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Pb-induced toxicity in plants: disruption of cellular structure and cell membrane

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Abstract

Lead (Pb) is the most abundant heavy metal contaminant in the environment. Pb accumulates in plants and affects human health. Pb exposure causes oxidative stress and affects growth and physiology of plants; and disrupts various biochemical attributes. Pb causes oxidative stress in plant roots and produces free radicals, which in turn act on the unsaturated lipids in the membranes, leading to an autocatalytic chain reaction called lipid peroxidation and damages cell membrane. The current review focuses on how Pb disrupts cellular structure, damages cell membrane, alters the number and structure of mitochondria and disrupts nuclear integrity. Pb exposure induces structural anomalies in the plant roots. Root surface exhibit withered cells and dense growth of root hairs. Primarily Pb moves into apoplast, however, higher concentrations of Pb may interrupt the casparian strips of the endodermis allowing Pb ions to move into the vascular tissue of the plant. Increase in the number of mitochondria can be attributed to the enhanced demand of ATP generation to combat Pb-induced stress. The alterations in ultrastructure of nuclei relates directly to the decrease in transcriptional and translational activity.

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Introduction

Lead (Pb) is the most abundant heavy metal contaminant in the environment (Sengar *et al.*, 2008). It is released through a variety of anthropogenic activities such as automobile exhausts, chimneys/ stacks of factories, additives in gasoline and paint, fertilizers and pesticides, effluents from storage battery industry, metal plating and finishing operations, smelting of ores (Kaur *et al.*, 2012). Being largely immobile in nature, Pb persists and pollutes the soil. Once released into the environment, it gets easily absorbed and accumulated in the soil, thereby inhibiting plant growth and development (Sharma and Dubey, 2005). Pb accumulates in plants and affects human health. Pb exposure causes oxidative stress and affects growth and physiology of plants; and disrupts various biochemical attributes (Singh *et al.,* 2011).

Pb has been reported to cease root growth along with growth inhibition at the root tips (Eun *et al.*, 2000). Pb causes oxidative stress in plant roots and produces free radicals, which in turn act on the unsaturated lipids in the membranes, leading to an autocatalytic chain reaction called lipid peroxidation and damages cell membrane (Kumar *et al.*, 2013).

The primary consequence of the damage to the cell membrane is the loss of sodium ion pump and the cell becomes flaccid. This results in permeability of the membrane, occurrence of abnormal calcium flux leading to mitochondrial damage and activation of phospholipases enzyme. Pb disrupts the membranes in Rough Endoplasmic Reticulum and dissociates the ribosomes, which in turn affects the protein synthesis. Further, damage to mitochondrial membranes starves the cell of energy in the form of ATP. Thus, the current review focuses on how Pb disrupts cellular structure, damages cell membrane, alters the number and structure of mitochondria and disrupts nuclear integrity.

Pb disrupts cellular structure

Pb exposure induces structural anomalies in the plant roots. Several morpho-anatomical changes have been observed in roots of plants grown under Pb exposure. Root surface exhibit withered cells and dense growth of root hairs (Kaur *et al.*, 2012). The damage caused in the superficial cells of roots may result from the direct effect of Pb on the membranes. Root hairs are responsible for the conduction of water from soil into the roots of plants. It ultimately affects the conduction of water into xylem tissue cells; whereas, nutrients enter directly from water into root but distorted root structures indicate inhibition of nutrients and water entry into the plant (Askari *et al.*, 2007). The increased number of root hairs possibly enables plant roots to acclimatize for absorption of other minerals and nutrients by increasing the area of absorption of the root.

Pb treated cells lost their turgidity and became flaccid (Kaur *et al.*, 2014) because Pb solutions serve as hypertonic solution (solute concentration outside the cell is higher than that inside the cell) for the root cells resulting in plasmolysis. Thus, the cells lose water and shrink. A prolonged plasmolysis can even lead to cell death (Ashraf, 2004). In fact, the accumulation of salts/osmolytes, reduce osmotic potential and help the plants to adjust to environmental conditions by alleviating the adverse effects of heavy metals or other stresses (Kamenova-Yuchimenko *et al.*, 1995; Ashraf and Foolad, 2007; Azhar *et al.*, 2009). Scanning electron microscopic studies revealed clogging of lumen with some depositions upon Pb exposure (Kaur *et al.*, 2014). This can be probably due to the alterations in root structure which might influence metal accumulation in different root tissues. Similarly, Marschner *et al.* (1990) reported depositions of amorphous silica in cell lumens, cell walls and intercellular spaces. In roots treated with Pb, some depositions were found in the cortical and vascular tissue of the root (Kaur *et al.*, 2014). Abnormal cortical cells and malformation of stelar cells upon Pb exposure were also reported by Kuno (1984). Distortions in vascular bundle indicated that Pb damaged growth through disrupting vascular tissue and deposition of unknown debris, which blocked water and nutrient translocation flow.

Pb-exposure damages cell membrane

Cell wall is an important site for metal ion storage and it is one of the favorable and necessary locations for Pb accumulation and sequestration. It also acts as an excretory organ for heavy metals and prevents their movement into the protoplasm (Yang *et al.*, 2005). Pb-induced toxicity results in formation of protuberances and consequently damages cell wall. Reports suggest that Pb damages cell structure in internodal segments of *Chara vulgaris* (Heumann, 1987) and *Elodea canadensis* (Sergio *et al.*, 2007). Likewise, Kurkova *et al.* (2002) opined that these protrusions increase the contact surface area of the cell and provide physiological advantage to the plant for enhanced transport of ions and water. Pb has been reported to disrupt the arrangement of cell wall microfibrils (Heumann, 1987; Sergio *et al.*, 2007) and affect microtubule assembly (Basile *et al.*, 1995), which manifest in the formation of folds / protrusions in the cell wall. Heumann (1987) suggested that Pb does not directly disrupt the cell wall, but indirectly by interfering with the various metabolic processes linked to synthesis, translocation and disposition of cell wall materials. Basile *et al.* (2009) documented that heavy metal pollution damages cell membrane and induces formation of membrane pits in the moss *Scorpiurum circinatum*.

The distortion, withering and irregularities in the root surface cell could be due to interference of Pb with cell wall and cell membranes (Nishizono *et al.*, 1987; Kaur *et al.*, 2012). Wierzbicka (1998) and Kaur *et al.* (2012) reported thinning of the cell walls upon Pb treatment (Fig 1 a,b) and Pb exposure is also known to induce breaks and nicks in the cell wall, thereby interfering with the synthesis, translocation, and disposition of cell wall materials (Heumann 1987).

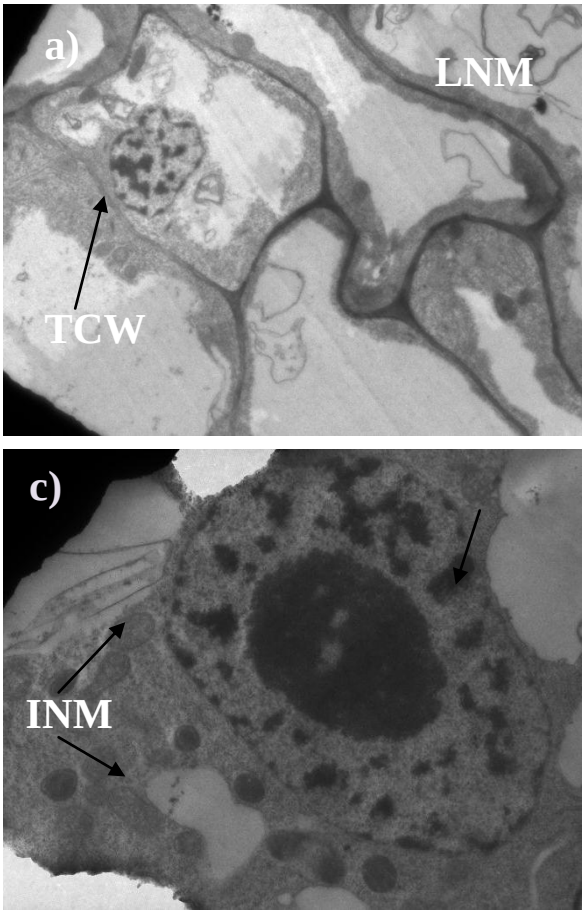

Fig. 1. Electron micrograph showing the effect of Pb on the cell wall of 4-day-old wheat roots: a) and b) thinning of cell wall (TCW); c) increased number of mitochondria (INM) and loss of nuclear membrane (LNM).

Alkhatib *et al.* (2013) reported the primary localization of Pb in the vascular tissue and cell walls of the plant roots. Primarily Pb moves into apoplast, however, higher concentrations of Pb may interrupt the casparian strips of the endodermis allowing Pb ions to move into the vascular tissue of the plant. Lane and Martin (1977) opined that endodermis act as a partial barrier and it allows the movement of some of the Pb ions through the vascular tissues. Moreover, Pb binds strongly to the carboxyl groups of carbohydrates in cell walls of roots thereby reducing its movement via apoplast (Rudakova *et al.*, 1988). Further, the excessive metal ions are also found to be sequestered in the vacuole as Silverberg (1975) reported the presence of metal ions in the vacuoles of *Stigeoclonium* .

Kaur *et al.* (2014) observed damaged cortical cells in light micrographs of Pb-treated roots. Damage in the cortical cells might be responsible for the disrupted diffusion of materials into the central cylinder of root and interrupted storage of food in the form of starch.

Pb exhibits a higher potential to bind the carboxyl groups of the carbohydrates such as galacturnic acid and glucuronic acid in the root cell wall (Rudakova *et al.*, 1988). Inoue *et al.* (2013) opined that the amount of carboxyl groups in the polysaccharides determine the tendency of divalent metal cations to bind with the cell walls. Pb-galacturonic acid fragments were reported in the roots of *Arabidopsis thaliana* treated with Pb (Polec-Pawlak *et al.*, 2007). Pb ions have a greater affinity for pectin in cell walls and it easily replaces Ca ions, thereby reducing the Ca content. Brunet *et al.* (2008) reported reduction in the content of Ca ions in the treated roots of *Lathyrus sativus* as compared to control. In the root cells of *Oryza sativa*, Ca ions were reported to compete with Pb ions for entry into the root cells. Further, supplementation of Ca ions in the medium reduced the uptake and toxicity of Pb indicative of the pathway of Pb entry into the root cells via Ca^{2+}/Mg^{2+} gated channel (Kim *et al.*, 2002). According to Sunkar *et al.* (2000), heavy metals like Pb probably enter the plant cells via essential cation transporters. A non-selective cation channel such as *AtCNGC* was recommended to facilitate Pb^{2+} entry in to the root cells but the over-expression of the truncated gene resulted in Pb^{2+} tolerance (Sunkar *et al.*, 2000).

Earlier, researchers have demonstrated that toxic ions interact with anionic contents in plasma membrane and accumulate in apoplast (Carrier *et al.*, 2003), and /or form bonds with proteins and phospholipids, cause membrane disintegration and disperse in cytoplasm (Cha and Lee, 1996; Liu *et al.*, 1996). The apparent origin of lesion within the cell wall observed in the present study suggests that Pb-treatment probably causes activation of certain enzymes associated with cell wall. It is strengthened by an earlier study wherein it has been reported that victorin (a pathotoxin)- induced lesions in cell wall of oat roots are due to activation of wall degrading enzymes (Hanchey *et al.*, 1968). Kaur *et al.* (2012) observed dark precipitates in the cell wall as Pb deposits which suggested that Pb movement occurs in apoplast and it parallels the earlier observations (Broyer *et al.*, 1972). Khatib *et al.* (2008) documented that Pb deposits occur as dark crystalline particles along cell wall in tobacco roots. Kaur *et al.* (2012) demonstrated Pb-induced significant alterations in wheat root ultrastructure analyzed by transmission electron microscopy.

Pb-exposure alters the number and structure of mitochondria

The increased number of mitochondria, as reported by Kaur *et al.* (2012), in response to Pb parallels the earlier observation that the number of mitochondria increases in response to abiotic stresses, including herbicides (Stoynova *et al.*, 1997), and heavy metals (Konarska, 2008; Gzyl *et al.*, 2009). Increased mitochondria possibly serve to meet enhanced ATP generation required for adaptation to Pb-induced stress (Fig. 1c). However, the destructive phenomenon observed at 500 µM Pb can be attributed to irreversible changes / damage induced by toxic concentration of heavy metal, which did not

allow adaptation (Kaur *et al.,* 2012). Earlier, the ultrastructural changes and degradation of mitochondria have been related to ionic (Fe, P, Mg, P, S, K and Ca) deficiency in the cytoplasm (Vázquez *et al*., 1992; Čiamporová and Mistrík, 1993; Koyro, 1997). Whether Pb-induced degradation / alteration of mitochondrial structure were due to deficiency of ions / uptake of essential oils is still unexplored.

Pb disrupts nuclear integrity

Effect on Nucleus

The structure of nucleus and nucleolus closely relates to metabolic capability and expression of genetic information (van Assche and Clijsters, 1990). The changes in the nucleolus ultrastructural organization in response to Pb resembled those caused by chorosulfuron on the nucleus of pea plant cells (Stoynova *et al*., 1997). Earlier, a parallel nucleolar segregation has been observed in root tip cells of *Allium cepa* by adenosine-3'-deoxyriboside (Fernändez-Gómez *et al*., 1972). Simard (1970) opined that chemicals which induce nucleolar segregation do so by binding directly to DNA, and interfering with its template activity and not by inhibiting RNA synthases. The alterations in ultrastructure of nuclei relates directly to the decrease in transcriptional and translational activity (Giménez-Martín *et al*., 1977). A study by Kaur *et al.* (2012) demonstrated loss of nuclear membrane in 4-days old wheat roots (Fig.1 c)

Conclusion

Pb exposure induces structural anomalies in the plant roots. Pb inhibits root growth and disrupts cellular structure, damages cell membrane, alters the number and structure of mitochondria and interrupts nuclear integrity.

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