

# Lead toxicity in plants

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Contamination of soils by heavy metals is of widespread occurrence as a result of human, agricultural and industrial activities. Among heavy metals, lead is a potential pollutant that readily accumulates in soils and sediments. Although lead is not an essential element for plants, it gets easily absorbed and accumulated in different plant parts. Uptake of Pb in plants is regulated by pH, particle size and cation exchange capacity of the soils as well as by root exudation and other physico-chemical parameters. Excess Pb causes a number of toxicity symptoms in plants e.g. stunted growth, chlorosis and blackening of root system. Pb inhibits photosynthesis, upsets mineral nutrition and water balance, changes hormonal status and affects membrane structure and permeability. This review addresses various morphological, physiological and biochemical effects of Pb toxicity and also strategies adopted by plants for Pb-detoxification and developing tolerance to Pb. Mechanisms of Pb-detoxification include sequestration of Pb in the vacuole, phytochelatin synthesis and binding to glutathione and aminoacids etc. Pb tolerance is associated with the capacity of plants to restrict Pb to the cell walls, synthesis of osmolytes and activation of antioxidant defense system. Remediation of soils contaminated with Pb using phytoremediation and rhizofiltration technologies appear to have great potential for cleaning of Pb-contaminated soils.

**Key words:** antioxidant defense system, detoxification, lead, osmolytes, toxicity, phytochelatin, phytoremediation, rhizofiltration, tolerance

**Toxicidade de chumbo em plantas:** A contaminação de solos por metais pesados é de ocorrência generalizada como resultado das atividades humana, agrícola e industrial. Entre os metais pesados, o chumbo (Pb) é um poluente potencial que, prontamente, se acumula nos solos e sedimentos. Apesar de não ser um elemento essencial para as plantas, é facilmente absorvido e acumulado em diferentes partes delas. A absorção de Pb é regulada pelo pH, tamanho de partículas e capacidade de troca de cátions dos solos, assim como pela exsudação e outros parâmetros físico-químicos. Excesso de Pb causa vários sintomas de toxicidade em plantas, por exemplo: redução de crescimento, clorose e escurecimento do sistema radicular. Pb inibe a fotossíntese, altera a nutrição mineral e o balanço hídrico, modifica o estado hormonal e afeta a estrutura e permeabilidade da membrana. Esta revisão aborda vários efeitos morfológicos, fisiológicos e bioquímicos da toxicidade de Pb e também as estratégias adotadas pelas plantas para a destoxificação e o desenvolvimento de tolerância ao Pb. Mecanismos de destoxificação ao Pb incluem o seqüestro de Pb no vacúolo, síntese de fitoquelatinas e o acoplamento a glutathione e aminoácidos, etc. A tolerância ao Pb está associada à capacidade das plantas a restringir o metal à parede celular, síntese de osmólitos, e ativação do sistema antioxidante de defesa. Remediação de solos contaminados com Pb usando as tecnologias de fitorremediação e rizofiltração parecem ter grande potencial para a limpeza de solos contaminados com esse metal pesado.

**Palavras-chave:** chumbo, destoxificação, fitoquelatina, fitorremediação, osmólitos, rizofiltração, sistema de defesa antioxidante, tolerância, toxicidade.

## INTRODUCTION

Lead (Pb) is one of the major heavy metals of the antiquity and has gained considerable importance as a potent

environmental pollutant. Apart from the natural weathering processes, Pb contamination of the environment has resulted from mining and smelting activities, Pb containing

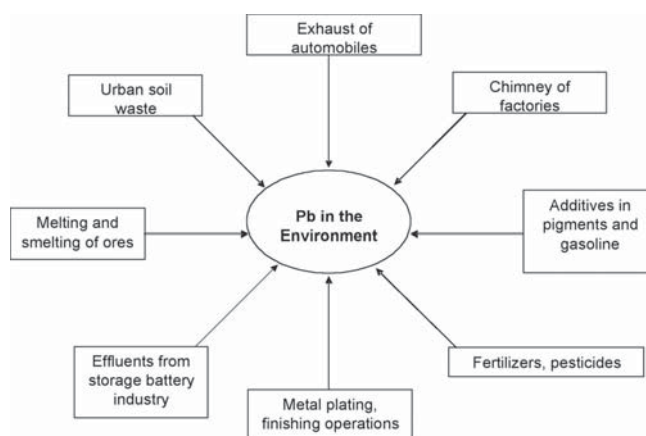
paints, gasoline and explosives as well as from the disposal of municipal sewage sludges enriched in Pb (Chaney and Ryan, 1994). Despite regulatory measures adopted in many countries to limit Pb input in the environment, it continues to be one of the most serious global environmental and human hazards. As many of the Pb pollutants are indispensable for modern human life, soil contamination with Pb is not likely to decrease in the near future (Yang et al., 2000).

Significant increases in the Pb content of cultivated soils has been observed near industrial areas. Pb tends to accumulate in the surface ground layer and its concentration decreases with soil depth (de Abreu et al., 1998). It is easily taken up by plants from the soil and is accumulated in different organs. Pb is considered a general protoplasmic poison, which is cumulative, slow acting and subtle. Soils contaminated with Pb cause sharp decreases in crop productivity thereby posing a serious problem for agriculture (Johnson and Eaton, 1980). The present review focuses on the sources of Pb; its uptake and transport within plant; physiological, biochemical and ultrastructural changes due to Pb toxicity; Pb tolerance in plants as well as possible remediation measures for Pb contaminated soils.

**Sources of lead:** Pb is a major pollutant in both terrestrial and aquatic ecosystems. Besides natural weathering processes the main sources of Pb pollution are exhaust fumes of automobiles, chimneys of factories using Pb, effluents from the storage battery, industry, mining and smelting of Pb ores, metal plating and finishing operations, fertilizers, pesticides and additives in pigments and gasoline (Eick et al., 1999). Figure 1 depicts various sources, which contribute to Pb pollution in the environment. Tetraethyl and tetramethyl Pb are added to gasoline to increase the octane rating. In urban areas automobile exhaust contributes substantially to the atmospheric pollution. Pb compounds are major pollutants emitted by automobiles. Plants growing near highways are usually exposed to more Pb than other localities. Sewage sludge containing large quantities of Pb and other metals is regularly discharged on to field and garden soils due to increasing trends in urbanization (Paivoke, 2002). Pb-affected soils contain Pb in the range of 400-800 mg Kg<sup>-1</sup> soil whereas in industrialized areas the level may reach upto 1000 mg Pb.Kg<sup>-1</sup> soil (Angelon and Bini, 1992). Half of the Pb-containing particulate matter falls to the ground within 100 feet of roadways and is then washed away and dispersed in the atmosphere and may be carried a considerable distance by air movements before it is eventually deposited. The ac-

cumulated Pb on the street and highways is transported to surface streams by rain water and consequently pollutes others surface waterways and soil (Laxen and Harrison, 1977). Compounds of Pb used as agricultural chemicals such as Pb arsenate, which is used as a pesticide, contaminate agricultural soils.

In a long term field experiment (41 years) involving regular application of mineral fertilizers to crops of sunflower or barley followed by oat-winter rye in rotation, revealed that the fertilizers increased the level of mobile forms of Pb in the soil and also its uptake by the crops (Stefanov et al., 1995). Mine water also transports a large amount of fine-grained sediments contaminated with Pb (Laxen and Harrison, 1977).



**Figure 1.** Sources of lead pollution in the environment.

**Uptake, transport and localization:** Pb is available to plants from soil and aerosol sources. Pb uptake studies in plants have demonstrated that roots have an ability to take up significant quantities of Pb whilst simultaneously greatly restricting its translocation to above ground parts (Lane and Martin, 1977). This notion was overturned by Miller and Koeppel (1971) who demonstrated that *Zea mays* L. plants could translocate and accumulate significant quantities of Pb in the leaves in a concentration dependent manner. The extent to which Pb enters plants via the leaves depends on the ability of leaves to absorb Pb from aerial sources, which in turn depends on the specific leaf morphology. Downy leaves absorb heavy metals from the atmosphere (Godzik, 1993). However it is agreed that the bulk of the Pb taken up by plants remains in the roots (Kumar et al., 1995). Pb accumulates in the surface layers of soils and therefore it is difficult to reliably measure the portion of soil Pb directly available to plants. Its availability depends highly on soil conditions. Pb binds to organic material

in the soil. Soil particle size and cation exchange capacity as well as plant factors such as root surface area, root exudates, mycorrhization and rate of transpiration affect the availability and uptake of Pb (Davies, 1995). The absorption of Pb (as Pb) in soil follows the Langmuir relation and increases with increasing pH between 3.0 to 8.5 (Lee et al., 1998). However Blaylock and coworkers (1997) reported that in soil with a pH between 5.5 and 7.5 Pb solubility is controlled by phosphate or carbonate precipitates and very little Pb is available to plants even if they have the genetic capacity to accumulate it. Pb in soil is classified as a weak Lewis acid, which implies a strong covalent character to many of the ionic bonds it forms in soils and plants. Pb present in the soil is nearly always tightly bound to organic or colloidal material or in a precipitated form, all of which serve to reduce the uptake of Pb by plant roots.

At the root surface Pb binds to carboxyl groups of mucilage uronic acids. Mucilage binding restricts metal uptake into the root and establishes an important barrier protecting the root system. Some of the bound metals are released when mucilage is biodegraded (Morel et al., 1986). Soil microorganisms may affect heavy metal availability by the process of biosorption, bioaccumulation and solubilization. It was shown by Marschner and coworkers (1996) that ectomycorrhiza could influence uptake, transport and toxicity of Pb in Norway spruce plants.

Pb retention in the roots is based on binding of Pb to ion exchangeable sites on the cell wall and extracellular precipitation, mainly in the form of Pb carbonate deposited in the cell wall. The addition of synthetic chelates, such as H-EDTA or EDTA, in combination with low pH, effectively prevents cell wall retention of lead, making it available for translocation to shoots (Jarvis and Leung, 2002). After being taken up by roots, the localization of Pb is greater in roots than in other parts of the plants. Pb binds strongly to the carboxyl groups of the carbohydrates galacturonic acid and glucuronic acid in the cell wall, which restricts its transportation via apoplast (Rudakova et al., 1988). In general dicots accumulate significantly higher amounts of Pb in the roots than monocots (Huang and Cunningham, 1996). Pb transported from the soil to the root cells has to cross the root-cell plasma membrane. One possible transport pathway of Pb across the plasma membrane (PM) appears to be through PM cation channels, such as Ca-channels. A voltage gated Ca-channel in the root cell PM has been characterized using right-side-out PM vesicles isolated from roots of wheat and corn plants (Marshall et al., 1994; Huang et al., 1994). Huang

and Cunningham (1996) found that Pb significantly inhibited voltage gated Ca-channels activity in the PM of wheat roots. The inhibition of the Ca-channel by Pb could arise from Pb blockage of the channel or due to competitive transport of Pb through the Ca-channel. While monitoring Pb entry into isolated cells, Tomsig and Suszkiw (1991) observed permeation of Pb through Ca-channels. These workers also found that voltage gated Pb transport was blocked by nifedipine (a Ca-channel blocker) and enhanced by BAY K8644 (a Ca-channel agonist).

Pb moves predominantly into the root apoplast and thereby in a radial manner across the cortex and accumulates near the endodermis. The endodermis acts as a partial barrier to the movement of Pb between the root and shoot. This may in part account for the reports of higher accumulation of Pb in roots compared to shoots (Jones et al., 1973; Verma and Dubey, 2003). When rice (*Oryza sativa* L.) seedlings were raised in sand cultures for 10 and 20 days in nutrient medium containing 500  $\mu\text{M}$  and 1000  $\mu\text{M}$   $\text{Pb}(\text{NO}_3)_2$ , root growth was reduced by 22 to 42 % and shoot growth by 25 %, whereas localization of absorbed Pb was 1.7 to 3.3 times higher in roots compared to shoots (table 1). The limited transport of Pb from roots to other organs is due to the barrier of the root endodermis. It appears that casparian strips of the endodermis are the major limiting factor restricting Pb transport across endodermis into the central cylinder tissue (Seregin and Ivanov, 1997). According to Lane and Martin (1977) the endodermis appears to act as a partial barrier since some of the Pb moves up through the vascular tissues and diffuses out into the surrounding tissues. This provides evidence that Pb moves into the symplast. That movement of Pb in the root is primarily via the apoplast is also supported by the report that a large proportion of Pb is readily extractable in water (Broyer et al., 1972). The possibility of symplastic transport of Pb has been demonstrated in onion roots and garden cress hypocotyls (Wierzbicka, 1987). Higher concentrations of Pb cause cell injury and disturb the barrier function of the plasmalemma as well as the selective permeability of the plasmalemma and tonoplast. Seregin and coworkers (2004) demonstrated that a significant amount of Pb was retained at the surface of plasmalemma rather than in the cell walls (Seregin et al., 2004). Pb enters the injured cells together with compounds such as the procion dyes, which do not enter the undamaged cells (Seregin et al., 2004).

The pattern of distribution of Pb in the roots considerably differs depending on whether the concentrations of Pb are lethal or non-lethal (Seregin et al., 2004). At lower concentra-

tions, Pb ions predominantly flow in the apoplast, whereas at higher concentrations, the barrier function of plasmalemma is damaged and a greater amount of Pb enters into the cells.

In general, the apparent concentration of Pb in aerial parts of the plant decreases as the distance from the root increases. This occurs due to greater localization of Pb in cell walls of the root than in other parts of the plant. Further, binding of Pb occurs more in lignified rather than non-lignified tissues. Suchodoller (1967) found that in barley much of the applied Pb was retained in the root epidermis while a small amount could be detected in the vascular tissues. This suggests that the extent of localization of Pb in different tissues of the plant is also dependent on the plant species. In seeds, the testa prevents entry of Pb into the internal tissues until it is ruptured by the developing radicle. Once the testa is ruptured, Pb is taken up very rapidly, with notable exceptions occurring in the meristematic regions of the radicle and hypocotyls (Lane and Martin, 1977). In the cotyledons, Pb moves through the vascular tissues and tends to accumulate in discrete areas in the distal parts (Lane and Martin, 1977).

The content of Pb in various plant organs tends to decrease in the following order: roots>leaves>stem>inflorescence>seeds. However this order can vary with plant species (Antosiewicz, 1992). In onion (*Allium cepa*) plants, absorbed Pb is localized in highest concentration in the root tips followed by proximal parts of the root, while its lowest concentration is found in the root base (Michalak and Wierzbicka, 1998). Leaves differ in their abilities to accumulate Pb depending on age. Maximum Pb content is found in senescing leaves and least in young leaves (Godzik, 1993). Ultrastructural studies have revealed that variable amounts of Pb deposits are present mainly in the intercellular space, cell wall and vacuoles, whereas small deposits of this metal are seen in the ER, dictyosomes and dictyosome derived vesi-

cles. The cell wall and vacuole together account for about 96 % of absorbed Pb (Wierzbicka and Antosiewicz, 1993). The fact that Pb is found in the ER and dictyosome is apparently related to metal secretion of the cell surface into the vacuole. A small quantity of Pb reaches nuclei, chloroplasts and mitochondria and exerts its toxic effects on these organelles. In leaf cells of *Potamogeton* spp. it was shown that the electrochemical potential gradients between cell vacuoles and the outside bathing solution ranged from -150 to -240 mV (Denny and Weeks, 1968) which could favour a passive influx of Pb into vacuoles during Pb treatment. Of particular interest is the invagination of the plasmalemma to form pinocytotic vacuoles in many plant species. In *Stigeoclonium* the formation of such vacuoles is important for the sequestration of excess metal ions, as these vacuoles could protect the cell contents from toxic effects of Pb (Silverberg, 1975). Sometimes, particularly in close proximity to the plasmodesmata, the larger Pb particles appear to occupy much of the volume of the cell wall. In the other regions where the cell wall is much thicker and more substantial, smaller Pb particles accumulate within the cell wall towards its periphery. The deposition of these smaller Pb particles occurs possibly through the action of pinocytotic vesicles (Ksiazek et al., 1984).

While considerable information is available on Pb distribution the cells of terrestrial plants, little is known about Pb localization in the cells of aquatic plants. *Lemna minor* L. (duck weed) plants when treated with Pb for one hour showed maximum concentration of Pb in small vacuoles (Samardakiewicz and Wozny, 2000). After 6 and 12 h of Pb treatment, the content of in cell walls gradually increased. The localization of Pb between vacuoles and cell walls possibly results due to redistribution of Pb and it reflects increased apoplastic transport (Samardakiewicz and Wozny, 2000).

**Table 1.** Lead content and lengths of roots and shoots of rice seedlings grown for 10 and 20 days in presence of 500  $\mu\text{M}$  and 1000  $\mu\text{M}$   $\text{Pb}(\text{NO}_3)_2$  in sand cultures.

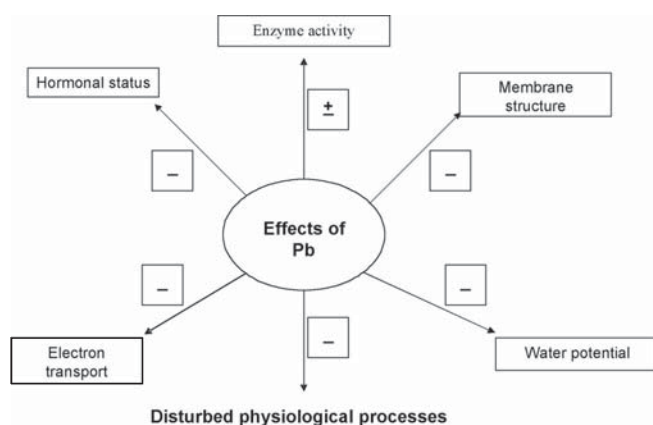
Age of seedlings (Days)	Concentration of Pb ( $\mu\text{M}$ )	Length (cm)		Pb content ( $\mu\text{mol Pb.g}^{-1}$ DW)	
		Root	Shoot	Root	Shoot
10 d	0	8.84	9.23	0	0
	500	6.92	8.84	0.16	0.06
	1000	6.15	6.92	0.72	0.22
20 d	0	11.92	17.69	0	0
	500	9.23	16.15	0.80	0.38
	1000	6.92	13.84	1.22	0.72

From Verma and Dubey (2003)



The presence of Pb in small vesicles in *Lemna minor* suggests that endocytosis plays an important role in Pb uptake in these plants.

*Physiological, biochemical and ultrastructural effects of lead:* The visual non-specific symptoms of Pb toxicity are rapid inhibition of root growth, stunted growth of the plant and chlorosis (Burton et al., 1984). When Pb enters inside the cells even in small amounts it produces a wide range of adverse effects on physiological processes. A generalized view of the effects of Pb toxicity on key physiological processes in plants is presented in figure 2. Pb phytotoxicity leads to inhibition of enzyme activities, disturbed mineral nutrition, water imbalance, change in hormonal status and alteration in membrane permeability. These disorders upset normal physiological activities of the plant. At high concentrations Pb eventually may Pb to cell death (Ernst, 1998; Seregin and Ivanov, 2001). At the cellular level Pb inhibits the activities of enzymes containing sulphhydryl (-SH) groups necessary for their activity (van Assche and Clijsters, 1990). Pb toxicity inhibits germination of seeds and retards growth of seedlings. Pb decreases germination percent, germination index, root/shoot length, tolerance index and dry mass of roots and shoots (Mishra and Choudhari, 1998). High concentrations of Pb (1 mM) caused 14 to 30 % decreased germination in rice seeds and reduced the growth of seedlings by more than 13 to 45 % (Verma and Dubey, 2003). In *Lupinus*, Pb reduced the number of germinating seeds and caused shortening of hypocotyl as well as roots (Wozny et al., 1982).



**Figure 2.** A generalized view of lead toxicity in plants. Pb phytotoxicity involves decreased water potential, alteration in membrane permeability, decrease in hormonal status and electron transport activities, whereas activities of enzymes are either increased or inhibited. These events ultimately result in overall disturbed physiological processes. '+' and '-' signs indicate positive and negative effects respectively.

Plant roots rapidly respond to absorbed Pb, through a reduction in growth rate and change in branching pattern. Several workers have reported the inhibition of root growth at  $10^{-2}$  to  $10^{-6}$  M Pb concentration or at a soil Pb content above 10 mg/kg (Breckle, 1991). At lower concentrations of Pb, development and extension of the main root is affected much more than the lateral roots (Obroucheva et al., 1998). Godbold and Kettner (1991) reported that *Picea abies* plants when exposed to Pb at concentrations of 0.1 to 2  $\mu$ M showed increased Pb content in roots with increasing Pb supply. A four week exposure of the growing plants to 0.5  $\mu$ M Pb reduced the growth of primary, secondary and tertiary roots. The initiation of lateral roots appeared to be more sensitive to Pb than the growth of primary roots. In *Zea mays* seedlings, Obroucheva et al. (1998) observed strong inhibition of primary root growth and a shorter branching zone with more compact lateral roots occupying a position much closer to the root tip compared with roots grown in the absence of Pb. It appears that the inhibition of root growth under Pb toxicity is as a result of Pb-induced inhibition of cell division in root tips (Eun et al., 2000). When the effect of different concentrations of Pb nitrate was studied on root growth, cell division, chromosome morphology and the nucleolus of root tip cells of onion (*Allium cepa*), a reduction in root growth, mitotic irregularities and chromosome stickiness were observed (Wierzbicka, 1994). The interphase cells possessed micronuclei, irregularly shaped nuclei and nuclei with decomposed nuclear material under Pb treatment (Wierzbicka, 1994). Pb treatment perturbs the alignment of microtubules in a concentration dependent manner beginning at 10  $\mu$ M and this may, at least in part, contribute to the inhibition of root growth seen in the presence of Pb (Yang et al., 2000). Pb destroys microtubules of the mitotic spindle causing characteristic c-mitoses to occur by blocking cells in pro-metaphase. This blocking however is not permanent (Wierzbicka, 1994). Microtubules of different regions of the root meristem and in different stages of the cell cycle show differential susceptibility to lead. These effects do not appear to be a general phenomenon common to toxic metals since Al and Cu at a concentration that decreases root growth to a comparable level did not show similar detrimental effects on microtubules (Eun et al., 2000). Based on such observations it is suggested that the damage to microtubules by Pb is an important component of Pb-induced injury in plants (Eun et al., 2000).

In corn seedlings Pb toxicity causes leakage of K ions from root cells (Malkowski et al., 2002). In seedlings treated with  $10^{-4}$  to  $10^{-3}$  M Pb, growth of mesocotyl and coleoptyl

was similarly affected, although the concentration of Pb was 3 fold higher in the mesocotyl tissues than in the coleoptyl. It is proposed that in corn seedlings depression of shoot growth is not as a consequence of potassium leakage or Pb accumulation but due to an unknown signal induced in roots, as a response to exposure of Pb which is transmitted to the shoots (Malkowski et al., 2002). A considerable decrease in dry weights of plant parts is observed under Pb treatment (Kosobrukhov, 2004). However in certain cases, as in corn seedlings, an apparent increase in dry weight of plant organs was reported which was due to an increase in the synthesis of cell wall polysaccharides resulting from Pb exposure (Wierzbicka, 1998). Pb toxicity lowers the protein content of tissues and causes significant alterations in lipid composition (Przymusiński et al., 1991; Stefanov et al., 1995). In *P. vulgaris* and *Z. mays* plants substantial changes were observed in the level of glycolipids, especially monogalactosyl diacylglycerols, which are associated with membrane permeability in chloroplasts (Stefanov et al., 1993). Incubation in presence of Pb salts invariably results in a decreased level of saturated fatty acids and an increased level of unsaturated fatty acids (18:3) (Stefanov et al., 1995). Synthesis of DNA, RNA and protein were greatly reduced in the embryo axis and endosperms of germinating rice seedlings with increasing concentration of Pb (Maitra and Mukherji, 1977).

**Enzymes activities:** Like various heavy metals, Pb treatment influences the activity behaviours of a wide range of enzymes of different metabolic pathways. Increasing attention has been paid to understand the action of Pb on plant enzymes. Pb at a concentration of  $10^{-5}$  to  $2 \times 10^{-4}$  M produces about 50 % inhibition of many enzymes. This concentration is defined as the inactivation constant ( $K_i$ ). In most, the inhibition exerted by Pb on enzyme activity results from the interaction of Pb with enzyme -SH groups (Levina, 1972). Pb interacts with free -SH groups that are present in the active site of the enzyme and essential for enzyme activity as well as with -SH groups that are necessary for the stabilization of enzyme tertiary structure. Besides the reaction with -SH groups, blockage of -COOH groups with Pb ions also appears to play a major role in inhibition of enzyme activity under Pb treatment. Pb forms a mercaptide with the -SH group of cysteine and also forms complexes with phosphate groups. Inhibition of metalloenzymes under Pb treatment appears to be due to displacement of the essential metal by Pb. The inhibition of enzyme activity due to Pb does not appear to be specific for Pb, as such inhibitions are also evident with other cations having comparable affinities for protein functional groups.

Table 2 presents an overview of the effect of Pb treatment on the activities of various enzymes reported from different plant species. The key enzyme of chlorophyll biosynthesis,  $\delta$ -amino laevulinate dehydrogenase, is strongly inhibited by Pb ions (Prasad and Prasad, 1987). Pb also inhibits the activities of enzymes of the reductive pentose phosphate pathway (Hampp et al., 1973). In leaf homogenates of spinach the activity of ribulose-bis-phosphate carboxylase/oxygenase was inhibited even at a Pb nitrate concentration of 5  $\mu$ M (Vallee and Ulmer, 1972). Lactate dehydrogenase showed a similar but a less pronounced decrease in activity. However, the activity of pyruvate kinase was enhanced by Pb nitrate (Vallee and Ulmer, 1972). Pb acetate has been shown to considerably decrease the activities of protease and  $\alpha$ -amylase in rice endosperms after 4 days of germination, whereas RNase and DNase activities are not much affected (Mukherji and Maitra, 1976). A decrease in activity of glutamate dehydrogenase and a low content of nitrate have been observed in leaves of Pb-treated soybean plants whereas no significant change could be observed in malate dehydrogenase activity (Lee et al., 1976). Pb was found to be highly effective in inhibiting ATP synthetase/ATPase (Tu Shu and Brouillette, 1987).

Activities of several enzymes are reported to be enhanced by Pb treatment. Such apparent enhancement results from changes in enzyme synthesis, immobilization of enzyme inhibitors, or as a result of effector molecules, which are synthesized under Pb phytotoxicity. Soybean plants grown in culture media containing 20-100 mg.L<sup>-1</sup> Pb showed increased activity of acid phosphatase,  $\alpha$ -amylase and peroxidase in leaves (Lee et al., 1976). Increased activity of hydrolytic enzymes as well as of peroxidase in soybean leaves under Pb treatment parallels with the senescence of leaves. An increase in the activity of the RNA hydrolyzing enzyme ribonuclease and of protease has been observed in submerged aquatic angiospermic plants growing in presence of Pb acetate (Jana and Choudhari, 1982).

As Pb promotes the formation of reactive oxygen species in plants leading to oxidative stress, an increase in the activity of certain antioxidative enzymes has been observed in Pb-treated plants. Rice plants grown for 20 days in sand cultures containing 0.5 mM and 1 mM Pb(NO<sub>3</sub>)<sub>2</sub> showed increased activities of the antioxidative enzymes superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase and glutathione reductase in roots and leaves (Verma and Dubey, 2003). However, activities of the antioxidative metalloenzymes decline when Pb displaces metals that are an essential part of enzyme.

Plant species differing in Pb tolerance show varying behaviour of certain enzymes under Pb treatment. Igoshina and Kositsin (1990), while studying the effect of Pb on carboanhydrase activities in the tolerant and sensitive species of melic-grass (*Melica nutans*), observed that in tolerant melic-grass population, Pb activated carboanhydrase activity whereas in the sensitive plants the activity of this enzyme remained unaffected.

**Photosynthesis:** The process of photosynthesis is adversely affected by Pb toxicity. Plants exposed to Pb ions show a decline in photosynthetic rate which results from distorted chloroplast ultrastructure, restrained synthesis of chlorophyll, plastoquinone and carotenoids, obstructed electron transport, inhibited activities of Calvin cycle enzymes, as well as deficiency of CO<sub>2</sub> as a result of stomatal closure. *Ceratophyllum*

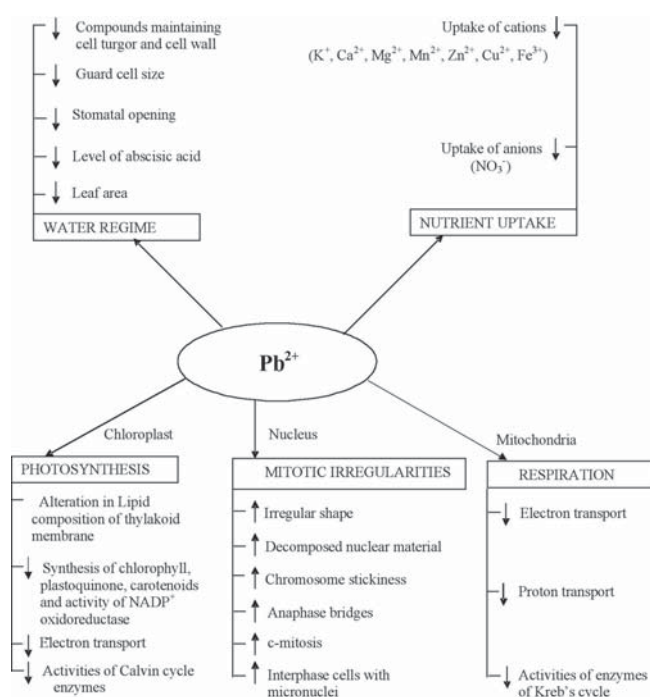
*demersum* plants when grown in aquatic medium containing Pb(NO<sub>3</sub>)<sub>2</sub> showed distinct changes in chloroplast fine structure (Rebechini and Hanzely, 1974). Leaf cells of such plants exhibited a reduction in grana stacks together with a reduction in the amount of stroma in relation to the lamellar system as well as absence of starch grains. Pb treatment also changes the lipid composition of thylakoid membranes (Stefanov et al., 1995). Effects of Pb on various components of photosynthesis, mitotic irregularities, respiration, water regime and nutrient uptake are shown in figure 3.

Pb inhibits chlorophyll synthesis by causing impaired uptake of essential elements such as Mg and Fe by plants (Burzynski, 1987). It damages the photosynthetic apparatus due to its affinity for protein N- and S- ligands (Ahmed and Tajmir-Riahi, 1993). An enhancement of chlorophyll degradation occurs in Pb-treated plants due to increased

**Table 2.** Effect of Pb on activities of enzymes of different metabolic processes.

Metabolic processes	Enzymes	Plant species	Effect of Pb	References
Chlorophyll synthesis	δ-Aminolaevulinate	<i>Pennisetum typhoideum</i>	-	Prasad and Prasad 1987
CO <sub>2</sub> fixation	Ribulose-1,5 bis phosphate	<i>Avena sativa</i>	-	Moustakas et al. (1994)
	Phosphoenol pyruvate Carboxylase	<i>Zea mays</i>	-	Vojtechova and Leblova (1991)
Calvin cycle	Glyceraldehyde 3-phosphate dehydrogenase	<i>Spinach oleracea</i>	-	Vallee and Ulmer (1972)
	Ribulose 5-phosphate kinase	<i>Spinach oleracea</i>	-	Vallee and Ulmer (1972)
Pentose phosphate pathway	Glucose 6-phosphate dehydrogenase	<i>Spinach oleracea</i>	-	Vallee and Ulmer (1972)
N <sub>2</sub> assimilation	Nitrate reductase	<i>Cucumis sativus</i>	-	Burzynski (1990)
	Glutamine synthetase	<i>Glycine max</i>	-	Lee et al. (1976)
Nucleolytic enzymes	Deoxyribonuclease	<i>Hydrilla verticillata</i>	+	Jana and Choudhary (1982)
	Ribonuclease	<i>Hydrilla verticillata</i>	+	Jana and Choudhary (1982)
Protein hydrolysis	Protease	<i>Hydrilla verticillata</i>	+	Jana and Choudhary (1982)
Phosphohydrolase	Alkaline phosphatase	<i>Hydrilla verticillata</i>	+	Jana and Choudhary (1982)
	Acid Phosphatase	<i>Glycine max</i>	+	Lee et al. (1976)
Sugar metabolism	α- amylase	<i>Oryza sativa</i>	-	Mukherji and Maitra (1977)
Energy generation	ATP synthetase	<i>Zea mays</i>	-	Tu Shu and Brouillette (1987)
	ATPase	<i>Zea mays</i>	-	Tu Shu and Brouillette (1987)
Antioxidative metabolism	Catalase	<i>Oryza sativa</i>	-	Verma and Dubey (2003)
	Guaiacol peroxidase	<i>Glycine max</i>	+	Lee et al. (1976)
	Ascorbate oxidase	<i>Phaseolus aureus</i>	+	Rashid and Mukherjee (1991)
	Ascorbate peroxidase	<i>Oryza sativa</i>	+	Verma and Dubey (2003)
	Glutathione reductase	<i>Oryza sativa</i>	+	Verma and Dubey (2003)
	Superoxide dismutase	<i>Oryza sativa</i>	+	Verma and Dubey (2003)

Signs '-' and '+' represent inhibition or increase in enzyme activity, respectively, in Pb treated plants.



**Figure 3.** Effect of Pb on photosynthesis, mitotic irregularities, respiration, water regime and nutrient uptake. '↑' and '↓' signs represent enhanced and decreased activities respectively.

chlorophyllase activity (Drazkiewicz, 1994). Chlorophyll *b* is reported to be more affected than chlorophyll *a* by Pb treatment (Vodnik et al., 1999). Pb also inhibits electron transport (Rashid et al., 1994). Pb effects have been reported for both donor and acceptor sites of PS II, the cytochrome *b/f* complex and PS I. It is largely accepted that PS I electron transport is less sensitive to inhibition by Pb than PS II (Mohanty et al., 1989; Sersen et al., 1998).

Pb also causes strong dissociation of the oxygen evolving extrinsic polypeptide of PS II and displacement of Ca, Cl<sup>-</sup>, Mn from the oxygen-evolving complex (Rashid et al., 1991). Ahmed and Tajmir-Riahi (1993) found conformational changes in light-harvesting chlorophyll (LHC II) subunits, following binding with Pb *in vitro*. It is proposed that conformational changes induced by Pb treatment might lead to incomplete assembly followed by degradation (Ahmed and Tajmir-Riahi, 1993).

A strong relationship exists between Pb application and a decrease in photosynthesis of the whole plant and is believed to result from stomatal closure rather than a direct effect of Pb on the process of photosynthesis (Bazzaz et al., 1975). According to Kosobrukhov and coworkers (2004), the photosynthetic activity of plant is governed by many factors including stomatal cell size, number of stomata, stomatal

conductance, leaf area etc. While studying the effects of Pb on the development of thylakoid of cucumber and poplar plants Savari and coworkers (2002) observed increased chlorophyll content either in the PS II core or LHC II at low concentrations of Pb treatment, whereas a strong decrease in chlorophyll level of seedlings was seen at the 50 mM Pb. At 50 mM Pb treatment level the concentration of Pb inside the leaf might have been high enough to directly inhibit chlorophyll synthesis (Sengar and Pandey, 1996).

**Respiration and ATP content:** Pb exerts a significant effect on respiration and ATP content of photosynthetic organisms. *In vitro* application of Pb to mitochondrial preparations from plant cells revealed a decrease in respiration rate with increasing Pb concentrations (Reese and Roberts, 1985). Using isolated chloroplasts and mitochondria in different plant species it has been shown that Pb affects the flow of electrons via the electron transport system (Miles et al., 1972; Bazzaz and Govindjee, 1974). The inhibitory effect of Pb at higher concentrations appears to be due to uncoupling of oxidative phosphorylation (Miller et al., 1973). At lower concentrations, however, a stimulation of respiration is observed in whole plants (Lee et al., 1976), detached leaves (Lemoreaux and Chaney, 1978), isolated protoplasts (Parys et al., 1998) and mitochondria (Koeppel and Miller, 1970). The exposure of detached leaves of *C*<sub>3</sub> plants (pea, barley) and *C*<sub>4</sub> plants (maize) to 5 mM Pb(NO<sub>3</sub>)<sub>2</sub> for 24 h caused stimulation of the respiratory rate by 20-50 % (Romanowska et al., 2002). Mitochondria isolated from Pb-treated pea leaves oxidized substrates (glycine, succinate, malate) at higher rates than mitochondria from control leaves (Romanowska et al., 2002). The respiratory control and the ADP/O were not affected by Pb treatment. Pb caused an increase in ATP content as well as an increase in the ATP/ADP ratio in pea and maize leaves (Romanowska et al., 2002). Rapid fractionation of barley protoplasts incubated at low and high CO<sub>2</sub> conditions, indicated that the increased ATP/ADP ratio in Pb-treated leaves resulted mainly from the production of mitochondrial ATP. The activity of NAD<sup>+</sup>-malate dehydrogenase in protoplasts of barley leaves treated with Pb was 3-fold higher than the protoplasts from control leaves (Romanowska et al., 2002). The activities of photorespiratory enzymes NADH-hydroxypyruvate reductase and glycolate oxidase as well as of NAD-malic enzyme were however, not affected by Pb treatment (Romanowska et al., 2002). The mechanism underlying the stimulation of respiration by Pb is not clear. According to Ernst (1980) the higher respiration rate observed under Pb



toxicity could be due to an increased demand for ATP production through oxidative phosphorylation.

The key enzyme of CO<sub>2</sub> assimilation in C<sub>3</sub> plants ribulose-bisphosphate carboxylase, is sensitive to Pb whereas the oxygenase activity remains relatively unaffected (van Assche and Clijsters, 1990). Therefore, it is quite possible that after Pb treatment when photosynthesis is significantly reduced, photorespiration could continue at a similar rate. This would increase the relative rate of photorespiration to photosynthesis. The inhibition of photosynthesis observed after Pb treatment leads to decreased utilization of ATP for CO<sub>2</sub> fixation. In leaf extracts of Pb-treated plants higher in ATP/ADP ratios have been observed compared to untreated plants (van Assche and Clijsters, 1990). Leaves of Pb-treated plants show increased respiration, which appears to be a result of oxidation of excess photosynthetic reducing equivalents, which are produced under conditions of limited CO<sub>2</sub> fixation (Poskuta et al., 1996).

At higher concentrations of Pb, inhibition of respiration is observed. Respiration of corn root tips decreased by 10-17 % after 1 h treatment with 20 mM Pb and by 28-40 % after 3 h treatment (Koepe, 1977). Pb is regarded as one of the most potent metal ions for the inhibition of chloroplastic ATP synthetase/ATPase activity and for the destruction of the membranes (Tu Shu and Brouillette, 1987). Although the sensitivity of photophosphorylation to heavy metal ions is well documented, there is no general agreement regarding their site of action nor on the underlying mechanism. Some experiments suggest that different mechanisms exist for the action of heavy metal ions on chloroplastic ATPase activity when these metal ions are applied under *in vivo* and *in vitro* conditions.

**Nutrient uptake:** High concentrations of Pb in the soil environment causes imbalance of mineral nutrients in growing plants. Many of the observed actions of Pb appear to be indirect as a result of mineral imbalance within the tissues. Significant changes in nutrient contents as well as in internal ratios of nutrients occur in plants under Pb toxicity (Kabata-Pendias and Pendias, 1992). In most cases Pb blocks the entry of cations (K<sup>+</sup>, Ca, Mg, Mn, Zn, Cu, Fe<sup>3+</sup>) and anions (NO<sub>3</sub><sup>-</sup>) in the root system (Figure 3). Two mechanisms for decreased uptake of micro and macronutrients under Pb toxicity have been suggested. The first mechanism, termed physical, relies on the size of metal ion radii, whereas the second mechanism, which is a chemical one, relies on the metal-induced disorder in the cell metabolism leading to changes in membrane

enzyme activities and membrane structure. The efflux of K<sup>+</sup> from roots, apparently due to the extreme sensitivity of K<sup>+</sup>-ATPase and -SH groups of cell membrane proteins to Pb, is an example of the second type of mechanism.

Pb physically blocks the access of many ions from absorption sites of the roots (Godbold and Kettner, 1991). Although Pb levels in root tips and the basal root may appear to be similar, Pb alters the levels of mineral elements in the roots. In root tips the levels of Ca, Fe, Zn decrease after exposure to Pb. In root tips of Norway spruce 40 % of the Ca taken up was used in root tips growth. The inhibition of root growth after exposure to Pb may be due to a decrease in Ca in the root tips, leading to a decrease in cell division or cell elongation (Haussling et al., 1988). In Norway spruce needles, the level of Ca and Mn decrease with Pb treatment, which could be a result of a decrease in number of root tips and sites for apoplastic solute flux through the endodermis. In *Picea abies* Pb treatment lowered the Mn level of the needles (Sieghardt, 1988). In *Cucumis sativus* seedlings Pb decreased the uptake of K, Ca, Mg, Fe and NO<sub>3</sub><sup>-</sup> and in *Zea mays* the uptake of Ca, Mg, K and P (Walker et al., 1997). Pb influences the overall distribution of nutritional elements within the different organs of the plant. The overall distribution ratio of Mn and S changed in favour of root over shoot under Pb toxicity, which may represent retention of these ions in root. Phosphorus content was found to be negatively correlated with soil Pb (Paivoke, 2002). Root nitrogen content is significantly reduced under Pb toxicity. Nitrate uptake declines in plants under exposure to Pb with a concomitant lowering of nitrate reductase activity and disturbed nitrogen metabolism (Burzynski and Gabrowski, 1984). The decline in nitrate uptake due to Pb may be as a result of moisture stress created by Pb (Burzynski and Gabrowski, 1984). In certain plant species like *Pisum sativum*, elevated nitrogen content is observed in roots at a Pb treatment level of 2 mM kg<sup>-1</sup> soil which probably occurs due to inhibitory effects of Pb on NR activity (Paivoke, 2002).

**Water status:** A decline in transpiration rate and water content in tissues occurs in plants growing under Pb exposure. Various mechanisms have been suggested for the Pb-induced decline in transpiration rate and water content. Pb treatment causes growth retardation, which results in a reduced leaf area, the major transpiring organ (Iqbal and Moshtaq, 1987). Guard cells are generally smaller in size in plants treated with Pb. Pb lowers the level of compounds that are associated with maintaining cell turgor and cell wall plasticity and thus low-

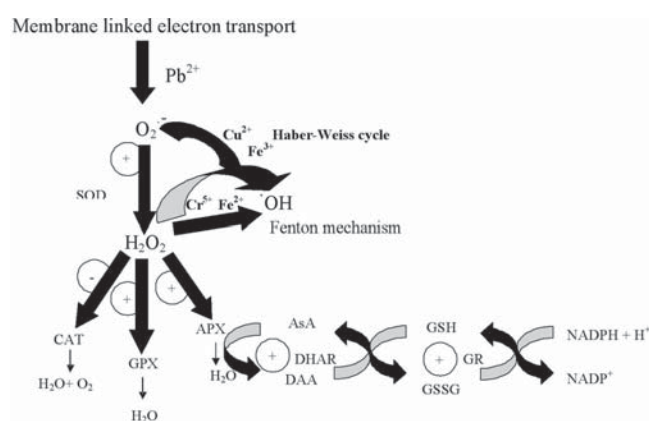
ers the water potential within the cell. Metal ions including Pb increase the content of ABA and induce stomatal closure (Figure 3). Disordered respiration and oxidative phosphorylation observed under Pb toxicity may also cause disarray in the plant water regime. Experiments using epidermal peels floating on Pb solutions have shown that Pb induces stomatal closure (Bazzaz et al., 1974). Experiments using excised leaves have indicated that metals increase stomatal resistance not only when directly applied to guard or epidermal cells but also when they reach the cells via xylem (Bazzaz et al., 1974). A unified hypothesis regarding Pb-induced stomatal closure indicates that such effect is due to the inhibition of an energy system or due to alterations of  $K^+$  fluxes through membranes (Bazzaz et al., 1974).

**Oxidative metabolism:** One of the phytotoxic effects of Pb appears to be induction of oxidative stress in growing plant parts due to enhanced production of reactive oxygen species (ROS) resulting in an unbalanced cellular redox status. A number of different ROS, including the superoxide anion ( $O_2^{\cdot-}$ ), singlet oxygen ( $^1O_2$ ), hydrogen peroxide ( $H_2O_2$ ) and the hydroxyl radical ( $\cdot OH$ ) are produced during normal oxidative metabolism in aerobic organisms, but these ROS can pose a severe threat when produced in larger amounts. Although some of the ROS may function as important signaling molecules altering gene expression and modulating activity of specific defense proteins, all ROS can be extremely harmful to organisms at high concentrations.

Pb induces production of ROS within plants and such production depends on the intensity of the stress, repeated stress periods, species and plant age (Asada, 1994; Chaitnya and Naithani, 1999; Verma and Dubey, 2003). Lipid peroxidation, which is regarded as indicator of oxidative damage, involves oxidative degradation of polyunsaturated fatty acyl residues of membranes (Girotti, 1990). Pb ions induce lipid peroxidation, decrease the level of saturated fatty acids and increase the content of unsaturated fatty acids of membrane in several plant species (Halliwell and Gutteridge, 1999). Although the ROS generating processes are slow under normal conditions, Pb accelerates them (Verma and Dubey, 2003). When rice (*Oryza sativa*) seedlings were grown in sand culture under 500 and 1000  $\mu M$   $Pb(NO_3)_2$  in the medium, during a growth period of 5-20 days about 21 to 177 % increase in the level of lipid peroxides was observed, indicating that Pb induces oxidative stress in these plants (Verma and Dubey, 2003).

A wide range of protective mechanisms exist in plants that serve to remove ROS before they can damage sensitive parts of the cellular machinery. These mechanisms can be conveniently divided in two groups, that is, non-enzymic antioxidants such as glutathione, ascorbate, tocopherols, carotenoids, etc. and the enzymic antioxidants like catalase, peroxidases, superoxide dismutases (SOD) as well as enzymes of ascorbate-glutathione cycle such as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) (Verma and Dubey, 2003).

Pb is not a oxido-reducing metal like iron, therefore the oxidative stress induced by Pb in plants appears to be an indirect effect of Pb toxicity leading to production of ROS, enhancing pro-oxidant status of cell by reducing the pool of reduced glutathione (GSH), activating calcium-dependent systems and affecting iron-mediated processes (Pinto et al., 2003). Figure 4 shows the sequence of events leading to production of ROS by Pb during membrane-linked electron transport and the role of antioxidative enzymes in scavenging ROS and maintaining the level of antioxidants ascorbate (AsA) and glutathione (GSH). Among the antioxidative enzymes, catalase decomposes  $H_2O_2$  to water and molecular oxygen. A decline in the activity of catalase has been observed in Pb-stressed plants (Verma and Dubey, 2003). Such

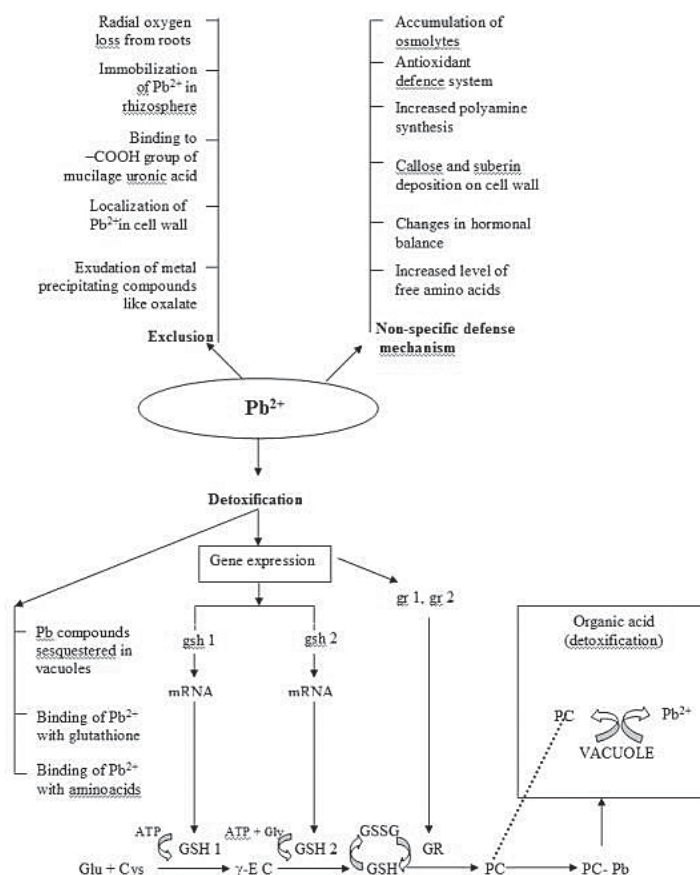


**Figure 4.** Effect of Pb on generation of reactive oxygen species and activities of antioxidative enzymes. Pb induces increased formation of reactive oxygen species ( $O_2^{\cdot-}$ ,  $H_2O_2$ ,  $\cdot OH$ ), increases the activities of antioxidative enzymes superoxide dismutase (SOD), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR) and NADPH dependent glutathione reductase (GR) but decreases the activity of catalase (CAT). The compounds ascorbic acid (AsA) and glutathione (GSH) are important non-enzymic antioxidants within the cell. Their oxidized forms are dehydroascorbic acid (DHA) and GSSG. Haber-Weiss cycle and Fenton mechanism generate hydroxyl radical ( $\cdot OH$ ) from superoxide anion ( $O_2^{\cdot-}$ ) and  $H_2O_2$ . The signs '+' and '-' denote induction or inhibition due to Pb, respectively.

a decrease appears to be due to a decline in enzyme synthesis or a change in the assembly of enzyme subunits (Hertwig, 1992). Verma and Dubey (2003) observed decreased intensity of two isozymic forms of catalase in shoots of Pb-stressed seedlings, consistent with decreased activity of the enzyme under Pb treatment. These isoforms are under the control of different genes (Frugoli et al., 1996). Pb has been reported to induce peroxidase activity in soybean, rice seedlings etc. (Lee et al., 1976; Verma and Dubey, 2003). The role of peroxidase as a stress enzyme (Gaspar, 1982) in plants has been widely accepted (Subhashini and Reddy, 1990). Increased peroxidase activity with Pb treatment can be correlated with the release of peroxidase localized in the cell walls (Gaspar, 1982). Increased activity of the superoxide scavenging enzyme has been reported in *Lupinus luteus* and *O. sativa* plants (Przymusiński et al., 1995; Verma and Dubey, 2003). The increase in the activity of SOD in response to Pb appears to be due to *de novo* synthesis of enzymatic protein (Lozano et al., 1996). Pb-stressed rice seedlings also show increased activities of APX and GR (Verma and Dubey, 2003). Though APX is considered a key enzyme of the antioxidative defense mechanism (Sharma and Dubey, 2004) which directly determines the cellular concentration of  $O_2^-$  and  $H_2O_2$ , the two enzymes SOD and GR appear to play a pivotal role in combating Pb-induced oxidative injury in rice plants (Verma and Dubey, 2003). Increased GR activity under Pb toxicity helps in recycling oxidized glutathione to reduced glutathione to maintain the ratio of oxidized glutathione to reduced glutathione (GSH/GSSG) and the total glutathione pool (Foyer et al., 1997). The increase in the activity of GR under stress has been attributed to *de novo* synthesis of the enzyme protein (Baisak et al., 1994).

**Lead tolerance in plants:** The two basic strategies of metal uptake related to tolerance in plants, as suggested by Baker (1981), involve (i) the 'excluder' strategy in which the concentration of heavy metals is maintained at a constant low level until critical soil concentration is reached when toxicity ensues and unrestricted metal transport results and (ii) the 'accumulator' strategy in which metals are actively concentrated within the plant tissues over the full range of soil concentration implying a highly specialized physiology. Berry (1986) also suggested three basic strategies of response: avoidance, detoxification and biochemical tolerance each of which affects tissue metal concentrations in different ways. Figure 5 describes various responses of cells, when plants are exposed to Pb. These responses include exclusion, detoxification mechanisms and non-specific defense systems.

It was shown by Ye and coworkers (1997) that *Typha latifolia* can colonize in both uncontaminated and metal-polluted areas and that the concentrations of Pb in the leaves are maintained at low levels. Similar results have been reported for this species by other authors. Collectively these observations indicate that *T. latifolia* tolerates Pb and that this tolerance ability depends mainly on metal exclusion ability. Metal tolerance and metal exclusion ability of this species appears to be related to its oxygen transport capability and radial oxygen loss from the roots resulting in a greater ability to modify the rhizosphere and immobilize metals in the rhizosphere



**Figure 5.** Response of plant cell to toxic levels of Pb. Exclusion capacity of plants is related to radial oxygen loss from root, efficiency to immobilize metals in rhizosphere, localization in cell wall, binding to -COOH groups of mucilage uronic acid and its precipitation by oxalate, whereas detoxification mechanisms include sequestration of Pb in the vacuoles by the formation of complexes, Binding of Pb to glutathione (GSH), amino acids (AA), phytochelatin (PC). The synthesis of PCs accompanies with decrease in cell glutathione pool and increase in the activities of glutamyl cysteine synthetase (GSH 1), glutathione synthetase (GSH 2) as well as glutathione reductase (GR). The elevated activities of GSH 1, GSH 2 and GR is correlated with enhanced expression of corresponding genes *gsh 1*, *gsh 2*, *gr1* and *gr 2*. Non-specific defense mechanisms induced due to Pb include accumulation of osmolytes, antioxidants, callose and suberin, amino acids and changes in hormonal balance.

(Ye et al., 1997). Rice roots have been reported to synthesize oxalate via the oxidation of glycolate to glyoxylate and then oxidation of glyoxylate to oxalate. Tolerant rice varieties up-regulate the synthesis and secretion of oxalate, a compound that precipitates Pb thereby reducing its uptake by root (Yang et al., 2000). Binding of Pb to the carboxy groups of mucilage uronic acids also restricts uptake of Pb into the root (Morel et al., 1986).

Strong binding of Pb to the carboxyl groups of carbohydrate in cell walls leads to its diminished transport via apoplast (Rudakova et al., 1988). An electron microscopic study of root tips from tolerant plants reveals the presence of Pb in the cell wall as well as the cytoplasm. It is suggested that the tolerance mechanism in *Anthoxanthum odoratum* plants is associated with its capacity to restrict the localization of Pb to the cell walls (Qureshi et al., 1986). Within the cell the major part of Pb is sequestered in the vacuole in the form of complexes. This may represent another mechanism of Pb detoxification in plants. Pinocytosis is observed in leaf cells of many plants treated with Pb salt solution. Through pinocytotic vesicles, Pb particles could be discharged into the vacuole (Wierzbica and Antosiewicz, 1993). Accumulation of excess total amino acid in response to Pb can be regarded as an important adaptive response of plants to avoid Pb toxicity. Several workers have emphasized the importance of the synthesis of metal chelating compounds such as amino acids like proline to avoid heavy metal toxicity (Alia and Saradhi, 1991).

Plants exposed to Pb and certain other heavy metal pollutants like Cd, Zn, Cu, Hg synthesize cysteine-rich low molecular weight polypeptides called phytochelatins (Cobbett, 2000). Phytochelatins (PCs) form a family of structures with increasing repetitions of the  $\gamma$ -Glu-Cys dipeptide followed by a terminal Gly;  $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ , where  $n$  has been reported to be high as 11, but is generally in the range of 2 to 5 (Zenk, 1996). Among the common metal pollutants, Cd and Pb are inducers of phytochelatin synthesis and the binding of these metals with PCs has been demonstrated in many plant species (Grill et al., 1987). Binding capacity of phytochelatins to different metals differs. It has been shown that Pb compounds bind less strongly to PCs than Cd, due to larger ion radius (Pb, octahedral) and high coordination number (Pb, 6-8). Induction of PC synthesis was shown in cell cultures of *Rauwolfia serpentina* when 1mM Pb was added to the medium. Such synthesis was accompanied by a decrease in the cell glutathione pool (Grill et al., 1987). It is believed that PCs are synthesized from glutathione and such synthesis

is due to transpeptidation of  $\gamma$ -glutamyl cysteinyl dipeptides from glutathione by the action of a constitutively present enzyme, PC synthase (Chen et al., 1997). Phytochelatin binds to Pb ions leading to sequestration of Pb ions in plants and thus serve as an important component of the detoxification mechanism in plants.

Several non-specific defense systems are also activated when plants are exposed to Pb. These include synthesis of osmolytes (like proline) and polyamines (putrescine), changes in the chemical composition of the cell wall (callose and suberin deposition), and changes in hormonal balance (primarily that of ethylene and ABA) (Seregin and Ivanov, 2001). There are different opinions regarding mechanisms by which osmolytes (proline) alleviate metal toxicity effects within the cell. It has been shown that free proline acts as an osmoprotectant (Paleg et al., 1984), protein stabilizer (Sharma and Dubey, 2004), metal chelator (Farago and Mullen, 1979), inhibitor of lipid peroxidation (Mehta and Gaur, 1999), free radical scavenger (Alia et al., 2001), etc.

*Remediation of lead contaminated soils:* The remediation of Pb-contaminated soils represents a significant challenge to many industries and government agencies. To date Pb-contaminated sites have been remediated through a relatively narrow range of engineering based technologies (Salt et al., 1995). Heavily contaminated soils have primarily been excavated, stabilized with cement and then placed in secured landfills. This process is expensive and requires additional site restoration.

During recent years the concept of using plants to remediate heavy metal contaminated sites (called phytoremediation) has received greater attention (Raskin et al., 1994; Vassil et al., 1998; Jarvis and Leung, 2002). Phytoremediation may involve either phytostabilization or phytoextraction. Phytostabilization reduces the intrinsic hazards posed by the contaminant without removing it from the site by reducing the bioavailability in the soil, whereas phytoextraction involves the use of plants to remove the contaminant from contaminated soils. The concept of using plants to accumulate metal for subsequent processing is both technically and economically attractive.

For practical reasons, the shoot Pb concentration is the most important physiological parameter for evaluating Pb-phytoextraction potential of plants (Huang and Cunningham, 1996). It has been shown that Pb accumulation in roots is significantly higher than in shoots, possibly because of the low Pb translocation from roots to shoots (Cunningham et



al., 1995; Verma and Dubey, 2003). Plant species with higher shoot/root Pb concentrations are more efficient in Pb translocation. *Thlaspi rotundifolium* plants have been reported to accumulate 130-8200 mg Pb.kg<sup>-1</sup> shoot dry weight (Reeves and Brooks, 1983). Because of its slow growth rate and small biomass however, this species is not suited for phytoextraction of Pb from contaminated soils. Some cultivars of *Brassica juncea* have been shown to accumulate as high as 1.5 % Pb in the shoot when grown in nutrient solution containing 760 µM Pb (Kumar et al., 1995). It was reported by Huang and Cunningham (1996) that the Pb concentration in the shoot of corn was significantly higher than that of the best Pb-accumulating *Brassica juncea* cultivars when the plants were grown on a Pb contaminated soil.

Results from chelation experiments indicate that Pb concentration in the shoot can be increased dramatically when the soil Pb concentration is increased by adding a synthetic chelate to the contaminated soil. The synthetic chelate EDTA forms a soluble complex with many metals, including Pb (Kroschwitz, 1995) and can solubilize Pb from soil particles (Vassil et al., 1998). Application of EDTA to Pb-contaminated soils has been shown to induce the uptake of Pb by plants causing Pb to accumulate more than 1 % (w/w) of the shoot dry biomass (Huang and Cunningham, 1996; Huang et al., 1997). Large Pb particles cannot easily cross the casparian strip due to their size and charge characteristics but once they form a complex with chelators such as EDTA, their solubility increases, the particle size decreases and they become partially 'invisible' to those processes that would normally prevent their unrestricted movement such as precipitation with phosphates and carbonates, or binding to the cell wall through mechanisms such as cation exchange (Jarvis and Leung, 2002). The mechanism by which solutes that have moved symplastically from root epidermal cells to the parenchyma cells and on to the vascular cylinder, enter the vessels or tracheids of the xylem is postulated to be some type of highly selective active-carrier transport, as opposed to facilitated diffusion (Raven et al., 1999). Although it is certain that this mechanism does not involve transport of lead, and that the movement of charged Pb particles through this route would be impeded to some extent, it is likely that a Pb chelate complex could pass through this route more successfully. It is important to point out that the addition of chelates to the soil has to be done in a carefully controlled manner so as not to mobilize Pb into ground water or otherwise promote its off-site migration (Huang and Cunningham, 1996).

Another promising clean-up technology appears to be rhizofiltration, which involves use of plant roots to remove contaminants such as heavy metals from contaminated water (Dushenkov et al., 1995). A high level of Pb deposition is seen in corn root tips as revealed by histochemical and electron microscopy studies. When the seedlings are incubated in a medium containing 0.66 mM Pb for 19 days (Tung and Temple, 1996), a strong deposition of Pb occurs on the surface as well as in internal tissues within the first 2 mm of tap root and secondary root branches in the seedlings (Tung and Temple, 1996). Malkowski and coworkers (2002) also showed that corn plants treated with 10<sup>-3</sup> M Pb accumulated 138,430 mg of Pb per kg of dry weight in root tips compared to 26,833 mg in the root basal part. Since the first 8 mm of the apical root accounts for approximately 50 % of the Pb accumulated by the entire root system (Malkowski et al., 2002), it appears that the plant with a more branched root system will take up more Pb and other heavy metals compared to plants with longer and less branched root systems. Though phytoremediation and rhizofiltration technologies are still in developmental stages, for the future these technologies appear to have great potential for the clean-up of soils contaminated with Pb and other heavy metals.

**Conclusion:** Pb has gained considerable attention as a potent heavy metal pollutant due to the growing anthropogenic pressure on the environment. Pb contaminated soils show a sharp decline in crop productivity. Pb is taken up by plants mainly through the root system and partly, in minor amounts through the leaves. Inside the plants Pb accumulates primarily in the root but a part of it is translocated to the aerial portions. Soil pH, soil particle size, cation exchange capacity as well as plant factors such as root surface area, root exudation and mycorrhizal transpiration rate affect the availability and uptake of lead. Limited translocation of Pb occurs from root to other organs due to the barrier function of the root endodermis. At lethal concentrations this barrier is broken and the flux of Pb enters the vascular tissues. Pb deposits of various size are present mainly in the intercellular spaces, cell walls and vacuoles. Small deposits of this metal are also seen in the endoplasmic reticulum, dictyosome and dictyosome derived vesicles.

After entering the cell, Pb inhibits activities of many enzymes, upsets mineral nutrition and water balance, changes the hormonal status and affects membrane structure and its permeability. Visual non-specific symptoms of Pb toxicity are stunted growth, chlorosis and blackening of the root sys-

tem. In most cases inhibition of enzyme activities due to Pb results from the interaction of the metal with enzyme –SH groups. The activities of metalloenzymes may decline due to displacement of an essential metal by Pb from enzyme active sites.

Pb decreases photosynthetic rate by distorting chloroplast ultrastructure, diminishing chlorophyll synthesis, obstructing electron transport and inhibiting activities of Calvin cycle enzymes. At low concentrations Pb stimulates respiration and increases ATP content whereas higher concentrations are inhibitory to respiration and decrease ATP. Pb causes the imbalance of the minerals K, Ca, Mg, Mn, Zn, Cu, Fe within the tissues by physically blocking the access of these ions to the absorption sites of the roots.

Pb exclusion capacity of plants is related to oxygen transport ability, radial oxygen loss from the root, efficiency to immobilize Pb in the rhizosphere, binding capacity to –COOH groups of mucilage uronic acid, its precipitation by oxalate, etc. Mechanisms of Pb detoxification include sequestration of Pb in the vacuole by the formation of complexes, binding of Pb by phytochelators, glutathione and amino acids. Biochemical tolerance to Pb is related to the capacity of the plants to restrict Pb to the cell walls, synthesis of osmolytes and activation of the antioxidant defense system. Further studies are needed to specify the parameters associated with Pb exclusion and Pb detoxification capacity of plants as well as those biochemical parameters associated with tolerance to Pb in plants. Such studies will enlighten the mechanism of the genetic and biochemical basis of Pb tolerance in crops and based on biotechnological tools it should be possible to produce plants with enhanced Pb tolerance. Emerging clean-up technologies for remediation of Pb such as phytoextraction and rhizofiltration have the potential to provide environmentally sound and economically viable remedies for the cleaning of Pb-contaminated soils.

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