grown in a rain fed site in four years. The increased growth may be linked to sufficient availability of water and better status of nutrients in soil. Since municipal waste water contains plant nutrients and organic matter, it may improve the properties of soil for an increase in growth and biomass production (Lopez *et al.*, 2007).

The increase in growth indicates that the waste water application influenced the physiological processes, facilitated early needle initiation and resulted in a net increase in the number of needles. An increase in needles could have captured more solar energy for metabolic use, fixed more CO_2, and produced greater photosynthesis, and growth. As a whole, the use of municipal effluent in irrigations can be an overflowing resource from the nutrient elements. As a matter of fact, high nutrient concentrations in effluent compared to those in well water, cause the nutrient accumulation in the soil thereby makingplants an easy access to the high nutrient concentration (macro and micro elements) and increases their growth.

CONCLUSION

An increasing shortage of fresh water is shifting the trend of farmers to use alternate sources of irrigation for their crop. Sewage water being applied near peri-urban areas contains many beneficial as well as harmful elements. Due to robust growth of crop in sewage water, there is negligence by societies to think about heavy metals inside those crops. These metals cause serious cancer problems in human, restarted growth or silent cell death in plants. Breeding for crops which have some tolerance mechanism or escape mechanism is only possible economic solution to address these problems. Moreover, selection in existing germplasm could also be considered at an initial phase of breeding programs for screening against these heavy metals.

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