J. Phytol. Res. **26**(1&2) : 77-81, 2013

EFFECT OF TOXIC HEAVY METALS ON THE GERMINATION AND SEEDLING PERFORMANCE OF *AMARANTHUS SPINOSUS* L.

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The present study focussed on the effect of different concentrations of toxic heavy metals such as Lead, Cadmium and Chromium on the seed germination and seedling growth of *Amaranthus spinosus* L. under laboratory conditions. The growth parameters under investigation include germination percentage, germination speed, germination index, seedling length, vigour index, mean germination time, coefficient of germination velocity and tolerence indices. All the heavy metals showed toxic effects on all growth indices as compared to control. Inhibition in the seed germination and seedling performance was noticed under higher concentrations of heavy metals. The level of phytotoxicity recorded a trend of $Cd^{2+}>Cr^{6+}\geq Pb^{2+}$.

Keywords : *Amaranthus spinosus;* Germination; Heavy metal; Seedling performance.

Introduction

Heavy metal contamination is a serious environmental problem that limits plant productivity and threatens human health. In plant sciences the term heavy metal is so widely used that it is hardly possible to eliminate it[1]. It is almost impossible to visualize a soil without trace levels of heavy metals and most of the heavy metals are essential elements for living organisms, but their excess amounts are generally harmful to plants, animals and human health[2,3]. Currently, contamination of soil with toxic heavy metals has emerged as a new threat to agriculture. Some of them like cadmium, lead and chromium are of primary importance and are dangerous to health or to the environment[4,5].Inorder to remove the excess heavy metals from contaminated soils, hyperaccumulators from weedy species will be more effective compared with agricultural crops[6]. As a result of long-term natural selection, most of the weed species have an extensive adaptive capacity, and play an important role in water and soil conservation and in the improvement of soil fertility and these weeds can thrive under severe growth conditions[7].

Several studies have been conducted by various researchers in order to evaluate the effect of different heavy metal concentrations on the germination and seedling performance[8-14]. *Amaranthus spinosus* L. commonly known as spiny amaranth, Prickly amaranth or thorny amaranth is an invasive weed common on the waste lands, abandoned heaps and road sides were selected for the present investigation. It is native to the tropical America and present on most continents as an introduced species and sometimes a noxious weed[15]. The leaves and stem of the plant were used as spinach. The plant has many medicinal properties and is used as antidote, astringent, diaphoretic, emmenagogue, emmolient etc. Reports on the heavy metal tolerance of the seeds of *Amaranthus spinosus* remain few and fragmentary that prompted the present investigation.

Material and Methods

The seeds of *Amaranthus spinosus* were collected from natural habitat. The seeds were surface sterilized with 0.1% $HgCl_2$, for two minutes and repeatedly washed with distilled water. The seeds were scarified with 5% H_2SO_4 for one hour. The seeds were transferred into petriplates that were filled with 75µg sand sieved through 2mm sieve and moistened with 20 ml of treatment solution containing different concentrations of heavy metals. Lead nitrate and potassium dichromate (100,150 and 200µM) and cadmium sulphate (5, 10 and 15µM) were used as the heavy metal source. The experiment was continued till 10 days under controlled laboratory conditions and various growth parameters such as germination percentage (GP), germination speed (GS), germination index (GI), seedling length (SL), vigour index (VI), mean germination time (MGT), coefficient of germination velocity (CGV) and tolerance indices (TI) were documented.

Plant sampling and Analysis-The germination of the seeds were observed and various parameters like Germination percentage, Germination speed, germination index[16], Vigour index[17], Mean Germination Time[18], Coefficient of Germination velocity, Tolerance indices[19] were calculated. The shoot and root length were measured with the help of a meter rod[20]. All the parameters were subjected to statistical analysis. The parameters were calculated by using the following formulae:

Germination Percentage (GP) = (Number of Germinated Seeds/ Number of planted seeds) × 100

Germination Speed (GS) = Percentage of germination/Day of completion of germination

Germination Index (GI) = P/t (where p- final percentage of germination and t-time to reach 50% germination).

Vigour Index (VI) = (root length+ shoot length) × percentage of germination.

Mean Germination Time (MGT) = $\Sigma(Dn)/\Sigma n$; where n- number of seeds germinated on the day D, D-number of days counted from the beginning of the germination test.

Coefficient of Germination Velocity (CGV) = Total number of seedling/$A_1T_1 + A_2T_2 + A_xT_x$

(Where, A= the number of seedlings emerging on a particular number of days (T), and subscripts 1, 2....x are respective number of germinated seeds per respective number of days after sowing of the seeds)

Tolerance indices (TI) = (Mean root length in metal solution / Mean root length in Control) × 100

Results and Discussion

The present study indicated that nature and dose of heavy metals altered all the parameters under investigation.

Effects of heavy metals on seed germination- The effects of varying concentrations of Cd^{2+}, Cr^{6+}, and Pb^{2+} on seed germination of *Amaranthus spinosus* were presented in Fig.1. Seed germination was reduced as metal concentrations increased. The percentage of germination indicated that the Cd^{2+} treatments even at low concentrations seriously affected the germination of seeds compared with Cr^{6+} and Pb^{2+} treatments. These findings were in conformity with the reports of Shafiq *et al.*[21] that decrease in seed germination of plant can be attributed to the accelerated breakdown of stored food materials in seed by the application of Cd^{2+}.

Higher concentration of all the metals had drastic effect on germination speed (Fig.2), germination index (Fig.5) and coefficient of germination velocity (Fig.7). More than 50% reduction in the germination was observed with higher concentration of Cd^{2+}. The inhibitory effect on germination of *A. spinosus* seeds treated with the heavy metals followed a trend of $Cd^{2+}>Cr^{6+}\geq Pb^{2+}$.

Effects of heavy metals on Seedling growth- The effects of heavy metals on shoot and root length of *A.spinosus* was depicted in Fig.8. Seedling growth is considered as an indicator of metal stress on plant ability to survive. It was also observed that addition of heavy metals from lower to higher concentration remarkably reduced shoot length but maximum inhibition occurred at 15 µM of Cd^{2+}. A reduction in Seedling length from 5.1 to 2.85cm, 3.95 to 1.35cm, and 2.75 to 2.05cm was recorded in response to varying concentrations of Pb^{2+} and Cr^{6+} (100-200 µM) as well as Cd^{2+} (5-15 µM). The decrease in seedling length due to heavy metal stress remained in confirmity with the findings of other researchers[9, 10, 22].

Effects of heavy metals on Mean Germination Time- MGT (Fig.3) significantly increased at higher concentration of Cd^{2+} (50% increase) as compared to control while a reduction was noticed at lower concentration (5µM). Cd^{2+} stress enhanced MGT and emphasized its inhibitory effect on germination ability of seed. Similar response was reported by Ahmad *et al*[9]. A reduction in the rate of germination leads to an increase in the MGT[23]. MGT of a seed lot represents the mean of the lag period from the start of imbibition to physiological germination of radicle protrusion[24].

Effects of heavy metals on Vigour Index- The vigour index of the seedlings after heavy metal treatments was depicted in Fig.4. Seed vigour may be defined as the sum total of those properties of the seed which determine the level of activity and performance. The seed showing higher seed vigour index is considered to be more vigorous[17]. In the present study, with increase in the concentration of solution, seedling vigour started to decrease. Morethan 75% reduction was observed at higher concentration of heavy metals such as Pb^{2+} (150µM), Cr^{6+} (200µM) and Cd^{2+} (10 & 15µM).

Effects of heavy metals on Tolerance Indices- The tolerant indices of seedlings under heavy metal treatments were depicted on the Fig. 6. The seedlings of *A. spinosus* were tested for tolerance to heavy metals under different concentrations of Pb^{2+}, Cr^{6+} and Cd^{2+}. High percentage of tolerance indices was expressed at 100µM of Cr^{6+} as compared to control. A gradual reduction in tolerence index was observed with Increase in concentration of heavy metals *viz*., $Pb^{2+}$and Cr^{6+} (150-200 µM) Cd^{2+} (10-15 µM). The present investigation clearly depicted that even though the concentrations of Cd^{2+} treatments were very low as compared to Pb^{2+} and Cr^{6+}, tolerant capacity of seed was seriously affected which indicated that Cd^{2+} treatment had high toxic effect than other two heavy metals. The order of heavy metal toxicity can be represented as $Cd^{2+}>Pb^{2+}>Cr^{6+}$. These findings were corroborated with the findings of Shafiq *et al.*[21].

The Present investigation revealed that the lower

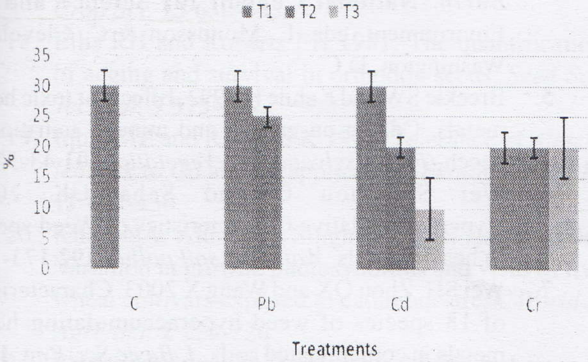

Fig.1. Germination percentage

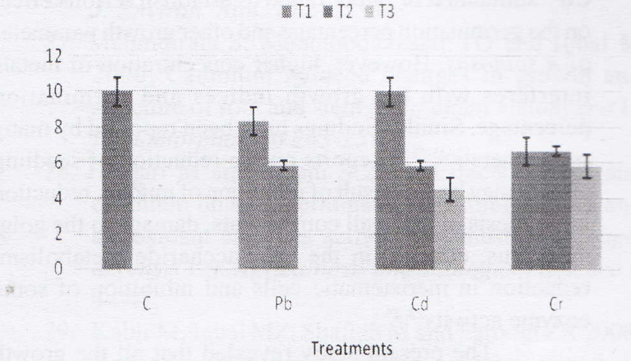

Fig.2. Germination speed

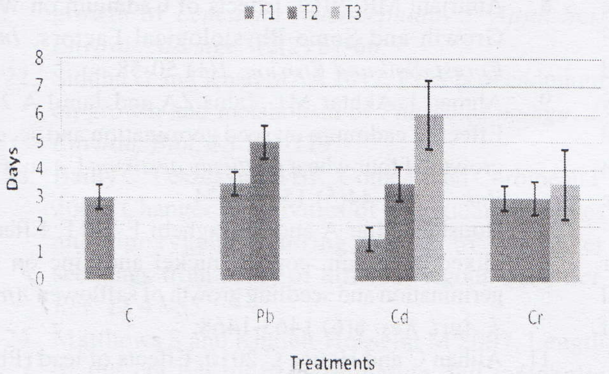

Fig.3. Mean germination time

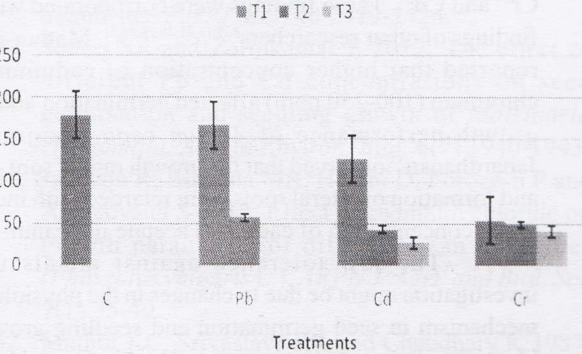

Fig.4. Vigour index

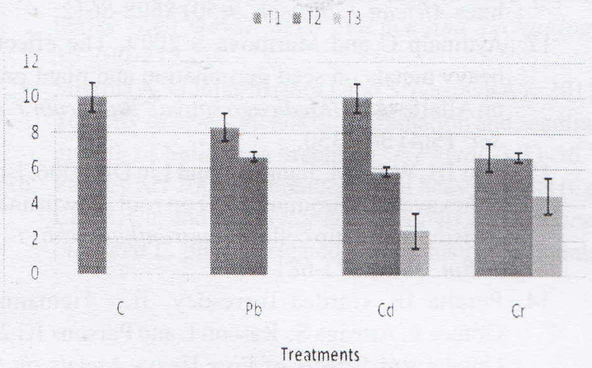

Fig.5. Germination index

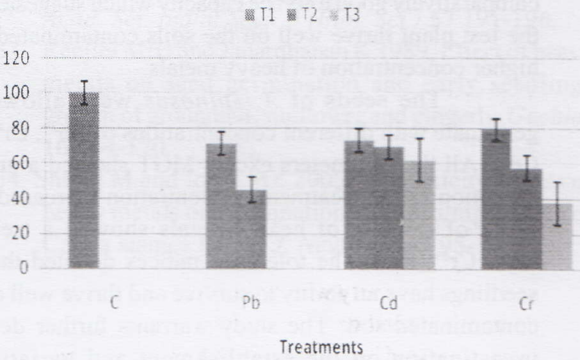

Fig.6. Tolerance indices

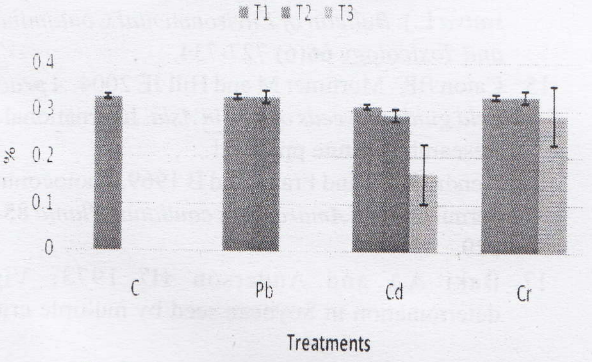

Fig.7. Coefficient of germination velocity

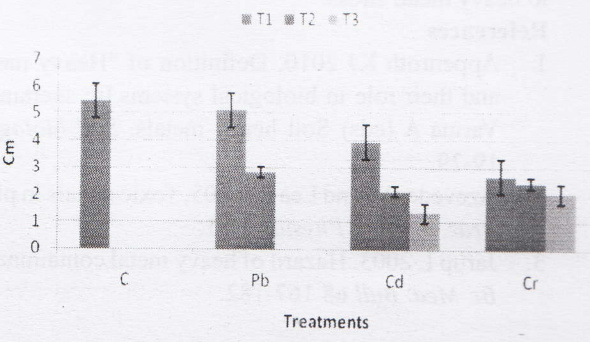

Fig.8. Seedling length

concentration of all the three metals *viz.,* Pb^{2+}, Cr^{6+} and Cd^{2+} stimulated or rather failed to establish serious effect on the germination percentage and other growth parameter of *A.spinosus*. However, higher concentration of metals interferes with the growth indices and germination percentage. Similar findings have been reported by many researchers[12, 25-27]. Reports on the reduction of seedling growth may be as a result of inhibition of mitosis, reduction in synthesis of cell wall components, damage to the golgi apparatus, changes in the polysaccharide metabolism, reduction in meristematic cells and inhibition of some enzyme activity[28, 29].

The present study revealed that all the growth parameters were sensitive to higher concentrations of Pb^{2+}, Cr^{6+} and Cd^{2+}. These findings were corroborated with the findings of other researchers[8, 9,12,21,25,28,30,31]. Mathur *et al.*[32] reported that higher concentration of cadmium and chromium (100-250 ppm) affected germination and early growth performance of *Allium cepa*. Renjini and Janardhanan[33] observed that the growth rate of root, shoot and formation of lateral roots were retarded with increase in the concentration of cadmium acetate in peanut.

The low tolerance against metals under investigation might be due to changes in the physiological mechanism in seed germination and seedling growth of plant. Similar results were reported by Shafiq and Iqbal[34]. In the present study the seeds of *A. spinosus* showed comparatively good tolernce capacity which suggested that the test plant thrive well on the soils contaminated with higher concentration of heavy metals.

The seeds of *A. spinosus* were allowed to germinate with different concentrations of Pb^{2+}, Cr^{6+} and Cd^{2+}. All the parameters except MGT showed a gradual reduction as the treatment concentration increased. The order of toxicity of heavy metals showed a trend of $Cd^{2+}>Cr^{6+}\geq Pb^{2+}$. The tolerence indices denoted that the seedlings have an ability to survive and thrive well on the contaminated soil. The study warrants further detailed investigation on the establishment and variation in metabolism as well as antioxidative defence of the plant to heavy metal stress.

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