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Research Article

EFFECTS OF HEAVY METALS ON *IN VITRO* SEED GERMINATION AND EARLY SEEDLING GROWTH OF *PENNISETUM GLACUM* (L.) R.Br.

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ABSTRACT

Crop yield mainly depends on the biotic and abiotic factors. Specifically abiotic stress such as drought, salinity, extreme temperatures and heavy metal (HM) contamination are the important factors that influence the crop growth and development worldwide. Plant responses to HM stress has been studying extensively for more than two decades. The present investigation demonstrates that the effects of different heavy metals such as chromium (Cr), cobalt (Co) and lead (Pb) on pearl millet [*Pennisetum glaucum* (L.) R.Br.] seed germination and seedling growth were studied in *in vitro* conditions using doses of 1, 10, 20, 50 and 100 ppm along with control cultures i.e. tap and distilled water media. Both Cr and Co proved decreased seed germination as well as seedling growth at increased concentrations. But lead treatments showed mixed results with respect to both seed germination and seedling growth pattern. These results may be useful in near future to know the morphological, phenotypical and ecological aspects of heavy metal stress on plants and ultimately know the plant growth and development in heavy metal polluted areas.

Key Words: *Pearl millet, Chromium, Cobalt, Lead, Seed germination*

INTRODUCTION

Besides heavy metals importance, they are one of the key soil pollutants and also a cause of environmental risk. Every heavy metal interacts with plant in a specific method which depends on different edaphic and growth factors including the surrounding environmental conditions (Abbassi *et al.*, 1998). The uptake, translocation and accumulation of heavy metals in plants are mediated by integrated network of physiological, biochemical and molecular mechanisms. Generally industrial wastes include heavy metals are one of the major threats for agriculture practices because above critical levels they may turn into toxins and cause inhibition of growth and development for the most of the plant species and at times leads to death also (Weiqiang *et al.*, 2005).

Heavy metal stress negatively affects the process associated with biomass accumulation and overall yield in almost all the major field grown crops by damaging several metabolic pathways and if not yield damage they may get incorporated in our food supply through harvested crops. However plants have some defense mechanism to deal with the excess of heavy metals in the soil by which they can prevent or restrict the uptake of metals or minimize the toxic effects through metal excluders, accumulators and indicators. They may localize selected metals mostly in roots and stems, or they may accumulate and store other metals in non-toxic forms for later distribution and use (Aydinalp and Marinova, 2009).

However the whole process is different from each plant group. Based on this process only some plants are growing in some regions and others not. In the present investigation we emphasize the seed germination pattern in different heavy metal doses by using pearl millet (*Pennisetum glaucum*). Pearl millet is the most widely grown type of millet and is well adapted to growing areas characterized by drought, low soil fertility, and high temperature etc. It performs well in soils with high salinity or low pH. Because of its tolerance to difficult growing conditions, it can be grown in areas where other cereal crops, such as wheat and maize, would not survive. Inspite of its tolerance, at present we focused on seed germination and seedling growth experiments in order to evaluate the effects of different heavy metal doses. Researchers have observed that some plants species are endemic to metalliferous soils and can tolerate greater than usual amounts of heavy metals or other toxic compounds 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. Previously Burhan *et al.,* (2001) reported that the different effects of cobalt on germination and seedling growth in *Pennisetum americanum* using traditional methods.

At present investigation, as a first step to understand how heavy metals affect the ability of seed germination and seedling growth of this crop by using Cr, Co and Pb at different developmental stages on *in vitro* conditions. For the best of our knowledge we are conducting heavy metal treatment experiment for the first time in *in vitro* conditions specifically with this crop. This study aims to standardize the growth and development of pearl millet in heavy metal contaminated regions.

MATERIALS AND METHODS

In the present work we have selected local varieties of pearl millet as source material which carries great nutritive value. Before going to start the experiments all the glassware and decontaminant vessels and test tubes (Borosil, India) were washed thoroughly with detergent solution and later it was then cleaned under running tap water, further rinsed with distilled water and oven dried.

Preparation and Sterilization of Media

The media used in the present work were distilled water agar media along with different concentrations of Cr (Potassium dichromate), Co (Cobalt chloride) and Pb (Lead nitrate) individually. As the experiment progressed, the amount of heavy metal present in the test tubes increased. In the present investigation we used 1, 10, 20, 50 and 100 PPM doses of heavy metals and tap and distilled water media as controls. The media employed are shown in Table A. pH (Elico limited, India) of the media was adjusted to 5.7 to 5.8 and before dispensing the equal amount of media into 25 x 150 mm test tubes, 0.8 per cent agar was added to the media and melted. Culture tubes containing media were autoclaved for 15 min at 15 lbs/in^2 in an autoclave [Forged Bail Valve, RBI (Italy)]. After the completion of sterilization, they were removed from the autoclave, and the tubes were placed in a slight slanting position to get more surface area.

Surface Sterilization of Pearl Millet Seed Material

Local varieties of pearl millet seeds were initially washed with tap water for 5-10 min to get them free from dust particles. Surface sterilization of the explants was performed by treated in 70% ethanol for 1 min and later seeds were treated with a solution of 0.1%. $HgCl_2$ (w/v) for 8-10 min. Surface sterilization was followed by 3 to 4 rinses in sterile distilled water each with 5 min interval. Then the seeds were blotted with sterile filter paper discs before inoculation to remove excess of water.

Inoculation and Culture Conditions

Before inoculation, the laminar air flow chamber (Hitech products, Chennai, India) was smeared with ethanol and all the requirements for inoculation were transferred inside the chamber. Sterilization of the chamber was done by switching on the ultraviolet lamp for half an hour before inoculation. Inoculation was carried out near the spirit lamp. Hands were frequently cleaned with ethanol from time to time to minimize contamination. All the inoculated cultures were incubated in a culture room at 25 ± 2°C with a relative humidity of 50-60% and around 16 h photo period at a photo flux density of 15-20 μEm^2S^{-1} of White fluorescent tubes.

Observations & Statistical Analysis

Visual observations of the cultures were done and were quantified on the basis of percentage of germination i.e. the number of germinations per culture and the length of shoot and root of the seedlings per culture. A minimum of three replicates were involved in each experiment and conducted thrice. The mean and excel programming techniques using personal computer for different parameters.

Table 1: List of the media combinations used in the present work

No.	Combinations	Heavy metal PPM
1	Tap water	Alone as control
2	Distilled water	Alone as control
3	Distilled water	1
4	Distilled water	10
5	Distilled water	20
6	Distilled water	50
7	Distilled water	100

0.8% agar was used for all the experiments, pH of the media adjusted to 5.7 to 5.8 in all the experiments

RESULTS AND DISCUSSION

We started all the experiments in *in vitro* conditions and an attempt has been made to standardize the optimum growth and development of pearl millet in different heavy metal doses. Percentage of seed germination, shoot length and root lengths results were recorded along with controls including observation of greenness.

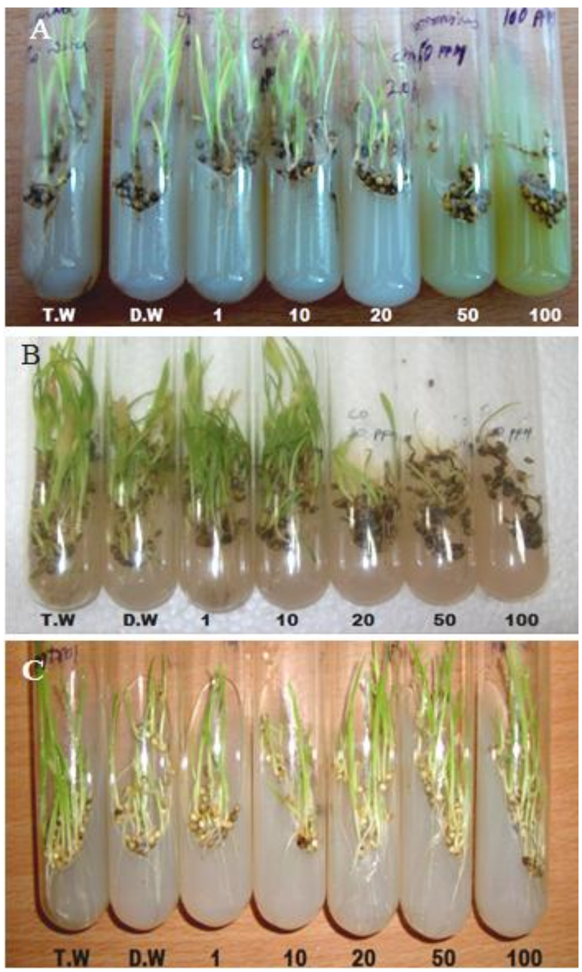

Figure A, B and C: Effects of chromium (A), cobalt (B) and lead (C) on seed germination and seedling growth of pearl millet

Abbreviations: T.W- Tap water sample, D.W-Distilled water sample, 1, 10, 20, 50, 100 PPM samples

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Figure 1 and Table 1 Effect of chromium on percentage of seed germination (figure 1) and early seedling growth (table-1).

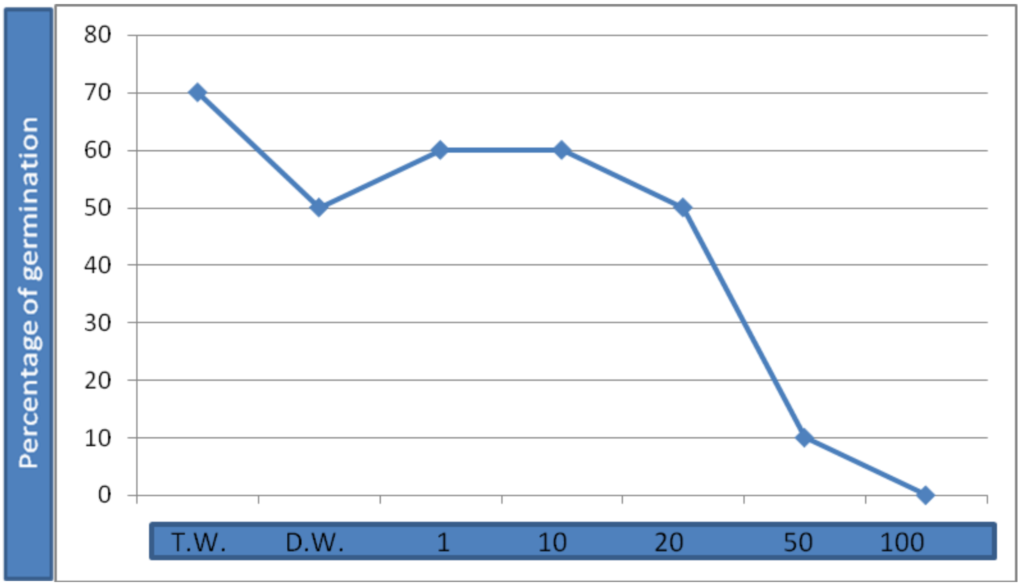

Figure 1: Effect of chromium on percentage of seed germination

Table 1: Effect of chromium on percentage of early seedling growth

S.No	**Concentration of chromium**	***Pearl millet* Seedling Morphology**	
		Shoot length (cm)	**Root length (cm)**
1	T.W	5.8	2.4
2	D.W	5.1	2.4
3	1ppm	4.9	2.2
4	10ppm	4.1	2.1
5	20ppm	3.5	1.8
6	50ppm	1.8	1.0
7	100ppm	0	0

The above data is collected between 2-3 weeks. Values above represented are mean of 3 replicates.

Effect of Heavy Metals on Seed Germination

Chromium will be available in all the phases of environment including air, water and soil. Percentage of seed germination was more in low concentration of chromium and there is continuous decreasing pattern with increasing the concentration and at 100 ppm there is no seed germination (Figure A and 1). Similar results i.e increasing concentration of Cr leads to decreasing seed germination was observed in *Hibiscus esculentus* and some important pulses (Amin *et al.*, 2013; Jun *et al.*, 2009). Similarly cobalt cultures were also more or less like chromium treatments. Percentage of germination was gradually decreased with increased concentration of cobalt (Figure B and 2). 50 and 100 ppm of cobalt proved less responsive, specifically there is negligible percentage of germination (5%) in 100ppm when compared to 1, 10 and 20 ppm concentrations and this is correlated with the works of Burhan *et al.*, (2001). Interestingly lead (Pb) did not show significant variations in all the concentrations indicating mixed results (Figure C and 3). Exposure to lead does not show any significant changes in percentage of germination with few exceptions in contrast with an inhibition in seed germination and growth as observed in wheat by Lamhamdi *et al.*, (2011). In conclusion it is to be noted that both chromium and cobalt showed more negative effect in germination of *P.glaucum* when compared with lead treatments.

Figure 2 and Table 2 Effect of cobalt on percentage of seed germination (figure 2) and early seedling growth (table 2).

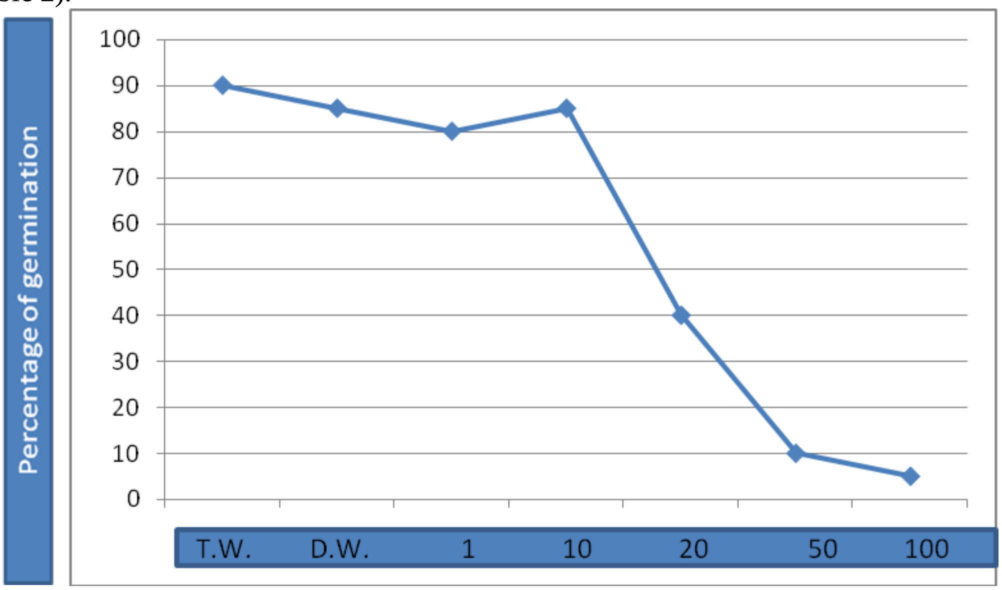

Figure 2: Effect of cobalt on percentage of seed germination

Table 2: Effect of cobalt on percentage of early seedling growth

S.No	**Concentration of cobalt**	***Pearl millet* Seedling Morphology**	
		Shoot length (cm)	**Root length (cm)**
1	T.W	6.1	4.4
2	D.W	5.8	3.9
3	1ppm	5.9	2.6
4	10ppm	5.5	2.6
5	20ppm	3.7	2.4
6	50ppm	2.5	1.1
7	100ppm	0	0

The above data is collected between 2-3 weeks. Values above represented are mean of 3 replicates

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Figure 3 and Table 3: Effect of lead on percentage of seed germination (figure 3) and early seedling growth (table 3).

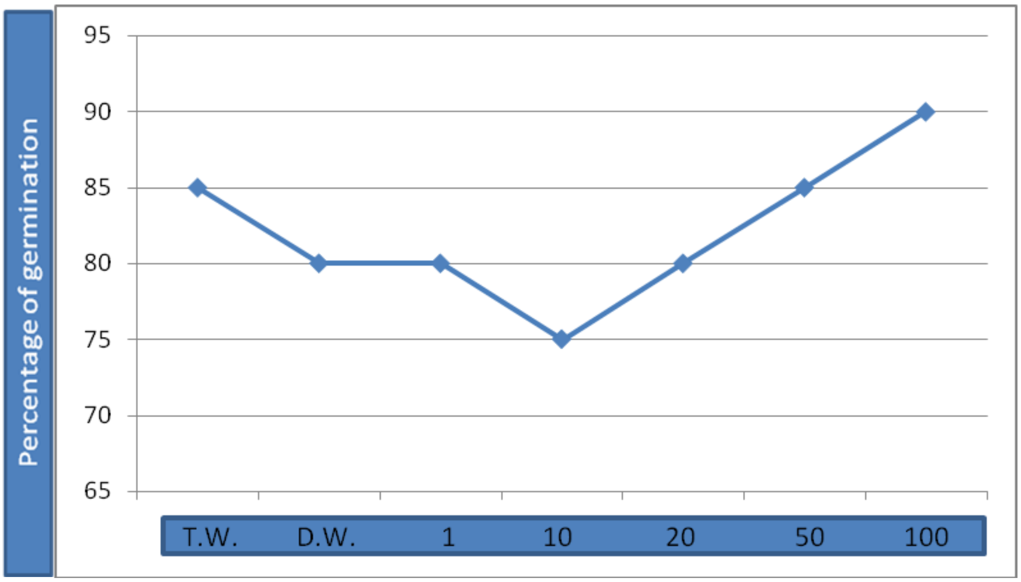

Figure 3: Effect of lead on percentage of seed germination

Table 3: Effect of lead on percentage of early seedling growth

S.No	**Concentration of Lead**	***Pearl millet*** **Seedling Morphology**	
		Shoot length (cm)	**Root length (cm)**
1	T.W	5.5	8.0
2	D.W	6.0	5.5
3	1ppm	5.8	6.5
4	10ppm	6.2	6.6
5	20ppm	7.3	6.8
6	50ppm	7.1	6.8
7	100ppm	7.6	6.9

The above data is collected between 2-3 weeks. Values above represented are mean of 3 replicates

Effect of Heavy Metal on Early Seedling Growth

In chromium treatments we noticed gradual decreased in shoot and root lengths (Table 1) as previously proved in melon plant (Akinsi and Akinsi, 2010). Similar results were observed in cobalt treatments i.e. gradual decrease in shoot and root lengths with increased cobalt concentrations (Table 2) and these results similar to observations of Khan *et al.,* (2010) in chick pea. Greenness (chlorophyll content) also reduced with increasing concentrations doses of both Cr and Co (data not shown) as observed by Ozdener *et al.,* (2011) in *Brassica oleracia*. As expected and mentioned above lead (Pb) did not show significant variations in shoot as well root length also (Table 3). In fact there is a slight increasing pattern of root length with increasing doses which is in contrast with Pourrat *et al.,* (2011) results.

Conclusion

In conclusion effect of both chromium and cobalt treatment shows decreasing germination and seedling growth with increasing concentrations (Figure A, B and C). This study may be useful to evaluate plant growth and development pattern in rich heavy metal areas.

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