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Review Article

The Roles of Plant Cell Wall as the First-line Protection Against Lead (Pb) Toxicity

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Abstract
Heavy metal contamination is one of the serious environmental problems. Among heavy metals, lead (Pb) is a non-essential metal with highly toxic to plants and animals. Due to the high atomic weight, Pb is largely accumulated in plant roots more than shoots. Pb absorbed along with water and other nutrient possibly pass through cell wall and cell membrane. A number of plants have been accumulated Pb in their cell wall and intercellular space. This review paper focuses on the role of plant cell wall as the first line protection of the plant cells against Pb toxicity. Upon Pb exposure, production of Reactive Oxygen Species (ROS) such as nitric oxide (NO) and hydrogen peroxide (H_2O_2) have been increased. These small molecules are normally used as signaling molecules in antioxidation system activating radical scavengers e.g. peroxidase and catalase. Sharply increased ROS may also acts as a signal for remodifying cell wall structure. Pectins and other polysaccharides in plant cells can bind and sequester Pb within the cell walls. The thick cell walls act as a barrier that limit Pb from entering into the protoplasm as well as serving as a compartment for storing the metal.

Keywords: Plant cell wall, Pectins, Lead (Pb), Toxicity, Heavy metal

1 Introduction

Lead (Pb) is a non-essential element with the atomic number of 82. It is one of the heavy metals that have very high atomic mass (207.2 g/mol). When exposed to moist air, Pb can form a covering layer of different elements. Lead carbonate is commonly found in the layers. Pb rarely presents in the metallic form, but generally forms compounds with sulfur and remains on the earth surface. Amounts of Pb in the earth crustal is approximated 10–14 ppm [1]. It is the 38th most abundant element found in the earth crust.

Humans have been using Pb since the Romans. Lead pipes were used as drains from the Roman baths. However, Pb contamination in the environment became widespread at the beginning of the Second Industrial Revolution (1850–1890) and the magnitude of Pb contamination significantly increased [2]. Pb is extensively used in battery industry as a major constituent of the lead-acid batteries. Pb is also used as a radiation shield in computer glasses and television screens. Contamination of Pb in the environment frequently is resulted from mining and smelting activities and disposal of municipal sludge enriched in lead. Although amounts of Pb have been reportedly declined in some areas, Pb contamination remains largely present and highly enriched in global ecosystems especially in developing countries e.g. Asia and

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South America [2]. Despite regulatory measurement, the overall Pb input in the environment continues to increase. Once introduced into the soil matrix, Pb is very difficult to remove. Pb is strongly bound to the soil particles through the process of adsorption, ion exchange, precipitation and complexation with organic matters [3]. Environment enriched with Pb has deleterious effects on ecological systems, plants and human populations.

2 Lead in Environment

Soil Pb can be categorized into 6 groups including soluble ions in soil water, exchangeable, lead carbonate, lead oxyhydroxide, organic bound and precipitation fraction [3]. Water soluble and exchangeable Pb are the only fractions readily available for plant uptake.

Oxyhydroxide, organic and precipitated forms of Pb are strongly bound to soil particles and largely remain within the topsoil (6–8 in). However, mobility of Pb in the soil matrix depends on pH. Pb ions are more mobile thus available for plant absorption under acidic conditions ($pH < 5.5$) Low availability of lead (Pb) fraction in soil matrix significantly reduces Pb accumulation in the plants [4].

With continuously increasing number of population, more lands are needed for agriculture and food production. It becomes more common that cultivated areas are located nearby industrial zones. Significant increases in Pb content have been observed near industrial areas [5]. Since Pb is in the surface ground layer, it is easily absorbed and accumulated in different plant organs. Soil with Pb contamination causes sharp reduction in crop productivity thus posing a serious problem for agriculture.

Although large amounts are probably deposited to soil and water, Pb can be widely dispersed into in the air. The amount of Pb in air varies from 2–4 $\mu g/m^3$ in large cities with heavy automobile traffic to less than 0.2 $\mu g/m^3$ in most suburban areas [6]. Atmospheric Pb can be introduced to human communities by deposition on terrestrial surfaces. There is insufficient evidence to indicate an airborne Pb hazard on terrestrial organisms. However, Pb concentrations in roadside soil can be increased up to 5,000 mg/kg comparing to 15–30 mg/kg of Pb concentrations in normal soils [6]. Fractions of Pb could be leached from highly contaminated soils or taken up by plants and eventually passed to animals and human in the area. Contaminated Pb can enter into the food chain causing serious health problems in the community. Children with younger ages are more susceptible to the effects of lead which include lower IQ and slowed growth [7].

3 Lead Accumulation and Toxicity in Plants

Although Pb has tendency to form insoluble complexes with anions and soils, portions of Pb in highly contaminated soils are readily for plant absorption via root system. Uptake and transportation of Pb can take place along with absorption of water and other required minerals. Adverse effects of Pb on plants include inhibition of seed germination and plant growth, reduction in plant fresh and dry weights, induction of oxidative stress, decrease in photosynthesis and respiration, imbalance of nutrient uptake [8], [9]. Among the broad range effects of Pb, root inhibition is the prominent response which has been observed in several plant species [10]. Roots are the main structure through which Pb enters the plant body. In most plants, translocation of Pb from root to shoot part is very low, thus large amounts of Pb are found in the roots (Table 1). The Pb ratios in shoot to root of these plants are extremely low and are classified as Pb excluders [11]. On the other hand, a group of plants called hyperaccumulator shows high capacity for absorbing and concentrating large amounts of Pb in their tissues especially above ground tissues. It is possible that the two groups of plants have different mechanisms to overcome Pb toxicity. In this review article, we basically focus on the Pb responses in the excluders which possibly contain a larger groups of plants.

Pb treatments also showed adverse effects on plant ultrastructure. Plants exposed to Pb show abnormal chloroplast ultrastructure with disorganization of thylakoids and stroma lamellae as well as enlarged starch grains [12]. When Pb enters cells even in small amounts, it causes a wide range of adverse effects. Pb treatment caused dilated nuclear envelope, disintegrated mitochondrial membrane and disappearance of endoplasmic reticulum leading to autophagy in root cells [13].

4 Plant Cell Wall as a Primary Site for Pb Accumulation

Plant cells are surrounded by a strong cell wall. This

Table 1: Pb concentrations (mg/kg dry wt) in root and shoot or leaf tissues of different plants species

Species	Class	Pb Conc. (mg/kg)		TF[1]	BCFR[2]	Growing Time (mo.)	Ref.
		Root	Shoot				
Potential Pb hyperaccumulators							
Jatropha curcas	eudicot	28.16	17.06	0.60	41.41	18	[14]
Brassica napus L.	eudicot	8.71	2.64	0.30	0.09	4.5	[15]
Hemidesmus indicus	eudicot	611	384	0.63	0.61	3	[16]
Potential Pb excluders							
Hydrocotyle vulgaris	eudicot	60 ± 3	4.6 ± 0.9	0.08	0.19	3	[17]
Cyperus alternifolius	monocot	82 ± 9	6.9 ± 0.9	0.08	0.26		
Echinodorus baothii	monocot	134 ± 6	9.2 ± 0.5	0.07	0.43		
Panicum repens	monocot	38 ± 1	3.3 ± 0.8	0.09	0.12		
Scirpus triqueter	monocot	84 ± 8	7.9 ± 1.3	0.09	0.27		

[1] Translocation Factor (TF) is calculated from the concentration of Pb in plant's aerial parts (stems and leaves) divided by the Pb concentration accumulated in the roots. These numbers represent the ability of plants on Pb translocation.

[2] Bioconcentration Factor of Roots (BCFR) represents ability of plants to accumulate Pb from the ambient environment in their roots. It is calculated by dividing Pb concentration in plant roots with Pb concentration from the soil.

wall contains a complex mixture of polysaccharides and other polymers. Composition of cell walls varies depending on species, cell type and developmental stages [18]. The primary cell wall of land plants is composed of cellulose, hemicellulose and pectin. Cellulose is a linear polymer of glucoses or glucans forming crystalline microfibrils stabilized by hydrogen bridges. The cellulose microfibrils are surrounded by a highly hydrated matrix of hemicelluloses and pectins. Pectins acts as hydrophilic filler in which the cellulose and hemicellulose network is embedded. Major polysaccharides found in pectins include xylans, mannans, galactomannans, glucomannans, beta-1,3 and beta-1,4-glucan. The pectic substances are a complex mixture of colloidal polysaccharides that can be extracted from the cell wall with water or chelating agents. In addition, pectins are abundant in middle lamella, the interface between adjacent plant cells, and glue them together. Primary wall is normally composed of approximately 25% cellulose, 25% hemicelluloses and 35% pectins on a dry-weight basis. However, the ratio can be diverse among species e.g. 20–25% cellulose, 60–70% hemicelluloses and only 10% pectins are found in grass coleoptiles [19].

Several ultrastructural studies have shown Pb localization in plant cell walls, intercellular spaces and vacuoles of several plants species [13], [20]–[22]. Pb appeared as large crystals or dense deposits in middle lamella and plant cell wall of root cells. Much lower amounts of fine deposit of Pb were observed in cytoplasm. It has been proposed that polysaccharides with negatively charged functional groups such as carboxyl and hydroxyl group play a crucial role in Pb binding and accumulation in plant cell walls [21]. The source of the carboxyl groups was suggested to be pectic compounds [23]. When treated cells with lead, Pb ions can replace calcium ions that cross-link between polymers of low-methylesterified pectins (Figure 1). Other cations such as Al^{3+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+} can also bind and replace calcium in pectin structure. However, Pb^{2+} is the most strongly bound bivalent to pectin polymers [24].

Pb treatment also affected levels of pectins in plant cell wall [25]. An increased level of low-methylesterified pectins was observed under Pb stress [26]. The higher amount of acidic pectins possibly increased cell wall capacity for Pb binding and sequestration. In addition to pectins, negatively charged molecules such as phosphates have been reported for binding with Pb into the wall [27]. A callose layer was also observed nearby the Pb deposits thickenings cell wall and protecting the metal from returning into the protoplast (Figure 1). These structural changes allow cell wall as an efficient barrier to Pb penetration.

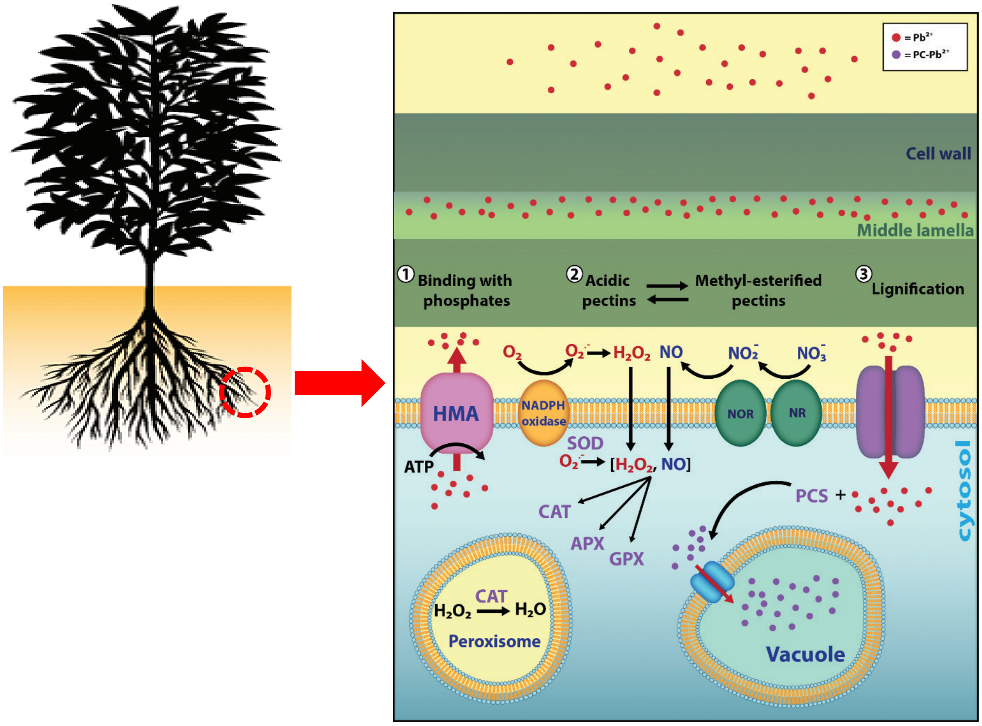

Figure 1: How plant cell wall response to lead (Pb) toxicity. Pb ion can bind to negative charges of phosphates (1), methylesterified pectins (2) or increase cross-linking of lignins (3) in plant cell wall. HMA: heavy metal ATPases, PCS: phytochelatin synthase, SOD: superoxide dismutases, CAT: catalase, GPX: glutathione peroxidase, APX: ascorbate peroxidase.

5 Signals Leading to Cell Wall Remodeling

After passing plant cell wall, Pb ions may compete with nutritional ions such as Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+} and enter into the cytoplasm through membrane carriers such as Fe transport Natural Resistance And Macrophage Protein 1 (NRAMP1), Zn/Fe Regulator Protein (ZIP), Iron-Regulated Transporter (IRT) or Heavy Metal transporting P-type ATPase (HMA) [28]. Pb ions in cytoplasm can be bound by metal chelators such as glutathione (GSH), phytochelatins (PCs) and metallothioneins (MT) and transported in vacuole. A number of carriers such as HMA have also been reported as metal effluxer pumping the metals out into the apoplast. However, an increase in metal concentrations in plant cytoplasm triggers series of physiological changes. One of the Pb toxicity is the induction of oxidative stress by enhancing production of Reactive Oxygen Species (ROS). Molecules such nitric oxide (NO), hydrogen peroxide (H_2O_2) and superoxide anion (O^{2-}) are produced when plants are under Pb stress. An excess production of ROS can damage lipids, proteins and nucleic acids causing membrane leakage, enzyme inactivation and DNA cleavage [29]. Plants exposed to Pb significantly increased in NO and H_2O_2 production within 6 h. after the treatment [30]. Accumulation of ROS is usually accompanied by up-regulation of the activities of antioxidative enzymes such as peroxidase (POD), catalase (CAT), superoxide dismutase (SOD) and glutathione-s transferase (GST) [31]. Activation of these antioxidative enzymes would be crucial for plant survival alleviating the metal toxicity. In addition, NO production could increase amino acids and phenolic compounds that possibly lead to more rigid plant cell wall [30].

ROS production is thought to act as a trigger for lipid peroxide induction and jasmonic acid formation [32]. Pretreat of JA prior to metal exposure could decrease heavy metal accumulation and activate

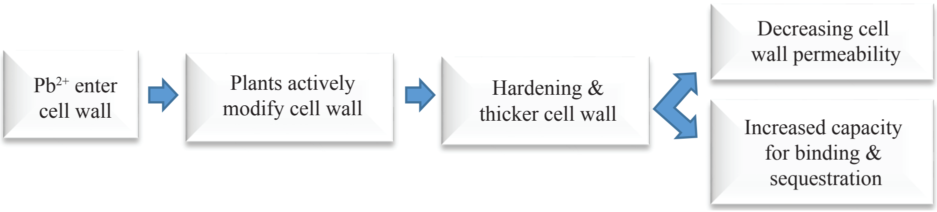

Figure 2: Plant survival mechanisms under heavy metal stress. Accumulation of Pb ions in plant cell wall results in cell wall remodeling and thickening. These changes neutralize Pb^{2+} toxicity and improve plant survival.

antioxidative system such as catalase, ascorbate peroxidase, NADH peroxidase. Other plant hormones have also been reported to interplay during plant responses to metal toxicity. Co-application of brassinosteriods and salicylic acid could improve Pb tolerance in *Brassica juncea* plants [33]. These phytohormones possibly enhance the oxidative defense generated by the Pb toxicity.

6 Cell Wall Remodeling as Survival Strategy under Heavy Metal Stress

Integrity of cell wall has been proved to be crucial for plant survival under various kinds of stress [34].

The presence of heavy metals in plant cell wall normally results in inhibition of cell elongation as well as cell wall stiffening [8]. The accumulation of Pb in the plant cell wall possibly increases the cross-linking of phenolic groups in plant cell wall making the wall more rigid and thicker. The hardening cell wall then limits more metals from entering into the plant cell and functions as an effective metal barrier (Figure 2). In addition, the thicker cell wall provides more compartment for active binding and sequestration of the metals. These changes at the cell wall play vital roles for plant survival under heavy metal stress.

7 Conclusions

With growing anthropogenic pressure on environment, plants are increasingly exposed to strong stress agents such Pb. In response to the toxicant, plants activate several programs for survival and further adaptation. These programs include reduced uptake of the metal, exclusion of the metal, segregation of the metal in metabolically inactive cell compartments such as plant cell wall. Figure 1 schematically outlines the responses to Pb when the metal enters the plant cells. Most plant species act as excluders by accumulating Pb in their underground tissues. When entering the roots, Pb moves by apoplastic route and largely accumulates in the cell walls. Plants comprise several functional barriers for reducing the uptake of Pb into the cytoplasm.

One of the barriers is restriction of plasma membrane for Pb uptake into the protoplast. In addition, plant cell walls act as a sink to accommodate of the toxic Pb. Pb ions possibly cross-link with the free carboxyl groups of low-methylesterified pectins in plant cell walls. Increasing amounts of Pb actively modify the plant cell wall thickening the structure. The thickened cell wall physically traps as well as chemically binds the Pb ions limiting the metal from penetrating the protoplast. These cell wall modifications essentially enhance the chance of plant survival and growth in the soils with highly contaminated with Pb.

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