ORIGINAL RESEARCH

Research on Crop Ecophysiology
Vol.13/1, Issue 1 (2018), Pages: 49 - 52

The Effect of Lead and Zinc Chlorides on Total Protein Content of Soybean

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Received: 20 Sebtember 2017 Accepted: 18 JANUARY 2018

ABSTRACT
Among heavy metals, lead is a powerful pollutant that can be easily accumulated in the soil, partly because it has been prominent in the debate concerning the growing anthropogenic pressure on the environment. Zinc is also a heavy metal but minimum requirement for plant growth. However, excessive amounts of these elements can become harmful to plants. Industrialization, urbanization, mining, and many other anthropogenic activities have resulted in the redistribution of lead from the earth's crust to the soil and to the environment. Lead forms various complexes with soil components, and only a small fraction of the lead present as these complexes in the soil solution are phyto available. In order to study the physiological traits, several concentrations of Lead (II) chloride (0, 0.5, 2.5, 4.5 and 6.5 mM) with different concentrations of Zinc chloride (5, 10, 15 and 20 mM) was conducted on soybean plant (*Glycine max* L.) growth biochemical parameters. Results were obtained from a factorial experiment in a completely randomized design with four replications under in vitro conditions. The results showed that the total protein contents decreased with increasing zinc and lead concentrations. This research concluded lead has a negative effect on protein content of soybean.

Keywords: Lead, Zinc, Total protein, soybean plant (*Glycine max* L)

INTRODUCTION

The use of sewage sludge compost with large heavy metals concentration could result in an increase in the metals accumulation in the plants roots and leaves Heavy metal bioaccumulation in the soil, water and atmosphere may be seriously hazardous to both human and animals with the contamination of food supply chain. Indeed, lead has been used by people since the dawn of civilization (Dong *et al.*, 2014). Recent studies show that, lead (Pb) is a nonessential toxic heavy metal with large solubility in water that alters the morphology and physiology of plants (Kamran *et al.,* 2015), which quickly absorb and compound into plant tissues and transfer to aerial organs where it can cumulate to high levels in contrast with mercury (Zhang *et al.,* 2014). The most typical symptom of Pb toxicity in plants is stunting, chlorosis and necrosis in the leaves, Chlorosis occurrence by the excess level of Pb appears to be due to a direct or an indirect interaction of Pb with Fe content, and induced deficiency of CO2 resulting from stomatal closure. Under lead stress, plants possess several defense strategies to cope with lead toxicity. Such strategies include reduced uptake into the cell; sequestration of lead into vacuoles by the formation of complexes; binding of lead by phytochelatins,glutathione, and amino acids; and synthesis of osmolytes. In addition, activation of various antioxidants to combat increased production of lead-induced ROS constitutes a secondary defense system. (Elavarthi, and Martin, 2010). Lead stress can considerably cause growth reduction, inhibition of seed germination, and lead toxicity causes inhibition of ATP production, lipid peroxidation, and DNA damage by over production of ROS. Also prevents photosynthesis activity, respiration, cell proliferation, plant water relationship and mineral nutrition, leading to poor growth and low biomass (Kamran *et al.*, 2015).

There are different methods by which plants respond to the toxic impacts of Pb. There are different methods by which plants respond to the toxic impacts of Pb. These includes, selective uptake of the element, Pb binding to the root surface, and formation of antioxidants such as proline, glutathione, cysteine, ascorbic acid and antioxidant enzymes, such as guaiacol peroxidase, superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase (Hansch and Mendel, 2009). Pb interacts with the cellular components and increases the cell wall thickness. Usually, the plant cell wall contains pectin and Pb can form a complex with the carboxyl group of pectin and this process is regarded as the corner stone of the plant cells resistance to Pb toxicity (*et al.*, 2009) and it acts as a physical barrier and restricted the movement of Pb through the plasma membrane. As the uptake of Pb from the soil increases, it can damage the ultrastructures of the organs, tissues, chloroplast, mitochondria, nucleus, cell wall, and cell membrane in the plants. This damage can cause a loss of organelle function, and can eventually affect the normal physiological functions that include photosynthesis, respiration, protein synthesis, cell division within the plant species (Salazar and Pignata, 2014).

Although soybean is a staple food in diets of many parts of the world as a source of polyunsaturated and saturated fatty acids, It has a complex mixture of triglycerides with vegetable proteins and minerals such as calcium, so the cultivation of this plant has been more widely considered among other legumes. Bojinova *et al.* (1994) reported that soybean and other beans belong to a group of crops that strongly accumulate heavy metals. Therefore, it is more important to recognize the elements that are involved in soil contamination of in order to reduce their absorption, as well as plant toxicity and growth (Arif *et al*., 2012). Zinc is an essential element for plants, but its excess can significantly damage plants. Zinc (Zn) is the second most common metallic metal in organisms and is the only metal found in all six enzymes (oxidoreductases, transferases, hydrolases, lysates, isomers, lignases) (Arif *et al.*, 2012). Dang *et al*. (2014), showed that although zinc at low concentrations can show positive effects on plant growth, but toxicities will occur when the Zn concentration exceeds (Arif *et al.,* 2012). This study indicated that increasing concentrations of Zn are responsible for increased toxicity in soybean plant. Therefore, the objective of this study was to investigate the effect of lead its application on soybean growth and yield and to evaluate the morphological characteristics of this plant.

MATERIALS AND METHODS

The seeds of soybean plants used in the present investigation were collected from the Agricultural Jahad Research Institute. Seeds were selected uniformly and disinfected with 5% hypochlorite for 1 min. After germination, uniform seedlings were transferred into plastic pots containing sand. After seven days, they were irrigated with half Hoagland solution and treatments were applied, 20 days after planting. Plants were treated with 5 concentrations of $PbCl_2$ (0, 0.5, 2.5, 4.5 and 6.5 mM) and $ZnCl_2$ (0, 5, 10, 15 and 20 mM) under controlled environment. In a completely randomized design with four replications. Plants were irrigated alternately with a nutrient. After 45 days, the plants were harvested for biochemical and physiological measurements. Analysis of variance and mean comparison were performed using SPSS software and Excel.

Total protein measurements

The aerial parts and fresh roots of the plants weighed and mixed with 2 ml phosphate buffer 0.1 molar (pH 6.8) homogeneously. After homogenization, each sample was transferred to 2 ml vials. Samples were centrifuged for 12 minutes at 4 ° C. The tap part of the extract was used to measure the total protein concentration. Protein concentrations were determined by a dye binding method (Bradford, 1976).

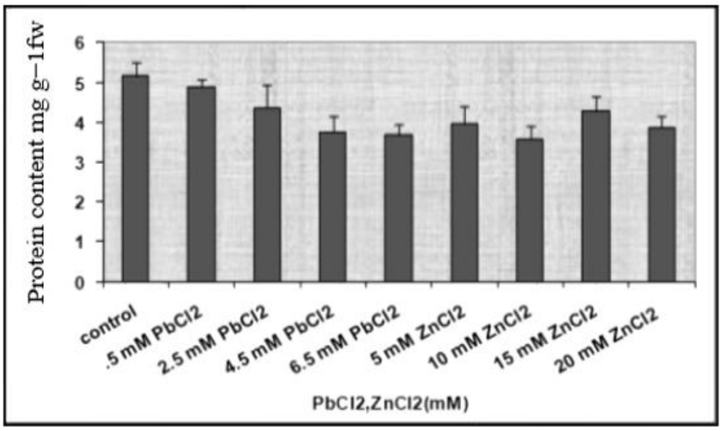

Figure 1. Changes in total protein content (mg g−1fresh weight) in different concentrations of $PbCl_2$ and $ZnCl_2$ in soybean seedlings

RESULTS AND DISCUSSIONS

Total protein content

Visual, nonspecific symptoms of lead toxicity are stunted growth, chlorosis, and blackening of the root system. Results revealed that, in the treatment of $PbCl_2$ with increasing concentrations, the total protein content of the leaf was 12.34%, 25.67%. 43.27% decreased compared to control treatment. Increasing zinc concentration also resulted in a significant decrease in protein content compared to control (Figure 1). The minimum amount of protein was observed in the treatment of 10 mM zinc, that decreased in 44.22% compared to the control. Reduced total protein at higher concentration of lead might be attributed to inactivation of enzyme by ROS, decrease in synthesis of enzyme, or change in assembly of its subunits. In most the inhibition exerted by zinc on enzyme activity results from the interaction of zinc with enzyme –SH groups (Auda, 2010). The results are obtained in the same effects of heavy metals such as cadmium and lead on the amount of soybean protein. The same results were shown in the Seppanen *et al*., 2003, Hussain *et al*., 2013 and Hansch and Mendel, 2009 research On the rice, maize and barely plants respectively. Also the effect of lead and zinc on the sunflower plant and the ability of absorb soil lead was considered (Agilera *et al*., 2013). Lead also inhibits the activities of enzymes of the reductive pentose phosphate pathway. In leaf homogenates of spinach, the activity of ribulose-bis-phosphate carboxylase/ oxygenase was inhibited even at a lead nitrate concentration of 5μM. Lead was found to be highly definite in inhibiting ATP syntheses ATPase (John *et al*., 2009). In vitro application of lead to mitochondrial preparations from plant cells exposed a decrease in respiration rate with increasing lead concentrations (Hansch and Mendel, 2009). Overall, the increase of zinc affected the protein amount in various organs of some plants, especially in their roots (Elavarthi and Martin, 2010). Previous studies reported a reduction in protein content in algae and *Brassica napus* (rapeseed) as Zn increased (Aldwairji *et al*., 2014). Zinc reduced plant biomass, because it led to a deficiency of macro-nutrients such as phosphorus. There are many reasons for the drop in protein content with heavy metals. A drop may be due to the accelerating degradation of protein with increasing protease activity or disturbance of nitrogen metabolism in the presence of heavy metals such as Cd and Pb. The protease activity increases in stress conditions like the presence of heavy metals in the growth medium. According to previous work heavy metals such as Cd and Pb disturb nitrogen metabolism, which further decreases the synthesis of protein. Heavy metals including Cd are responsible for the reduction in photosynthesis, which reduces the synthesis of protein (Aldwairji *et al*., 2014) but, Gamalero *et al* (2015) have stated that the process of changes in protein content is not always the same and depends on the species and environmental conditions. Increasing the amount of protein in high concentrations can be attributed to increased levels of antioxidant enzymes and other anti-stress proteins (Hansch and Mendel, 2009). Dang *et al*., (2014) showed that zinc at low concentrations can show its useful effects, but in high concentrations it has toxic impacts. The beneficial effects of this element have been reported on increasing resistance to non-biological stresses (Arif *et al*., 2012).

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