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Research Paper

Pb-INDUCED TOXICITY IN PLANTS: EFFECT ON GROWTH, DEVELOPMENT, AND BIOCHEMICAL ATTRIBUTES

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Abstract

Lead (Pb), a toxic element is harmful to plants; yet, the plants show the ability to accumulate large amounts of Pb without visible changes in their appearance or yield. Moreover, the daily consumption of Pb-contaminated foods poses a risk to human health, since it is harmful even in very small amounts. Pb contamination in plant environment affects germination of seeds and exerts deleterious effects on the growth and metabolism of plants. The plants growing in a medium or soil containing Pb accumulate the metal in all parts of plant body. However, a higher level is reported in roots than in shoots. In general, it has been postulated that growth inhibition and visible injury in the plants are the primary effects of heavy metals. This review updates the readers how Pb causes biochemical changes during early development and induces oxidative stress via reactive oxygen species (ROS) generation. Additionally, Pb induces lipid peroxidation and causes greater accumulation of H_2O_2 and $O_2^{\bullet -}$. Pb exposure not only alters ROS scavenging enzymes but also affects non-enzymatic antioxidants.

Key words: Pb toxicity, Reactive Oxygen Species (ROS), oxidative stress, non-enzymatic antioxidants, and macromolecules.

INTRODUCTION

Among heavy metals, Pb (Atomic Number: 82; Atomic mass: 207) is the heaviest of non-radioactive metals that occur naturally in substantial quantities on earth's surface. According to EPA (Environmental Protection Agency, USA), Pb is the most common heavy metal contaminant in the environment [1]. In fact, it is one of the major heavy metals of the antiquity and has gained considerable importance as a potent environmental pollutant in almost every ecosystem [2]. In the atmosphere, Pb mostly exists as lead sulphate and lead carbonate [3].

Pb is released into the natural environment from a variety of anthropogenic sources, including automobiles, mining, agricultural activities such as use of fertilizers and

pesticides, industrial activities such as metal plating, additives in pigments and as effluent from storage battery industry [4]. Pb is available to plants from the soil and aerosol sources and it is found in the form of Pb-phytochelatins, Pb-nitrate, Pb-acetate, Pb-sulfide and Pb-citrate type compounds [5]. Finally, this accumulated Pb enters surface streams, water ways and soil.

Various regulatory measures have been adopted to limit Pb in the environment, but still it continues to be a problem of worldwide concern. Pb pollution in the soil is of priority concern to agriculturalists and environmentalists. It is highly detrimental to plant and animal life due to high persistence in the environment [2,4]. It is known to greatly increase the soil metal burden [6]. Pb has the potential ability to react with various functional groups such as amine, carboxyl and sulfhydryl, thereby, altering the activity of various enzymes that are important for cell functions. Pb toxicity is an important environmental issue, as it cannot be degraded or transformed into other non-harmful forms. Pb is a general protoplasmic poison, cumulative, slow acting and subtle in nature [4]. This review highlights Pb induced toxicity in plants in terms of their growth, development, and biochemical attributes.

PB AFFECTS GERMINATION OF SEEDS

Pb has been reported to inhibit / retard seed germination. Hussain *et al.* [7] reported a continuous decrease in germination (10–100 %) of *Zea mays* with the increasing Pb concentration at 1–500 mM Pb, compared to the control. Pb has been found to decrease the seed germination in *Arachis hypogeae* L. [8].

PB-INDUCED CHANGES IN GROWTH AND DEVELOPMENT OF ROOTS

Generally, Pb toxicity is associated largely with roots of the plant as compared to other aerial parts of the plant. This can be attributed to higher accumulation of Pb in the cell walls of the root in contrast to other parts of the plant [4,9,10]. Plant roots counter Pb toxicity in three different ways; First, it creates a barrier by the synthesis and deposition of callose that prevents the entry of Pb. Secondly, roots can also uptake large amounts of Pb and sequester it in the vacuole, thereby affecting root growth. In third case, roots of hyperaccumulator plants can also translocate the heavy metal to the above ground parts.

Pb toxicity results in inhibited root elongation, and extensive root-hair development. Reduced or damaged root system limits water, mineral and nutrient uptake. Pb has also been reported to reduce growth and dry mass accumulation and negatively affect physiological processes in plants [4]. Pb has been reported to inhibit root growth in garlic [11], pea [12], *Z. mays* [13], wheat [10] and tobacco [14]. Hasnian *et al*. [15] reported a significant inhibition of root length of crop seedlings (*T. aestivum*, rice and barley) in response to Pb exposure.

Hussain *et al.* [7] reported a notable decrease in germination, early seedling growth, root and shoot length of *Zea mays* under Pb stress in a concentration-dependent manner. Such growth retardation was due to disturbance in various physiological and biochemical processes. Malecka *et al.* [16] observed a significant change in the appearance of the roots of *Pisum sativum* and their shape when treated with Pb. The root growth inhibition by Pb has been suggested to be due to non-selective suppression of both cell division and cell elongation of the seedlings [17]. Similarly, Kaur *et al.* [18] demonstrated reduction in root growth and increase in the number of root hairs of *T. aestivum* upon Pb exposure. Further, Scanning Electron Micrographs of the root of *T. aestivum* depicted morphological alterations and surface ultrastructural changes. Irregular and desiccated surface cells were observed in Pb^{2+} treated roots [18].

PB INDUCED BIOCHEMICAL CHANGES DURING EARLY DEVELOPMENT

Pb affects the content of macromolecules

Pb alters the content of macromolecules (proteins and carbohydrates) and activities of related hydrolyzing enzymes enzymes. Singh *et al.* [19] reported Pb-induced increase in protein and carbohydrates content in *B. campestris* roots. Suzuki *et al*. [20] suggested that these metal-induced proteins play a significant role either in detoxification and/or in the maintenance of heavy metal homeostasis. Kratovalieva and Cvetanowska [21] reported an increase in total sugars in tomato under the influence of Pb. Increased carbohydrates content indicated either failure of the plant to hydrolyze carbohydrates or *de-novo* synthesis of enhanced carbohydrates under Pb-stress.

Pb alters activity of protein and carbohydrate hydrolyzing enzymes

Previously, increased protein content under stress conditions has been attributed to decreased proteases activity [19,20]. Likewise, a decline in protease activity has been recorded in roots and shoots of rice plants [22] and in *Lemna minor* [23] upon exposure to excess Pb.

Pb-exposure alters the activity of amylolytic enzymes (α- and β- amylases). The decreased activity of amylases suggests the inability of the plant to meet the increased energy demands of the tissue in response to Pb-induced stress. Such an observation is not surprising, as earlier heavy metals, including Pb, have been reported to impair carbohydrate metabolism by inactivating amylases participating in their synthesis or hydrolysis [24, 25]. The activity of α- and β-amylases has been found to decrease upon Pb exposure [19]. Kaur *et al.* [26] correlated the enhanced the contents of water-soluble proteins and carbohydrates with the reduced activities of proteases and amylases in wheat radicle in a dose-dependent manner after 24 h of Pb exposure.

PB DISRUPTS OXIDATIVE METABOLISM

Pb induces lipid peroxidation

Kaur *et al.* [10] observed an increase in malondialdehyde (MDA) content in wheat roots upon Pb exposure (40 mg l^{-1}). Enhanced MDA content in response to Pb-exposure indicates a ROS-mediated damage to biological membranes and induction of oxidative stress [27]. Pb has also been reported to cause lipid peroxidation in *Z. mays* (at 200 μM; [28]), garlic (at 1000 μM; [11]) and pea (at ≥500 μM; [12, 29]) and induces oxidative damage. Pb-induced lipid peroxidation has been confirmed by increased Relative Electrolyte leakage (REL) [10]. It further suggested disintegration of the biological membrane. REL is an indicator of membrane damage and occurs due to membrane peroxidation resulting from an oxidative burst (Bajji *et al*., 2002). Enhanced REL suggests Pb-induced membrane damage. Pb-induced lipid peroxidation has been confirmed by in situ histochemical studies using Schiff's reagent [31].

Pb-exposure causes greater accumulation of hydrogen peroxide (H_2O_2)

Pb-exposure significantly enhanced H_2O_2 accumulation in wheat roots thereby further suggesting the induction of oxidative stress [10]. H_2O_2, one of the ROS, upon reaction with lipids, proteins, pigments and nucleic acids results in lipid peroxidation, membrane damage and alterations in enzymatic machinery; thereby ultimately affecting cell viability [32] and causing cell death [33]. In fact, H_2O_2 acts as a signaling molecule and serves a dual role in plant defense mechanism. It helps in tolerance and stress-acclimation at lower concentration, whereas, it acts as a ROS at higher concentrations and induces cellular damage leading to cell death (Stone and Yang, 2006). Earlier, a parallel increase in H_2O_2 content in response to Pb-exposure was observed in pea (at 500 μM Pb; [29]) and moss *Taxithelium nepalensis* (at 1000 μM Pb; [34]).

Pb induces the generation of $O_2^{\bullet -}$

Kaur *et al*. [10] noticed an increase in the levels of superoxide ions ($O_2^{\bullet-}$) in wheat roots at early hours of Pb^{2+} treatment. $O_2^{\bullet-}$ production was not only dose-dependent but was also affected by the duration of treatment. These observations are parallel to earlier reports of Kopyra and Gwozdz [35] that $O_2^{\bullet-}$ level increased in lupin roots treated with Pb. A similar trend of $O_2^{\bullet-}$ production was observed in the chloroplast of spinach in response to Pb [36]. Earlier, Fridovich [37] documented that $O_2^{\bullet-}$ and H_2O_2 interact with each other and result in the formation of $^{\bullet}OH$ and $^1O_2$, which being more destructive end products, cause greater peroxidation of the unsaturated lipids of the cell membrane.

Pb alters ROS-scavenging enzymes

Under normal metabolic conditions, cells have a well-developed antioxidant machinery to quench the ROS generated during physiological process. It is well-established that antioxidant machinery plays an important role in regulating cellular metabolism in response to heavy-metal induced stress in plant tissue [38]. It has been demonstrated that genes encoding these antioxidant enzymes are activated by heavy metal stress [39, 40]. Any disturbance in this mechanism upsets the redox state resulting in cellular oxidative stress. The genotoxicity of metals has been directly correlated to ROS generation induced by the metals [41,42].

Studies have revealed that Pb-exposure caused a significant upregulation in the SOD activity in a time- and concentration-dependent manner in wheat and onion roots [10,31]. SOD is a key enzyme in protecting cells against oxidative stress and dismutases $O_2^{\bullet-}$ to H_2O_2 and O_2 [43]. Its activity correlates positively to protection against peroxidation [44]. It provides a first line of defense against oxidative damage [38]. The increase in SOD activity can be attributed to *de novo* synthesis of enzymatic protein [45]. Previously studies have reported increase in SOD activity under Pb stress in moss [34], in horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengal gram (*Cicer arietinum* L.) [46], *Cassia angustifolia* [47], and maize [28], wheat and onion [10,31].

CAT, a universal oxido-reductase, scavenges H_2O_2 and converts it into O_2 and H_2O in peroxisomes and mitochondria. It plays a pivotal role in the removal of toxic peroxides. However, a significant decrease in CAT activity in response to Pb was noticed in *A. cepa* root tips [31], whereas an increase in CAT activity was noticed in *T. aestivum* [10] grown hydroponically. A decline in CAT activity suggested that this enzyme is not involved in providing protection against Pb toxicity in *A. cepa* root tips. Earlier, a similar reduction in CAT activity in response to Pb was reported in rice [48], mung bean [49], *A. cepa* root cells [50], and *Taxithelium nepalensis* [34]. In contrast, Reddy *et al*. [46] reported an up-regulation of CAT, glutathione reductases and peroxidases in horse gram (*Macrotyloma uniflorum* [Lam.] Verdc.) and bengal gram (*Cicer arietinum* L.) seedlings after 7-day exposure to 800 ppm Pb. Liu *et al*. [11] reported an increase in CAT activity in garlic roots exposed to 1000 μM Pb. The differential response of antioxidant enzyme CAT under Pb-stress in different plant species could be ascribed to Pb-concentration used and the variability among plant species towards Pb-toxicity. In fact, the combined activity of SOD and CAT is crucial in mitigating oxidative stress. Nevertheless, the observed decrease in CAT activity can be attributed to the activation of enzyme protein [51] or decrease in enzyme synthesis [52] or its degradation by induced peroxisomal proteases [53].

Within a cell, H_2O_2 levels are controlled by the scavenging enzymes, particularly CAT and peroxidases (both ascorbate- and guaiacol-). However, unlike CAT, GPX and APX have a high affinity to detoxify even low concentrations of H_2O_2 [50]. APX activity decreased in hydroponically grown *T. aestivum* roots upon Pb-exposure suggesting little role in scavenging H_2O_2 and providing protection against Pb-induced ROS-mediated

injury [10]. The greater levels of decrease in APX activity in *B. campestris* roots was further confirmed by greater endogenous H_2O_2 content observed in the present study. In contrast, Kaur *et al*. [31] reported a significant increase in the activity of APX in *A. cepa* suggesting its involvement in scavenging H_2O_2. It was in agreement to earlier observations regarding increased APX activity in *Oryza sativa* [48] and *Cassia angustifolia* [47] in response to Pb.

Excess H_2O_2 not scavenged by CAT and /or APX are scavenged by GPX and GR activity. Pb exposure increased GPX activity in *A. cepa* root tips, thereby preventing plant damage from $O_2^{\bullet-}$ and H_2O_2 to a great extent [31]. Similarly, a parallel increase in GPX activity has been reported upon Pb exposure in *Sesbania* seedlings [54] and *Glycine max* [55]. In contrast, GPX and GR activity declined in hydroponically grown *T. aestivum* suggesting its non-involvement in H_2O_2 scavenging [10]. It paralleled the reported decrease in GPX activity in *Ceratophyllum demersum* treated with 10–100 μM Pb for 7-days [56]. Despite up-regulation of SOD and GPX activities, there was a significant decline in *A. cepa* root growth [31]. It indicates that GPX is not involved in providing protection against oxidative damage by H_2O_2. Furthermore, enhanced GPX activity can be correlated with higher lignifications and consequent stunted growth, as an acclimation to stress [57].

Under Pb-exposure GR activity has been found to increase in *A. cepa* roots [31], rice [48], horse and Bengal gram [46], whereas a decline was noticed in *T. aestivum* [10] and in moss *Taxithelium nepalensis* [34] in response to Pb-toxicity. A higher GR activity has been related to enhanced production of GSH and better protection against ROS [58]. Earlier, a reduced GR activity has been correlated with enhanced paraquat sensitivity in *Tobacco* plants [59].

Pb alters non-enzymatic antioxidants

Exogenous Pb causes non-enzymatic antioxidant response in plants. Pb exposure stimulates non-enzymatic antioxidant machineryfor the regulation of ROS homeostasis. Oxidation of ascorbates affects the redox balance of other metabolites such as glutathione (a precursor of phytochelatins, produced by plants to immobilize heavy metals). Jin *et al.* [60] correlated the elevated glutathione level with the plant adaptation to extreme heavy metal stress; whereas reduced glutathione pool indicated marked alteration in response to heavy metal stress.

CONCLUSIONS

Pb induced toxicity in plants in terms of their growth, development, and biochemical attributes. Primary effects of Pb toxicity in plants include stunted root growth, probably due to inhibition of cell division in root tips. Secondarily, it induces oxidative stress via reactive oxygen species generation and results in cellular damage. Pb induces lipid peroxidation and causes greater accumulation of H_2O_2 and $O_2^{\bullet-}$. Pb exposure not only alters ROS scavenging enzymes but also affects non-enzymatic antioxidants.

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