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Research Article

SEED EMERGENCE IS MORE APPROPRIATE CRITERION FOR TESTING CADMIUM TOXICITY IN SOYBEAN

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ABSTRACT
Humans are continuously adding new contaminants in the environment, which are adversely affecting human, animal and plant life. Seed germination and root elongation tests are widely used as the simplest bio-monitoring methods for testing the toxicity of these pollutants. The present study investigated the impact of heavy metal cadmium (Cd) on seed germination, seed emergence and seedling growth in soybean. Seed germination in soybeans declined by 60% of control plants with Cd (40µM). Germination index also got affected severely (80%). The plumule/radicle ratio, an essential parameter of normal growth and development, increased nearly four times under Cd induced toxicity. The decline in vigour index of seedlings was up to 91%. The phytotoxicity index, an indicator of damaged seedlings, exhibited an increase of 94%. In all the cases, the emergence of seedlings reduced significantly (93% of control). The damage to the root system with higher concentrations of Cd was more as compared to shoots. The shoot/root ratio increased 435% in comparison to control seedlings. Although, the seeds were able to germinate in the present study but seedlings were unable to come out of the substratum due to loss of vigour index. So, if seed germination is the only criteria to test toxicity, the results can be misleading due to a larg gap in percentage of seed germination and their emergence rate. Hence, consideration of seedling emergence shall be more appropriate criterion for toxicity studies than percentage germination of seeds.

Keywords: *Soybean, Cadmium, Seed Germination, Seed Emergence, Vigour Index, Phytotoxicity*

INTRODUCTION
The growing consumerism, greed and urge for continuous development are adding new unwanted materials into the environment. These unwanted materials, known as pollutants, have become a major global problem these days and are adversely affecting the human beings, animals and plant life. Amongst the various contaminants, heavy metals are of more importance as they cause widespread contamination of soils and groundwater and are most hazardous to human and animal life (Lombi *et al.,* 2001). They have evoked a major concern these days because of their flow into the terrestrial food chain through crops grown in metal polluted agroecosystems (Krishnamurti *et al.*, 2006; Aulakh *et al.*, 2009) and subsequent biomagnifications (Nawrot *et al.*, 2008; Martin and Griswold, 2009). Cadmium (Cd) is one of the most toxic trace heavy metals (Günther and Kastenholz, 2005; Schulze *et al.*, 2005), primarily released into the environment through human activities (Wagner, 1993). Soybeans accumulate more concentrations of heavy metals than the recommended limit of 0.2 mg/kg by FAO and WHO (Shute and Macfie, 2006).
The heavy metals damage the root system of plants, which become gravi-irresponsive (Singh and Thakur, 2014). They interfere with uptake of water and mineral nutrients (Davies, 1991; Vernay *et al.,* 2007), disrupt the membrane permeability and impair enzyme functions (van-Assche and Clijsters, 1990; Ernst, 1998). The water relations of the plants get lopsided, affecting all major physiological processes like transpiration, stomatal conductance and CO_2 exchange (Poschenrieder and Barceló, 2004; Thakur and Singh, 2012).
Seed germination, in general, is affected adversely by heavy metals. Hence seed germination tests along with root elongation are conducted to assess toxicity of organic and inorganic compounds including heavy metals (Di Salvatore *et al.,* 2008). The present study was aimed at assessing the germination and emergence of soybean seedlings and their subsequent growth behavior under the influence of heavy metal Cd.

MATERIALS AND METHODS

Soybean (*Glycine max* (L.) Merr. cultivar. PK-416) seeds were procured from Punjab Agricultural University, Ludhiana, Punjab (India). They were surface sterilized with 0.1% mercuric chloride followed by thorough washing under running tap before various trials. The germination trials were conducted in petri plates in BOD incubator at 25±2ºC. The seedling emergence trials were conducted by sowing soybean seeds in small plastic pots containing 500g washed sand and kept in the growth chamber with controlled environmental conditions (photoperiod- 14h day^{-1}; photon flux density- 150 µmol m^{-2} s^{-1}; day/ night temperature- 25/20º±2ºC; relative humidity- 80%). They were exposed to different doses of cadmium ($CdSO_4.4H_2O$; MW- 208.47) as irrigation (50 ml) along with N-containing Minchin and Pate (1975) nutrient medium. Speed of emergence and germination index were measured according to Hall and Wiesner (1990). Vigour index (VI) of seedlings was calculated according to Abdul-Baki and Anderson (1973). Phytotoxicity Index was calculated according to Chou and Muller (1972). One way analysis of variance (ANOVA) was applied to all the results using the software BioStat Professional Package Release 5.2.5.0 (AnalystSoft, Robust Business Solutions, Vancouver, Canada) to statistically analyze the significance of treatments. The results of differences among treatments were considered as significant at $P<0.05$ according to widely followed Tukey's HSD multiple range test.

RESULTS AND DISCUSSION

Observations & Results

The germination percentage of seeds (Figure 1) in soybean was reduced to as low as 60% of the control plants with 40µM Cd treatment. There was a gradual decline in germination percentage with 4, 8, 12, 16, 20, 24, 28, 32, 36 and 40 µM of Cd. Speed of germination index was also affected severely (Figure 2). Even the lowest level of treatment (4 µM) reduced its value by 50%. The decline was more with higher concentrations.

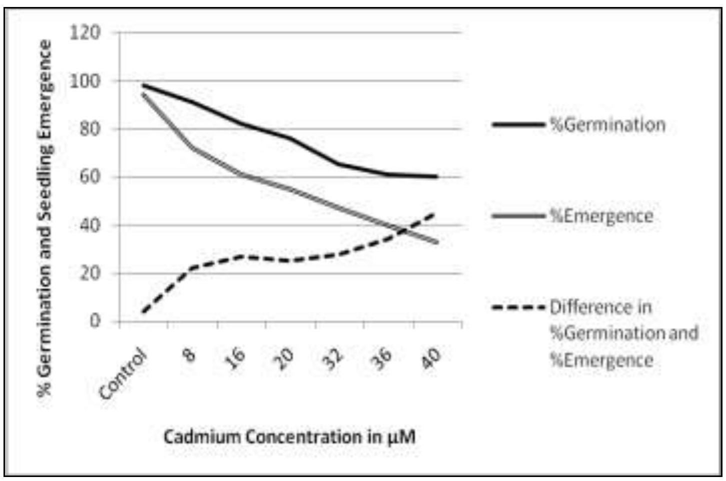

Figure 1: Seed germination, seed emergence and the difference in germination and emergence percentage of soybean in the presence of different concentrations of Cd

Plumule/radicle ratio (Figure 3), an essential parameter for the normal growth and development of plants, increased nearly four times with Cd induced toxicity (40 µM). Vigour index of the seedlings (Figure 4) got diminished to 136 (40 µM Cd) in comparison to starting at 1440 under control conditions, a decline of nearly 91%. Phytotoxicity index (Figure 5), an indicator of damage caused by environmental factors, also exhibited a gradual increase from 14 (4 µM Cd) to 94 (40 µM Cd). Many of the seedlings were unable to emerge out of the substratum even after their germination (Figure 1). The reduction in emergence was 18 and 57% with 1 and 40 µM Cd in comparison to control. Speed of emergence (Figure 2) was also reduced to 29, 52, 72 and 84% of control seedlings with 1, 10, 20, and 40µM Cd treatment, respectively. The length of shoot declined by 2, 14, 28 and 55% and that of root by 6, 27, 60 and 91% with cadmium concentrations of 1-40 µM. Shoot/root ratio was the worst affected parameter showing a phenomenal increase of 81 and 435% than control seedlings in 20 and 40 µM of Cd (Figure 3). Vigour index of the soybean seedlings (Figure 4) was reduced to 20, 39, 67 and 84% of the control. Phytotoxicity index increased to 6, 25, 60 and 91% in 1, 10, 20 and 40µM of Cd, respectively (Figure 5).

A comparison of the seedling emergence out of the substratum with the germination percentage showed contrasting results indicating that severity of stress had affected the behavior of germinating seeds and their seedlings. The difference in the germination and emergence (Figure 1) was only 4% with 4 µM Cd, and increased to 45% with 40 µM Cd. Emergence of the seedlings got appreciably affected and was very low in the presence of heavy metal cadmium.

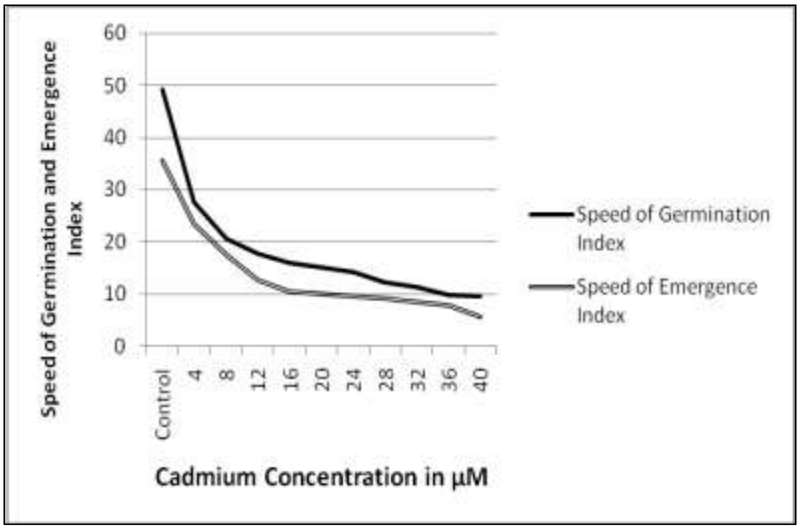

Figure 2: Speed of germination and emergence index of soybean in the presence of different concentrations of Cd

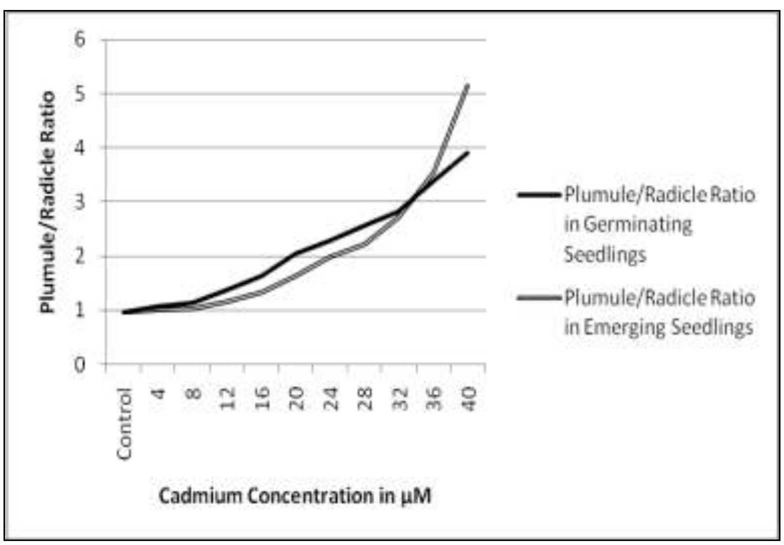

Figure 3: Plumule/Radicle ratio of germinating and emerging seedlings of soybean in the presence of different concentrations of Cd

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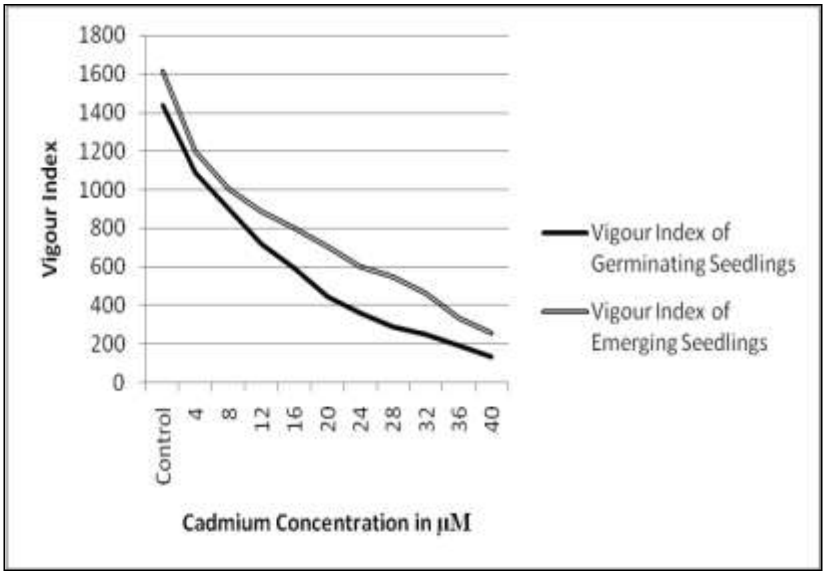

Figure 4: Vigour index of germinating and emerging seedlings of soybean in the presence of different concentrations of Cd

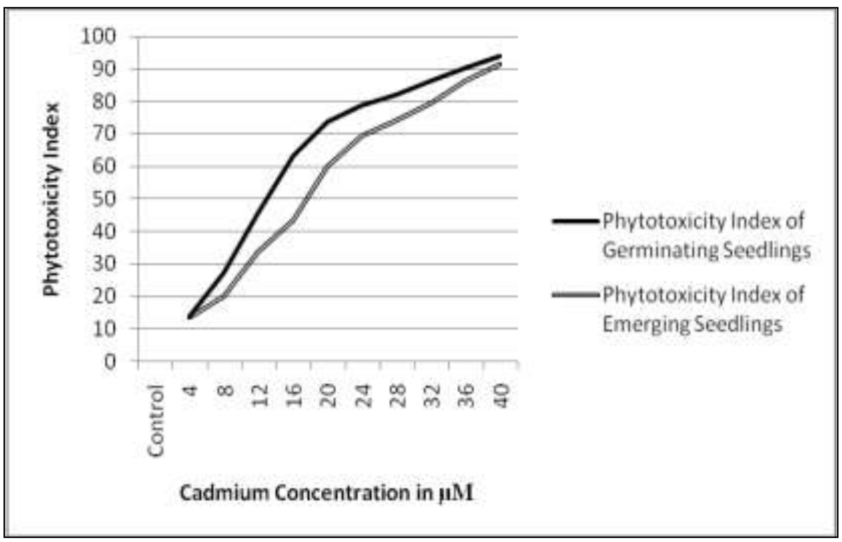

Figure 5: Phytotoxicity index of germinating and emerging seedlings of soybean in the presence of different concentrations of Cd

Discussion

Cd induced stress decreased the germination and emergence percentage, speed of germination and emergence index, shoot and root length and vigour index. The reduced rate of seed germination can be attributed to a number of metabolic processes. The hydration of seeds activates the existing proteins and enzymes and also promotes *de novo* synthesis of specific hydrolytic enzymes which function as trigger for germination and other metabolic processes. The toxicity of heavy metals expresses through binding of metals to sulphydryl groups in proteins leading to inhibition of enzyme activities and disruption of its structure (Hall, 2002). The binding of Cd with RNA in germinating seeds may render it non-functional (Satakopan and Rajendran, 1989). The abscisic acid titer of plants have also been reported to increase with heavy metal toxicity (Sharma and Kumar, 2002; Atici *et al.,* 2005). The higher shoot/root ratio indicates a reduced radicle length in Cd toxicity. It may be due to damage to the root tip (Singh and Thakur, 2014) and disrupted membrane functions (Cooke and Burden, 1990), which can be responsible for the non establishment of seedlings on the substratum and their inability to absorb water (Singh and Thakur, 2014).

All these factors directly or indirectly reduce the seed germination, seedling growth, vigour index and increase the phytotoxicity index. Vigour index, an indicator of the seedling emergence, decreased many folds with higher concentrations of Cd. The difference was significant in terms of seed germination and seedling emergence percentage. The seeds are normally considered germinated when their radicles are approximately 1 to 2 mm long (Deines *et al.*, 2007). The present study revealed that although the seeds were able to germinate but the seedlings were unable to come out of the substratum due to reduced vigour index caused by Cd induced toxicity.

So, if the germination of seeds is the only criteria for toxicity testing in plants, it can be misleading as there is a considerable gap in seed germination and seedling emergence. Hence, consideration of seedling emergence shall be more appropriate for toxicity studies than percentage germination of seeds.

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