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Effects of Heavy Metals on Seed Germination of Adzuki Bean Seed

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Abstract: The present study was conducted to evaluate effect of nickel chloride, zinc chloride and lead acetate on the germination response of Adzuki beans using pot experiments. Seeds were subjected separately to five levels (0, 100, 200, 500 and 1000 μM) of the metal salts. The sprout length decreased concomitantly with increasing lead, nickel and zinc up to 1000 μM compared to the control but it showed a slight increase at 100 μM of zinc concentrations. The sprout fresh weight decreased significantly with increasing lead, nickel and zinc concentration and the order of decreased values are lead, nickel and zinc. The germination index produced significant decreases by different lead, nickel and zinc concentrations compared to control except for 100 μM of zinc and nickel concentrations. Lead, nickel and zinc treatments at 1000 μM exhibited lowest value of vigor index but vigor index showed a slight increase by 6% compared to the control. The results of germination vigor showed significantly inhibition by tested metal concentrations except for 100 μM of zinc and nickel concentrations. The present findings suggested that the germination responses of Adzuki beans are relative to the changes of metal concentrations and types.

Key words: Heavy metal, seed germination, Adzuki beans, treatments, China

INTRODUCTION

Now-a-days, the heavy-metal pollution is one of the most important ecological problems on the world scale. Excessive heavy metals in contaminated soil can result in decreased soil microbial activity and soil fertility and losses in agricultural yield. Moreover, heavy metals accumulated in plants may either directly or indirectly, find their way into animals and human beings (Nagajyoti *et al*., 2010). Heavy metals, such as lead, nick and zinc are important environmental pollutants, particularly in areas where there is high anthropogenic pressure (Broadley *et al*., 2007; Pourrut *et al*., 2011; Yusuf *et al*., 2011). Among heavy metals, Lead (Pb) is a non-essential nutrients element in plant growth and development and the exposure to relative low concentrations results in serious toxicity. Lead can cause growth inhibition and even plant death due to its detrimental effect on many physiological processes (Pourrut *et al*., 2011). Nickel (Ni) and Zinc (Zn) as the essential micronutrients in plants are necessary for plant growth and development, since it is the active center of the enzyme urease required for nitrogen metabolism in higher plants. However, excessive nickel and zinc in plants can profoundly affect normal ionic homeostatic systems by interfering with the uptake, transport, osmotic and regulation of essential ions and results in inhibition of seed germination and seedling growth, photosynthesis, mineral nutrition, sugar transport and water relations, etc. (Broadley *et al*., 2007; Yusuf *et al*., 2011).

Earlier reports have observed that some plants species are endemic to metalliferous soils and can tolerate higher amounts of heavy metals. Several studies have been conducted in order to evaluate the effects of different heavy metal concentrations on live plants. Most of these studies have been conducted using seedlings or adult plants (Sengar *et al*., 2008; Solanki *et al*., 2011). In a few studies, the seeds have been exposed to the contaminants. The seed germination and early-seedling growth are important stages in the whole process of plant-growth and due to being the most sensitive stage in the plants changing of their environment have been widely used in environmental bio-monitoring (Di Salvatore *et al*., 2008; Kranner and Colville, 2011). Earlier reports suggested that many plants at seed germination stages are sensitive to environmental factors (Wang *et al*., 2001; Munzuroglu and Geckil, 2002; Ozdener and Kutbay, 2009). Therefore, the change of plant growth at the germination under heavy metal stress is often regarded as an important index to evaluate plant tolerance to heavy metals.

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Adzuki bean (*Vigna angularis*) has been grown and used for many centuries in the Orient. Adzuki bean are cooked and consumed as a dessert or snack food, especially during celebrations and traditional festivals, such as the Chinese New Year. The primary use of Adzuki bean also includes the product of bean sprouts (Redden *et al*., 2009). Thus, the present studies were therefore aimed to investigate the germinate responses of Adzuki bean to short-term exposure of different concentrations of lead, nickel and zinc. The biomarkers of germination responses, like sprout length, sprout fresh weight, germination index, vigor index and germination vigor are measured and analyzed to provide a basis for developing strategies for reducing the risks associated with heavy metals.

MATERIALS AND METHODS

Adzuki bean was purchased from Traditional Chinese Medicine Market, Chengdu, China and was used as plant material. Seeds were selected and stored at 4°C in a plastic box with labeled until using. Nickel chloride, zinc chloride and lead acetate were used heavy metals. Seeds were surface sterilized with 0.5% potassium permanganate solution for 10 min to avoid fungal contamination and then were rinsed several time with distilled sterile water. Seed germination and root elongation test on filter paper was carried out in glass Petri dishes (150 mm) with three layers of filter paper (150 mm Watman filter) on the bottom. Each dish contained 50 seeds and 10 mL of 1/10 strength Murashige and Skoog (MS) medium contained five different concentrations of Pb (0, 100, 200, 500 and 1000 μM), Ni (0, 100, 200, 500 and 1000 μM) or Zn (0, 100, 200, 500 and 1000 μM). Seeds were arranged in such a way that each seed did not touch each other nor touch the side of the dish. Three replicates of each test solution were prepared. Solutions were also changed every 24 h. These dishes were placed in growth chambers and maintained at 25/20°C with a 12 h photoperiod for 30 days. This temperature/light regime has been tested as optimal for germination in this species. Germinated seeds were counted every other day until the time in which no germination was observed over 3 days ever after. Seeds were considered germinated when the radical were extended to approximately >2 mm from their junction.

Germination percentage was recorded every 24 h for 7 days. Day 7th of experiment was considered as the final day because no more germination was observed and shoot length was measurable for all Petri dishes. At the end of the germination period, the parameters of germination under different metal concentrations were calculated. Five germination parameters were determined, namely sprout length, sprout fresh weight, germination index, vigor index and germination vigor. The germination percentage is the proportion, expressed as percentage of germinated seeds to the total number of viable seeds that were tested. Germination index was calculated using this equation: Germination Index (GI) = $\Sigma G_t/D_t$, where G_t is number of the germinated seeds in the t day, D_t is time corresponding to G_t in days. Vigor index was calculated using this equation: Vigor Index (VI) = GI × S, where GI is Germination Index and S is the length of sprout (cm). Germination Vigor (GV, 100%) was calculated using this equation: Germination Vigor (GV, 100%) = (ΣG_t/No. of seeds initiated)*100, where G_t is number of the germinated seeds in the 3 days.

All treatments were arranged in a completely randomized design with three replicates. Data were expressed as Mean±SD.

RESULTS

Effects of heavy metals on sprout length of Adzuki bean: The effects of different concentrations of Zn^{2+}, Ni^{2+} and Pb^{2+} on sprout length of Adzuki bean were presented in Fig. 1. The sprout length decreased by about 36.8, 45.8 and 60.9% at zinc concentrations of 200, 500 and 1000 μM compared to the control, respectively. However, it increased by about 8.29% at zinc concentrations of 100 μM. There were reductions in the sprout length as Pb^{2+} and Ni^{2+} concentrations in the solution increased in general. The 100, 200, 500 and 1000 μM of Pb^{2+} significantly reduced the sprout length by 44.2, 46.1, 73.9 and 82% compared to the control, respectively. Similarly, the sprout lengths were gradually inhibited with increasing Ni^{2+} concentrations up to 1000 μM compared to the control.

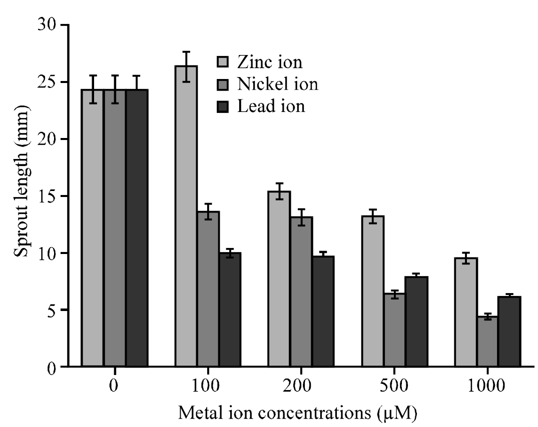

Fig. 1: Effects of different concentrations of Zn^{2+}, Ni^{2+} and Pb^{2+} on sprout length of Adzuki bean; bar represents Mean±SD

Effects of heavy metals on sprout fresh weight of Adzuki bean: The effects of different concentrations of Zn^{2+}, Ni^{2+} and Pb^{2+} on sprout fresh weight of Adzuki bean were presented in Fig. 2. Under zinc stress, the sprout fresh weight decreased significantly compared to the control and the highest decrements (about 44.9%) were observed at a zinc concentration of 1000 µM. The 100, 200, 500 and 1000 µM of Ni^{2+} may inhibited the fresh weight of sprout by 22.8, 27.9, 52.9 and 63.2%, respectively as compared to the control. Pb^{2+} at 100, 200, 500 and 1000 µM concentration also decreased the fresh weight over the the control by approximately 23.4, 36.8, 50.0 and 61.0% in this study.

Effects of heavy metals on germination index of Adzuki bean: The effects of different concentrations of Zn^{2+}, Ni^{2+} and Pb^{2+} on germination index of Adzuki bean were presented in Fig. 3. The Zn^{2+} concentrations of 200, 500 and 1000 µM may decrease the germination index by 6.25, 10.4 and 29.2, respectively related to the control. Similarly, 200, 500 and 1000 µM of Ni^{2+}, also reduced significantly the germination index as shown in the control plants. However, 100 µM of Zn^{2+} and Ni^{2+} showed no change compared to the control. Under Pb^{2+} stress conditions, the germination index were gradually inhibited up to 1000 µM and the minimum level decreased 47.1% compared to the control.

Effects of heavy metals on vigor index of Adzuki bean: The effects of different concentrations of Zn^{2+}, Ni^{2+} and Pb^{2+} on vigor index of Adzuki bean were presented in Fig. 4. The vigor index showed significant decreases at 200, 500 and 1000 µM of Zn^{2+} and the value decreased by 40.7, 51.4 and 72.3% compared to the control, respectively. Under different concentrations of Ni^{2+}, the value decreased gradually up to with increasing Ni^{2+} levels up to 1000 µM and the lowest value only represented 12.4% that of the control. With increasing in the concentration of Pb^{2+}, the value of vigor index decreased progressively up to 1000 µM and it decreased by 87% compared to the control.

Effects of heavy metals on germination vigor of Adzuki bean: The effects of different concentrations of Zn^{2+}, Ni^{2+} and Pb^{2+} on germination vigor of Adzuki bean were presented in Fig. 5. The values of germination vigor decreased about 2.08, 6.25, 10.4 and 29.2% at Zn^{2+} concentrations of 100, 200, 500 and 1000 µM, respectively. The values reduced by 20.8, 27.1 and 31.2% at nickel concentrations of 200, 500 and 1000 µM, respectively. Similarly, the values were gradually decreased with the rising concentrations of lead and the minimum value decreased by 47.9% compared to the control.

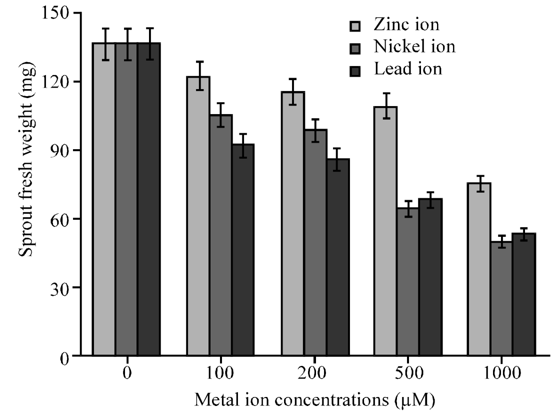

Fig. 2: Effects of different concentrations of Zn^{2+}, Ni^{2+} and Pb^{2+} on sprout fresh weight of Adzuki bean; bar represents Mean±SD

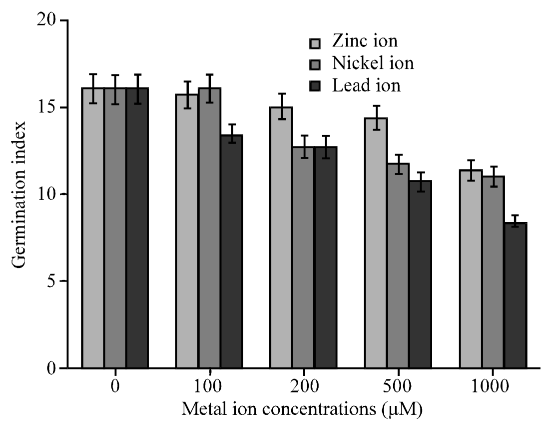

Fig. 3: Effects of different concentrations of Zn^{2+}, Ni^{2+} and Pb^{2+} on germination index of Adzuki bean; bar represents Mean±SD

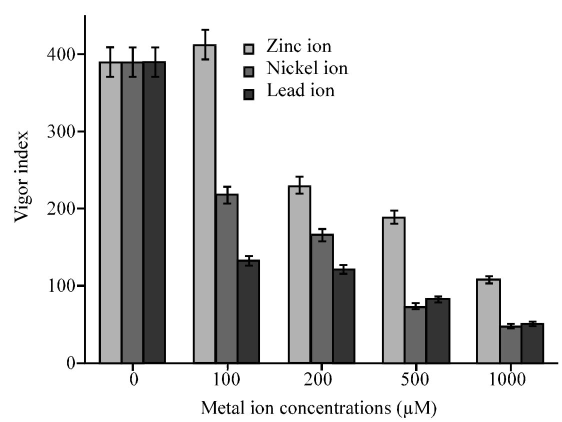

Fig. 4: Effects of different concentrations of Zn^{2+}, Ni^{2+} and Pb^{2+} on vigor index of Adzuki bean; bar represents Mean±SD

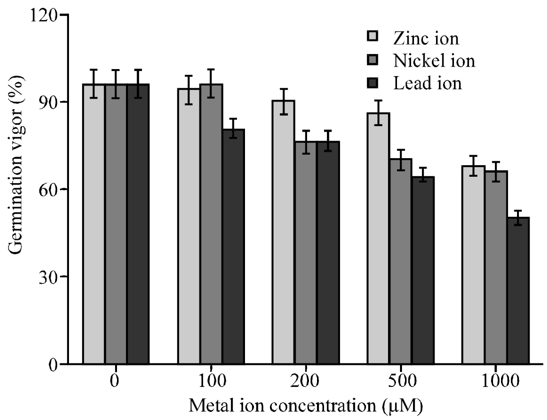

Fig. 5: Effects of different concentrations of Zn^{2+}, Ni^{2+} and Pb^{2+} on germination vigor of Adzuki bean; bar represents Mean±SD

DISCUSSION

Germination and early seedling development assay has been regarded as a basic experiment for evaluating the toxicity effect of any kind of metals or chemicals on plants (Di Salvatore *et al*., 2008; Kranner and Colville, 2011). Under heavy metal stress, the processes of germination, like the reduction of germination rate and seedling growth of different crops will be depressed (Ahmad *et al*., 2009; Farooqi *et al*., 2009; Curguz *et al*., 2012). In the present study, researchers choose Adzuki bean as tested seed for the germinate responses of it on heavy metals. Firstly, it is one of the most important food crops and also easier to culture in the laboratory conditions. Second, the germination percentage of Adzuki bean is very higher than other crops and the germination responses are more sensitive to heavy metals. The findings showed that the germination responses of Adzuki bean are different to different concentrations of zinc, nickel and lead.

Zinc (Zn), as one of the essential micronutrients in plants is necessary for plant growth. However, excessive Zn in plants can profoundly affect normal ionic homeostatic systems by interfering with the uptake, transport, osmotic and regulation of essential ions and results in the disruption of metabolic processes (Broadley *et al*., 2007). Seed priming with Zn can improve germination and seedling development, stand establishment and subsequent growth and yield (Ajouri *et al*., 2004; Farooq *et al*., 2012). Moreover, earlier reports showed that the newly-developed radicles and coleoptiles are much higher up to 200 mg kg^{-1} of zinc compared to no addition of zinc during seed germination. This is the reason that Zn is involved in physiological processes during early seedling development, such as protein synthesis, cell elongation membrane function and resistance to abiotic stresses (Cakmak, 2000; Ozturk *et al*., 2006; Broadley *et al*., 2007). However, the higher zinc level showed negative effect and led to a marked decrease in seed germination, seedling growth with the increase in metal concentrations (Ozdener and Kutbay, 2009; Solanki *et al*., 2011; Wei *et al*., 2012). In the present study when the zinc concentrations are >200 μM, the germination parameters of Adzuki bean were significantly affected with the rising zinc concentrations. Therefore, the present findings suggested that the germination of Adzuki bean showed a negative response to higher zinc toxicity, possibly through the enhancement of ROS production which in turn led to the oxidative damage to sprout and inhibited the germination. Further studies will need to clarify the overall importance of oxidative stress phenomena during the germination.

Nickel is an essential micronutrient for normal plant growth and metabolism and it has been demonstrated that excess nickel can inhibit of seed germination, young seedling growth, root elongation and cause damage to root epidermal cells and root cell membranes (Cempel and Nikel, 2006; Yusuf *et al*., 2011). Although, the toxic effects of excess nickel are evident through crop development, the germination stage is regarded as the most sensitive particularly to nickel toxicity. For example, the increasing concentration of Ni^{2+} has been shown to inhibit seed germination of different plant species (Leon *et al*., 2005, Farooqi *et al*., 2009). Such inhibitory effects of higher concentrations of Ni have been reported to be due to the inhibition of protein synthesis and activities of some key enzymes (Protease and α-amylase), carbohydrate metabolism and mobilization of food reserves (Lin and Kao, 2006; Maheshwari and Dubey, 2007). Moreover, higher concentrations of Ni might have strong interferences with uptake of mineral nutrients, thereby leading to their deficiency in germinating seed and further resulting in poor germination and seedling establishment (Cempel and Nikel, 2006; Yusuf *et al*., 2011). On the basis of these results, the finding suggested that the elevated nickel concentrations can inhibit the length and fresh weight of sprout as well as other germination parameters during the germination. From these results it is concluded that nickel has an adverse effect on germination of Adzuki bean and changes in germination parameters would occur after any visible symptom of toxicity appears. Thus, the endpoint based on biochemical parameters might be more sensitive or indicative than morphological observations in revealing eco-toxicity of nickel.

Pb is generally considered to be a highly toxic element and Pb toxicity has become an important issue due to their constant increase in the environment.

Inhibition of germination and retardation of plant growth are commonly observed due to lead toxicity in previous studies (Iqbal and Shazia, 2004; Pourrut *et al*., 2011). Negative effects of lead toxicity on seed germination has also been reported by many reports in many plant species, such as *Lythrum salicaria*, *Cassia siamea* and *Albizia lebbeck* (Uveges *et al*., 2002; Shafiq and Iqbal, 2005; Farooqi *et al*., 2009). These negative effects might be attributed to the accelerated breakdown of stored food material in seed by the application of lead during the seed germination (Pourrut *et al*., 2011). Radicle/root growth is known to be more sensitive than other parts growth to metal toxicity. More sensitivity of radicle/root length to Pb could be described by the fact that a plant root is the first point to contact with the toxicants in the growth media. Pb can easily penetrate the radicle/root cortex, consequently the radicle/roots are more likely to experience the Pb damage before any other part (Munzuroglu and Geckil, 2002; Araujo and Monteiro, 2005; Di Salvatore *et al*., 2008). In the present investigation, seed germination gradually decreased with the increase in concentration of lead. Lead treatments significantly decreased seed germination as compared to control. Although, results obtained with seed germination in the greenhouse condition can not be directly compared to those in field conditions, they are important in detailing germination response under controlled conditions.

CONCLUSION

In summary, it was revealed from the results that lead had more inhibitory effects on germination parameters of Adzuki bean than zinc and nickel salts. The germination index and germination vigor showed no significant changes at 100 μM of zinc and nickel salts. This was probably because of essential roles of zinc and nickel as micronutrient in plants. Thus, the results are hoped that these findings may contribute to a better understanding of the germination response of Adzuki bean to metal stress. In fact, the effects of heavy metals on seed germination are multiple heavy metals in natural environments and further in-depth studies will involve the effects of combination metals on the responses of germination and antioxidant defense mechanisms.

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