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LEAD UPTAKE, TOXICITY AND MITIGATION STRATEGIES IN PLANTS

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ABSTRACT

The issues of heavy metal adulteration are becoming common in world. Heavy metal toxicity cases are prevailing in mining industries, smelters, power plants based on coal burning, agriculture, etc. There are several heavy metals, such as Cd, Cu, Pb, Cr, Hg, Ar, etc. These heavy metals are major pollutants of environment, particularly in areas with increasing anthropogenic activities. The cumulation of heavy metal in soils is of great concern in agriculture because of the deleterious effects on food safety, crop growth and soil organisms' health. Heavy metals affect several physiological and biochemical processes in plants. They diminish crop yield by bringing toxic effects to several physiological processes in plants such as, seed chlorophyll reduced by the production of reactive oxygen species, affecting the redox balance and instigating oxidative stress. Lead (Pb) is one of the looming heavy metal which is neither essential nor plays any part in the course of cell metabolism. Pb has toxic effects on plant which may include inhibition of photosynthesis, disruption of mineral nutrition and water balance, and disturbs membrane structure and permeability. Its phytotoxicity can also affect human health and can prove detrimental through food chain. However, in order to combat the effects generated by heavy metal stress particularly by Pb, several amelioratives can be used. Pb phytotoxicity can be ameliorated by the application of certain phytohormones which can be a part of signal transduction pathway, or they may trigger reactions and causative agents to respond to stress. Various signaling molecules such as NO, H₂S, CO, etc. enhance the activity of antioxidant enzymes, level of secondary metabolites and osmolytes, hence scavenge the oxidative stress due to generation of free radicals in response to heavy metal stress.

Keywords: Heavy metal stress, Lead (Pb), signaling molecules, Nitric oxide, Phytohormone, secondary metabolite, antioxidant enzymes

INTRODUCTION

Poisonous elements are frequently described as “trace metals” or “heavy metals”. Heavy metals are often considered as the elements existing in the soils in small amounts. However, heavy metals are those toxic metals which cause hazardous effects to the plants when uptake excessively. Out of naturally occurring elements, 53 are stated as heavy metals and the maximum of these metals have no vital function in plants. Few heavy metals like Zn, Cu, Mn, Ni, Se, Co, Cr and Mo have vital biological functions, and consequently have beneficial effects in context of agricultural production (Salla *et al.*, 2011). Nevertheless, such metals and those lacking any vital metabolic activity such as Zr, Sb, As, Pb, Hg and Cd will significantly diminish agricultural production if their level increases to optimum concentrations (Xiong *et al.*, 2014; Pierart *et al.*, 2015).

Pb being one of the impending heavy metal is neither crucial element nor plays any part in the course of cell metabolism, but is effortlessly engrossed and accrued in several parts of a plant. It is extremely moldable, grayish-white trace element. Under standard atmospheric conditions, Pb occurs in solid state and in comparison, to other metals, it

is dense, brittle, and also very fragile with poor electrical properties. Pb is comprised of 0.002% of earth's crust and is extremely toxic and non-disruptive heavy metal. On the basis of rate of incidence, toxic effects, and risk for human susceptibility, it is considered as the second most harmful element, after arsenic (Agency for Toxic substances and Disease Registry 2003). The presence of Pb in natural environment is mostly the result of numerous current activities and natural events such as breakdown of rocks i.e. weathering, erosion of land, volcanic outbreaks, forest fires, and disintegration products of radioactive metals. Due to the fast-industrial development and various human activities like mining and casting of Pb ores, it has turned out to be the main atmospheric pollutant (Obiora *et al.*, 2016). Ayurveda related medicines are thought to contain more concentration of Pb. In latest research, Pb amount has been examined in blood samples of Ayurveda customers. Out of 115, 40 per cent consumers have been found to contain Pb approximately 10µg/dL in their blood. Most suitable consumption of this metal in human diet is around 25µkg⁻¹ of human body weight (Fang *et al.*, 2014). It is found in trace amount in virtually all crops. However, its intensity can be significantly increased by growing crops on soils polluted by Pb.

The accrual of this metal in plant tissues i.e. roots, shoots, and leaves have been established by Tangahu and colleagues. They proposed that numerous plants can accrue Pb in their tissues to about greater than 50 mg/g dry weight of plant. Even though, it is found in 3 components of our environment which is soil, water, and air in varying amounts. Soil pollution with Pb is measured as the most dangerous menaces to human and other life forms, and is comprehensively being monitored (Ma *et al.*, 2016). Pb is found to cause a large variety of adverse effects on living creatures which includes morphological, physiological and biochemical hazards. It negatively affects crop growth, root length, germination of seeds, seedling growth, transpiration, chlorophyll formation, chloroplast lamellar organization, and division of cell (Gupta *et al.*, 2009; Maestri *et al.*, 2010). The negative impact of Pb metal has been shown in *Eichhornia crassipes*, where the growth of plant is drastically affected by high concentration of Pb. Moreover, it has been found that in *Eichhornia sp.*, the activities of antioxidant enzymes are under high concentration of Pb (Malar *et al.*, 2014).

Uptake and accumulation of Pb in plants

Apart from the specific circumstances where plants are grown in proximity of metal concentration, the chief route through which plants acquire metals is via the soil root absorption (Uzu *et al.*, 2009). Pb is believed to possess low solubility and is less available to plant for absorption as it undergoes precipitation as phosphates and sulphates, which normally occur in the plant rhizosphere (Blaylock *et al.*, 2000). Portion of Pb which occurs in the soil water gets adsorbed on the roots of plants which then attaches itself to carboxyl groups of uronic acid, or directly to the rhizoderm cell surface polysaccharides (Seregin and Ivamov, 2001). After the adsorption of Pb on the rhizoderm cell surface, it penetrates the roots by passive method and pursue channels of water translocated (Fig. 1). But Pb uptake is not even through the roots of plant because of the concentration gradient of the metal that can be measured from root apex. However, larger percentage of Pb is observed in apices of root, since root cells present here are young and possess thin cell walls which promote absorption of roots (Seregin *et al.*, 2004).

There are some of the factors on which Pb uptake relies which includes total concentration of soil, physiological and chemical conditions of soil, the plant species and concerned genotypes (Alexander *et al.*, 2006). Researchers have described the pH variability effect in Pb uptake in several species of plants. In the soils with low pH of around 3.9, greater movement of Pb is observed leading to greater uptake. Corn, beans, *lactuca* species, and radishes are more prone to the toxic effects of lead when these are planted on calcareous soils (Bala and Setia 1990). However, Pb uptake can be diminished by

adding phosphate lime, organic matter, and chloride to soil (Liebhardt and Koske, 1974). Generally, it is found that dicots accrue considerably greater concentration of Pb in the roots as compared to monocots. The process by which this metal reaches the roots cellular level is unidentified. Pb can penetrate the roots by many routes, and ionic channel is one of the specific routes. Though, uptake of this metal is not a selective process, it still relies on the operation of an H^+ /ATPase pump in order to retain a high negative membrane potential in rhizodermal cells (Wang *et al.*, 2007). In case of Chinese sumac, it has been observed that Pb metal ions are absorbed via roots and they remain there, with limited transport to shoot and foliar parts of the plant.

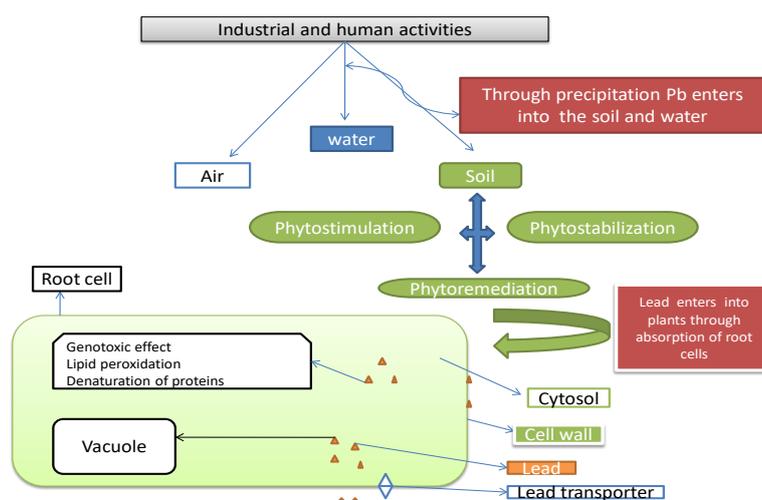


Fig. 1: Mechanism of Pb uptake by plants.

After uptake through roots Pb, some part of Pb gets accumulated and rest is translocated to the above ground parts of the plant. According to the previous studies it was reported that in majority of the plants like *Vicia faba*, *Pisum sativum*, and *Phaseolus vulgaris*, more than 95% of Pb gets accumulated in the roots and a major portion of it is translocated to the aerial parts (Malecka *et al.*, 2008; Shahid *et al.*, 2011; Johnson *et al.*, 1977). In the root endodermis, a barrier is present that limits the translocation of Pb from roots to other plant tissues. It is believed that casparian strips present in the endodermis are mainly responsible for limiting the transport of Pb through the endodermis into vascular tissue (Seregin *et al.*, 2004). Usually, in the endodermis, Pb can be precipitated by the casparian strip and then undergoes symplastic transport, followed by the separation of large fraction of Pb by the detoxification system of plants (Kaur *et al.*, 2013); Li *et al.*, (2016) explained that accumulation of Pb occurs in root cells close to the inner cell wall and the cell gap. Its occurrence has been confirmed by means of transmission electron microscope (Li *et al.*, 2016). Excessive concentration of Pb metal results in plasmolysis, disrupts structure of cell membrane, and causes membrane vacuolation. It has been reported that translocation of metal to above ground parts from roots may need transportation via the xylem vessels. Beside the xylem vessels, this metal transportation can

also occur through phloem (Rascia and Navari-IZZO, 2011). This has been confirmed via an X-ray mapping method, where it's been found that a larger proportion of Pb accumulation appears close to xylem and phloem cell of *Prosopis* plant (Zhenge *et al.*, 2012).

Effects of lead (Pb) in plants

Pb is known to have deleterious effect on the various aspects of plant such as germination rate of seed, growth of seedling, dry root and shoot mass, photosynthesis, respiration, plant-water relation, mineral nutrition, and several enzymatic activities (Munzuroglu and Geckil, 2002) (Fig. 2). Generally, Pb effects are more prominent at greater levels and perpetuation. However, even the less concentration of metal may trigger some biological processes (Gomes, 2011). These harmful effects are generally identified as certain symptoms such as chlorosis, necrosis on the surface of leaf, leaf senescence, and restricted growth. At greater levels, seed germination is severely affected. Root and shoot growth is also inhibited in plantlet stage, where roots are more sensitive to this effect. It deleteriously affects growth by diminishing the absorption and translocation of mineral nutrients in plants like, Ca, Fe, Mg, P, and Zn and by the attachment of the ions to ion-carriers which results in their unavailability for uptake and movement from roots to leaves (Xiong, 1997). Hence, various physiological and biochemical processes are greatly affected by Pb with photosynthesis as the most important (Gomes, 2011).

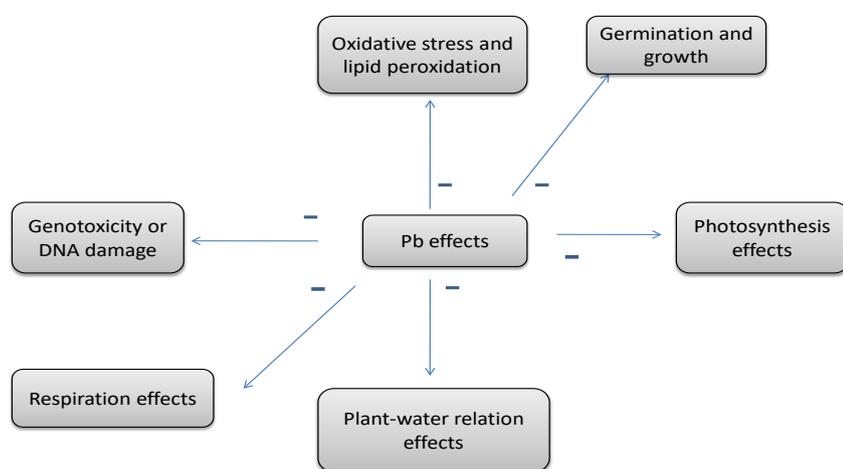


Fig.2: Diagrammatic representation of Pb toxicity on physiological and biochemical processes in plants.

Effects on germination and growth

Harmful effects on germination and growth of plant can take place when plants are subjected to Pb even at very small levels (Kopittke *et al.*, 2007). Very low levels of Pb greatly inhibits seed germination (Islam *et al.*, 2007) in several plants such as *Hordeum vulgare*, *Elsholtzia argyi*, *Spartina alterniflora*, *Pinus halepensis*, *Oryza sativa*, and *Zea mays*, seed germination inhibited by Pb has

been described (Senger *et al.*, 2009). However, at higher levels, Pb can increase the rate of seed germination and concurrently causes harmful effects on the radicle and hypocotyl length in *E. argyi* (Islam *et al.*, 2007). Intervention of Pb with protease and amylase enzymes can be the cause of decreased rate of seed germination (Senger *et al.*, 2009). Furthermore, Pb also results in the reduction of utilization of stored food which in turn causes reduced radicle formation, inhibition of proteolytic activities and destruction of osmoregulation in cells that inhibits seed germination and plantlet growth. Pb pollution damages early development of plants and harmful effect of Pb on development of radish plants (Tomulescu *et al.*, 2004). The biomass of plant is reduced on its contact with larger levels of Pb (Singh *et al.*, 2010). In *Prosopis* species also length of root is considerably repressed (Arias *et al.*, 2010). Furthermore, as the plants exposed to Pb even at lesser levels the development of above ground parts as well as plant roots is curbed (Kopittke *et al.*, 2007). But the rate at which growth due to this metal is inhibited in roots is much greater than rest of the parts of plants (Liu *et al.*, 2008). Moreover, harmful effects on seed germination and plant growth due to Pb vary from species to species, its concentration, developmental phase, and the amount of period a metal is exposed to plant (Gul *et al.*, 2018).

Photosynthesis effect

Decrease in the rate of photosynthesis is the main example of phytotoxicity due to Pb resulting in decreased crop production (Singh *et al.*, 2010). It has been established that this metal hinders the production of plastoquinone, carotenoids, and electron transport chain (Qufei and Fashul, 2009) and damage the working of enzymes involved in the fixation of carbon dioxide (Mishra *et al.*, 2006). In crops developed in Pb stress conditions, both stomatal as well as non-stomatal constraints are known for the reduction of fixation of carbon dioxide (Qufei and Fashul, 2009). Furthermore, toxic effects due to Pb stimulates oxidative stress in plants and further increases the production and action of enzyme chlorophyllase which is responsible for the cessation of chlorophyll resulting in reduction of photosynthesis process (Liu *et al.*, 2008). Usually, chlorophyll a is less susceptible as compared to chlorophyll b (Xiong *et al.*, 2006). Damage to the distinctive green color of chlorophyll takes place only when the breakdown of the porphyrin ring occur (Harpaz-Saad *et al.*, 2007). Pb results in less absorption of important minerals like Mg, Fe, etc. which in turn affects formation of chlorophyll molecules. The thylakoid structure is affected because of empathy of Pb to N- and S- ligand proteins (Ahmed and Tajmir-Rahi, 1993).

Respiration effects

Photosynthetic plants on its exposure to Pb, encounter deleterious impacts on respiration and ATP composition. The impact of Pb on respiration has been reviewed a little apart from the photosynthesis (Seregin and Ivanov, 2001). The impact of Pb on respiration has been conducted mainly on leaves while its impact on roots is not known. It is found to have an impact on ribulose-bisphosphate carboxylase action in C3 plants which is responsible for the acclimatization of CO₂, with no effect on its oxygenase activity. It has been found that divalent cations such as Pb, Zn, Cd, and Ni may get attached to membrane of mitochondria, causing the ET disruption which may result in the phosphorylation decoupling (Romanowska *et al.*, 2002, 2006). Pb has been observed to inhibit Hill reaction in chloroplasts of spinach, besides photophosphorylation. Furthermore, it has a greater impact on cyclic photophosphorylation as compared to non-cyclic photophosphorylation (Romanowska *et al.*, 2008). It has been shown that Pb attaches with membranes and this attachment results in some physiological impacts. Mitochondria from pea leaves with Pb treatment causes the oxidation of substrates such as glycine, succinate, and malate at greater rate as compared to mitochondria from the pea leaves not treated with Pb (Romanowska *et al.*, 2002).

Plant- water relation effects

Under Pb stress, plants experience changes in plant-water relations resulting in lowered turgor pressure (Rucinska-Sobkowiak *et al.*, 2013). In plants, Pb diminishes the elasticity of closes cell wall, as a result of which turgor pressure of guard cells is reduced (Pinho and Ladeiro, 2012). Plants exposed to Pb causes reduction in the levels of sugar, amino acids and other molecules which are responsible for maintaining turgor pressure of the cell (Barcelo and Poschenrieder, 1990). Furthermore, a plant hormone abscisic acid is responsible for closing the stomata and it has been stated that Pb stimulates the formation of abscisic acid which in turn decreases transpiration rate (Atici *et al.*, 2005). In several crop plants, changes in plant water-relation due to Pb stress has been described (Sharma and Dubey, 2005). For example, higher levels of Pb diminishes the rate of transpiration in Sunflower (*Helianthus annuus L.*), produces water deficit conditions and cause the formation of proline to manage water stress (Kastori *et al.*, 2008). Nevertheless, the plants having more stomatal density are able to cope up with such impacts (Elibieta and Miroslawa, 2005). In plants under Pb stress, respiration through leaves is also affected because of accumulation of waxy layer on leaves. It has been seen in soybean. Moreover, such changes in respiration and CO₂/O₂ inequity in plants results in oxidative phosphorylation which in turn affects water status (Elibieta and Miroslawa, 2005).

Genotoxicity or DNA damage

Components causing harm to the genetic material of cells that occur inside the nuclei or outside the nuclei, like DNA are known as genotoxic agents or mutagens. Pb is one such mutagen and causes cancer in humans (Shahid *et al.*, 2011). It acts as a strong toxic in mitosis which is responsible for causing spindle fibers disruption (Patra *et al.*, 2004). In plant cells, cytoskeleton and nucleus disruption, damage of DNA strand, formation of micronucleus (Kumar *et al.*, 2017), chromosomal abnormalities, variability in simple sequence repeats and depolymerization of micro tubule are some of the dangerous impacts suffered in the existence of Pb. Under low levels, mitosis is not affected by Pb, however causes certain abnormalities, damage to a centric DNA fragments during meiotic division, breaking of chromosomes, and micronucleus formation (Shahid *et al.*, 2011). Pb can make its entrance into the nucleus (Malecka *et al.*, 2008) and attach either to the DNA or to protein. When Pb attaches itself to DNA, it interrupts DNA repair and replication processes. It is not responsible for direct genotoxic effects, unless it binds to naked DNA. The conformation of enzymes responsible for polymerization of nucleotides in DNA synthesis is also disrupted as a result of which their role also gets affected (Pourrut *et al.*, 2011a). Newly, Cenksi *et al.* (2010) with the help of RAPD Assay described the effect of Pb on the constancy of template DNA strand in *Brassica rapa*.

Oxidative stress and lipid peroxidation

General cell metabolism in chloroplast results in the production of reactive oxygen species. The ROS like superoxide radicals and hydrogen peroxide are produced after being exposed to evident agents of environment. Among several ROS, hydrogen peroxide has the ability to pass via cell membrane, as result of which plays direct role in cell signaling (Pitzschke *et al.*, 2006). This generation of ROS in plant cells which causes oxidative stress is a common attribute of the toxic heavy metals, comprising Pb also (Grover *et al.*, 2010; Singh *et al.*, 2010). ROS after depleting the stores of cell antioxidants may quickly strike and oxidize various types of biomolecules like nucleic acids, proteins, and lipids (Wang *et al.*, 2007; Yadav, 2010). These strikes can cause permanent metabolic dysfunction and cell death. Pb is responsible for causing significant modifications in the composition of lipid of several cell membranes (Yan *et al.*, 2010; Singh *et al.*, 2010). The polyunsaturated fatty acid and the esters that occur in lipids are highly susceptible to ROS (Gupta *et al.*, 2010). Certainly, ROS causes the removal of hydrogen from unsaturated fatty acids and produces lipid radicals and aldehydes which are sensitive, eventually resulting in the disruption of the lipid bilayer (Mishra *et al.*, 2006). It has been seen that the activity of redox enzymes is decreased in presence of sufficient amount of Pb (Lamhamdi *et al.*, 2013). Pb results in rigorous modification of lipids of plasma membrane (Grover *et al.*, 2010; Yan *et al.*, 2010) which in turn leads to the development of anomalous cell

framework (Gupta *et al.*, 2009). In *Z.mays*, modifications in lipid composition and K⁺ ion seepage due to Pb have been described (Malkowski *et al.*, 2002). It has been found that Pb ions cause lipid peroxidation, lower the amount of saturated fatty acids, and higher the level of unsaturated fatty acid of cell membranes in various species of plant (Singh *et al.*, 2010).

Amelioration of Pb toxicity in plants

Amelioration by phytohormones

Plant hormones are the biological constituents which are responsible for controlling growth and development of plants. Various forms of hormones are produced by plants such as auxins, cytokinins, gibberellins, abscisic acid, salicylic acid, ethylene, jasmonates, and peptides. It has been believed that phytohormones take part in signal-transduction pathway or their existence can trigger reactions which are either signal or causal means for stress retorts (Leyser 2010; Qin *et al.*, 2011). Phytohormones in the form of signal molecules control cell processes in target cells as well as when transported to other parts of the plant. Hormones are very important for plants growth and development and also for several biotic and abiotic stress retorts. Moreover, it has been shown that the treatment of hormones exogenously increases stress forbearance to heavy metals in plants (Krishnamurthy and Rathinasabapathi, 2013; Srivastava *et al.*, 2013). Generally, auxin has intense impact on the growth; abscisic acid effects dormancy of bud, and closing of stomata; cytokinin defers senescence; gibberellic acid plays important role in germination of seed; and brassinosteroids control growth and differentiation in plants (Jaillais and Chory, 2010; Sun *et al.*, 2005). Moreover, whether the hormones like SA, ET, JA and ABA act synergistically or antagonistically, they help in regulating the different ecological stresses in plants (Fujita *et al.*, 2006). Ethylene, Jasmonic acid and Salicylic acid are mainly involved in providing resistance to several environmental stresses (Lorenza and Solano, 2005). SA also plays an important job in regulating reactive oxygen species, amount of antioxidant enzymes and stimulation of several genes to get expressed (Hossain *et al.*, 2012). Moreover, Sharaf *et al.* (2009) described the role of gibberellic acid in alleviating the harmful impacts of Cd and Pb on broad bean and lupin plants by controlling the proteases, catalases, and peroxidases activity (Sharaf *et al.*, 2009).

Amelioration by Antioxidant defense system

Plants cannot break out abiotic stresses as they are unable to move. Capability of larger plants to combat the lethal aspects of reactive oxygen species acts as vital factor to resist several abiotic stresses. The plants have antioxidant defense system to forage the lethal effects of reactive oxygen species and prevent any kind of harm due to oxidative stress in order to maintain the well-being of a plant (Kanazawa *et al.*, 2000). This antioxidant defense

system of plants is made up of enzymatic (superoxide dismutase, catalase, ascorbate peroxidase) and non-enzymatic (tocopherol, glutathione) antioxidants which efficiently forage reactive oxygen species produced due to oxidative stress (Gondim *et al.*, 2012). It has been seen that Superoxide dismutase acts as a major enzyme in safeguarding the plant from oxidative strain in several plants. Superoxide dismutase has a prominent job in protecting a plant from damage due to oxidative strain by capturing superoxide radical (Myouga *et al.*, 2008). It has been observed that the action of superoxide dismutase increases in barley which is resilient to cadmium stress than the one which is susceptible (Chen *et al.*, 2010a, b). The peanut varieties resulted in enhancing the level of antioxidative enzymes like superoxide dismutase, ascorbate peroxidase, Glutathione S-transferase and glutathione reductase in presence of Pb. Moreover, the intensity peaks of isoenzymes such as superoxide dismutase, ascorbate peroxidase has been observed to be constant if any changes occur in the actions of antioxidant enzymes (Nareshkumar *et al.*, 2015). In case of *Triticum*, reactive oxygen species amount is under the control of antioxidative defense system which contains both enzymatic and non-enzymatic antioxidative molecules as described above. Proline which belongs to non-enzymatic category has defensive role in response to Pb stress by inhibiting the peroxidation of lipids (Mehta and Gaur, 1999) and high levels of proline may be regarded to give protection under high Pb concentrations.

Amelioration by secondary metabolites and osmolytes

A wide range of secondary metabolites are generated in plants. Secondary metabolites such as flavanoids, alkaloids, phenols take part in directing the growth in plants, production of pigments, possess antioxidant role, and help in inhibiting enzyme action (Mikkelsen *et al.*, 2015). Among secondary metabolites, phenolics play very essential role under heavy metal stress. It has been found that the production of secondary metabolites in plants increases in presence of heavy metals. Common bean produces more phenolics on its exposure to Cd metal (Winkel-Shirley, 2002). Production and accrual of aminoalkanoic acid can combat the adverse effects generated due to several environmental stresses (Di Martino *et al.*, 2003). Therefore, accumulation of aminoalkanoic acid in plants under Pb stress can provide protection against adverse effects of Pb metal (Sharma and Dubey, 2005), that remained constant in *Prosopis* plant while the amino acid concentration enhances.

Osmolytes can be stated as solute molecules that provides protection for various environmental stresses together with heavy metal contamination (Dhir *et al.*, 2012). Glycinebetaine, trehalose and proline have been found increasing when a plant is exposed to Pb metal. Such increasing level of GB, proline and trehalose contents have also been reported in rice seedlings and runner

bean plants which were grown under cadmium, nickel and copper strain (Sharma *et al.*, 2013). Increasing level of osmolytes acts as an essential indicator of metal contamination. Elevation in concentration of osmolytes is considered as an important marker indicating heavy metal stress and therefore plays necessary part in alleviation of metal stress.

Amelioration by signaling molecules

Characteristically, ROS are generated in plants in response to several environmental strains (Jaspers and Kangasjarvi, 2010). When reactive oxygen species are generated, nitric oxide shows interaction with several signal molecules so that it can keep the amount of reactive oxygen species (Vranova *et al.*, 2002). NO plays major role in increasing the activity of antioxidant enzymes that further assist in controlling the undesirable effects of such heavy metal on plants, as a result enhancing the forbearance of plants to heavy metal stress (Singh *et al.*, 2016). It has been described that NO can oppose the toxic effects of Pb in plants. Treatment of cowpea seeds with SNP as donor of NO before exposing it to Pb proved to be effective against toxic effects of Pb resulting in enhancement of the chlorophyll level, RWC, and rate of photosynthesis by enhancing the activities of antioxidant enzymes (Sadeghipour, 2015). It has been shown that the use of Pb concentrations especially 500 µM is responsible for decreasing the dry weight of *Melissa officinalis*. But concurrent use of less concentration of NO (about 100 µM) increases the dry weight and height of *Melissa officinalis*. Thus, total dry weight of plant shows the inhibition of development due to Pb. Same response to the application of Pb has been previously described in several plants (Brunet *et al.*, 2009). The another signaling molecule that can help in mitigating the adverse effects of Pb stress is hydrogen sulphide (H₂S). Investigation have been carried out regarding the role of NaHS (donor of hydrogen sulphide) on germination and growth of cauliflower seed in presence of Pb (C₂H₃O₂)₂. Pb concentrations of about 0.25, 0.5 mM resulted in inhibiting the germination and development of cauliflower seed. However, the inhibitory effect can be suppressed with the treatment of sodium hydrogen sulphide (Chen *et al.*, 2018). In Rapeseeds, grown in Pb contaminated soil, the application of hydrogen sulphide resulted in increasing the growth of plant, accumulation of more micro and macro- nutrients and improved the activity of antioxidative enzymes (Ali *et al.*, 2014). Besides NO and H₂S, there are other signaling molecules like H₂O₂, CO, etc. that can be used to combat the effect of Pb toxicity in plants.

CONCLUSION

Heavy metal pollution in soil is one of the serious concerns regarding the environment and human health. Heavy metals are present naturally on earth however, human activities have reallocated them from earth's

crust to various components of atmosphere. After heavy metals get circulated in the soil, they have a great impact on quality of environment and damage crop production. Accrual of heavy metals in plant cells results in reduction of germination of seed, reduced elongation of root, lower biomass of plants, and stoppage of biosynthesis of chlorophyll. Whereas, Pb has a great impact on plants photosynthesis, respiration, enzymatic reactions, mineral nutrition, and various other physiological aspects. Among several effects of heavy metal toxicity in plants, generation of ROS is most common, which occurs because of the intervention of heavy metal with electron transport actions. Plants have developed various methods for detoxification in order to withstand the more ROS production. Moreover, in order to mitigate the toxic effects produced by heavy metals, various amelioratives can be used such as NO, hydrogen sulphide, plant hormones, secondary metabolites, etc. NO regulates antioxidant systems and associated expression of gene and activity of protein to improve heavy metal toxicity. Such visions may help us in future for better crop development and design plans, eventually resulting in the production of improved varieties of crops.

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