

## Effect of cadmium on germination, coleoptile and root growth of barley seeds in the presence of gibberellic acid and kinetin

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**Abstract:** Effect of cadmium on barley seeds treated with kinetin and gibberellic acid was investigated. As usual, cadmium has inhibited seed germination, and showed important inhibitory effects on roots and coleoptile growth after germination. In general, increase in cadmium concentration caused a greater inhibition of germination, root and coleoptile growth. The adverse effect of cadmium on root and coleoptile growth was more pronounced than that on germination. While testa was pierced by radicle (an indication of germination), no root or coleoptile development was observed above at concentration of 3-9.5 mM CdCl<sub>2</sub>.H<sub>2</sub>O. Low concentrations of cadmium have inhibited the root growth more than it did on coleoptile growth. Treatment of seeds with gibberellic acid and kinetin did not show any significant difference on the effect of cadmium in germination. However, inhibition of coleoptile elongation by cadmium has decreased a very much after kinetin application. The same result, although with lower rates when compared to kinetin, has been obtained for GA<sub>3</sub> as well. In addition, the inhibitory effect of cadmium on root growth increased even more after kinetin application. The results have been found statistically significant through the least significant different (LSD) test at levels of  $p < 0.05$  and  $p < 0.01$ .

**Key words:** Cadmium, Germination, Gibberellic acid, Kinetin, Root and coleoptile growth.

### Introduction

Different levels of heavy metals contained in soil, water and air cause pollution after reaching certain concentrations. There are different reasons for this kind of pollution. Metals are continuously released into the biosphere by volcanoes, natural weathering of rocks and by industrial activities such as mining and the combustion of fossil fuels and the release of sewage (Depledge *et al.*, 1994). Heavy metal contamination of agricultural soil has also been observed to be increased due to industrialization. Therefore, heavy metal contamination represents a risk for primary and secondary consumers and ultimately humans (Zeller and Feller, 1999).

Detailed studies indicate that heavy metals have many effects on plants. Several studies, carried out both under laboratory and natural conditions, with different plant species indicate that heavy metals have inhibitory effects on some important events such as root, stem and leaf growth (Liu *et al.*, 2000; Sresty and Madhava Rao, 1999), respiration (Fernandes and Henriques, 1991), photosynthesis (Prasad, 1995), cell division and biosynthesis of DNA, RNA and protein (Arduini *et al.*, 1994; Espen *et al.*, 1997; Mohan and Hosetti, 1997).

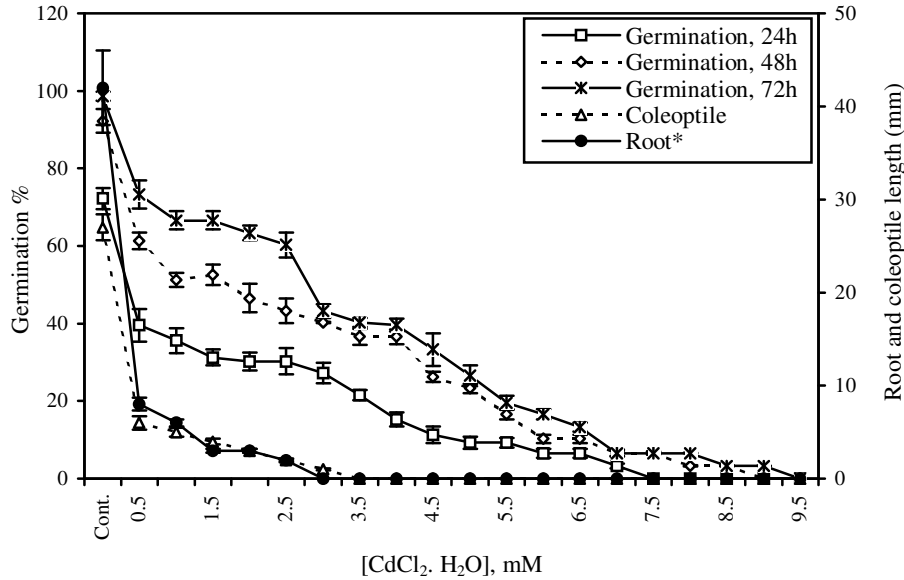
There are several reports demonstrating that heavy metals inhibit germination of seeds of different species of plants. Very low concentrations of copper stop the germination of rice seeds completely (Gupta and Mukherji, 1977). Higher concentrations of copper, mercury and cadmium decrease the germination of lentil seeds gradually (Ayaz and Kadioglu, 1997). Different metals cause different levels of inhibition of germination in different plant seeds. For example, effects of manganese on pea (Mukhopadhyay and Sharma, 1990); mercury and lead on rice (Mishra and Choudhuri, 1999); and

chromium and cobalt on bean (Zeid, 2001) have been reported.

In plants, both vegetative and reproductive developments and differentiation take place under the control of hormones. Plant growth is closely dependent on enzyme formation and the nucleic acids that control the process. Hormone formation in plants is partially under the effect of environment. And some of the effects of environment on plants are exerted via hormones. Hormones have very important functions in seed germination just like they have in almost every physiologic event in plants. For example, gibberellins promote the synthesis of some hydrolases by mobilizing stored material during germination (Wilkins, 1989). Because cytokinins have incremental effect on cell division and enlargement effect on cotyledons, they help the germination of seeds even in the absence of light (Wilkins, 1989). In many dormant seeds, germination is stimulated by cytokinins. Although many studies are there on the effect of heavy metals on plants, there are almost no studies regarding the hormonal side of this process. Especially in species *Hordeum vulgare* L., which is economically important to fed animals worldwide. The present study deals with the effect of cadmium on germination, root and coleoptile growth of barley seeds treated with gibberellic acid and kinetin.

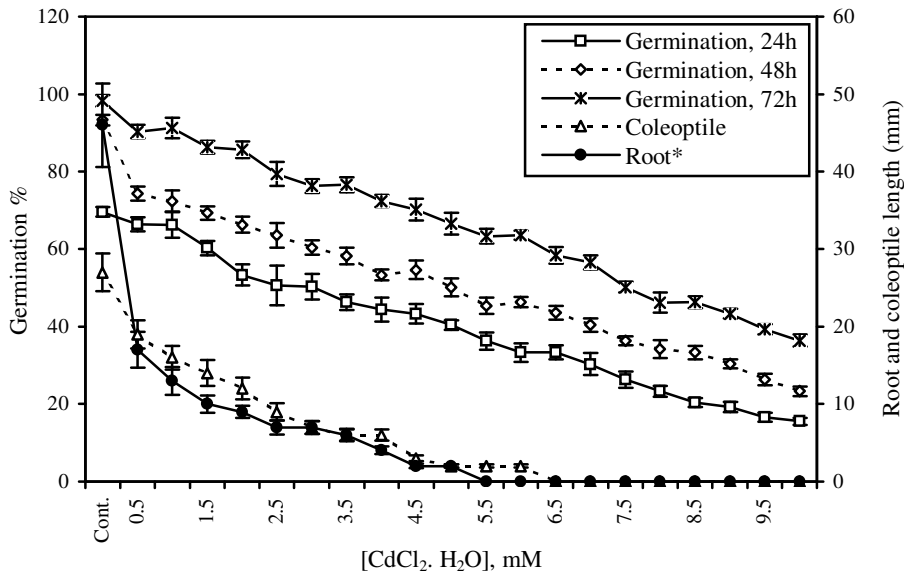
### Materials and Methods

In this study barley (*Hordeum vulgare* L.) seeds were used. Cadmium (as CdCl<sub>2</sub>. H<sub>2</sub>O) and gibberellic acid (GA<sub>3</sub>) were from Merck Chemicals and kinetin riboside (6-furfurylamino-purine riboside), was from Sigma Chemical Co. The application of gibberellic acid and kinetin riboside was 100 ppm each. Cadmium chloride was used at concentrations 0.5-10.0 mM (20 different concentrations with 0.5 increments). GA<sub>3</sub> was dissolved in 3-4 drops of 70% ethanol (Merck) whereas



**Fig. 1:** Germination rate and root and coleoptile growth of barley seeds imbibed and germinated in cadmium containing solutions. Values are averages of four trials on 30 seeds in each. Error bars are  $\pm$  SDEVs. Where no error bars are visible, they are smaller than the diameter of the points.

\*Root length is given as the sum of primary and secondary root lengths divided by number of roots.

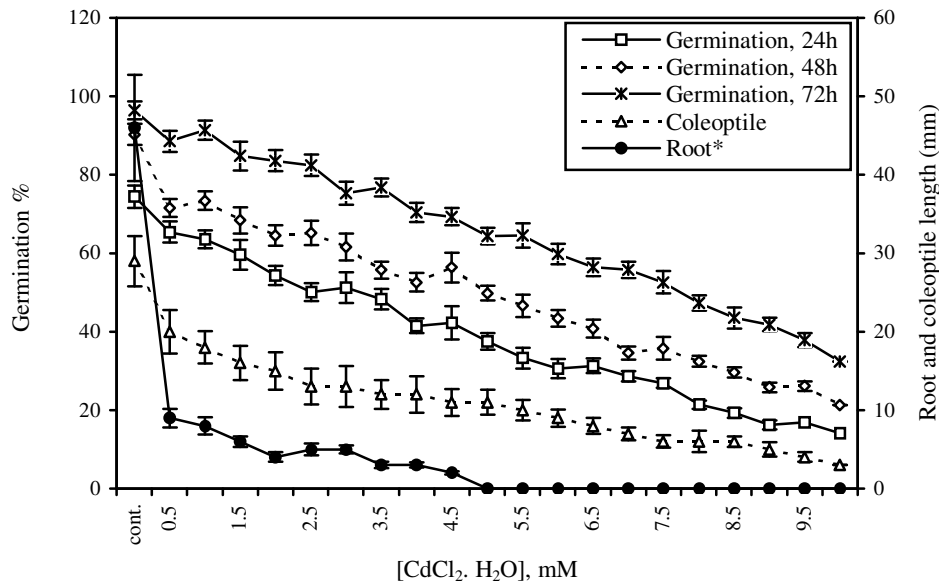


**Fig. 2:** Germination rate and root and coleoptile growth of barley seeds imbibed in cadmium and germinated in ddH<sub>2</sub>O. Values are averages of four trials on 30 seeds in each. Error bars are  $\pm$  SDEVs. Where no error bars are visible, they are smaller than the diameter of the points.

\*Root length is given as the sum of primary and secondary root lengths divided by number of roots.

kinetin was dissolved in 3-4 drops of diluted HCl (Merck) and the pH of both was adjusted to 6.3. All preparations were made in distilled-deionised water (ddH<sub>2</sub>O, pH = 6.3). pH adjustments were made by 1 N NaOH or 1 N HCl. The seeds were left to be imbibed (water uptake) in plastic beakers and were germinated in petri dishes. For imbibition and germination, a plant growth

cabinet (LEEC plant growth cabinet, Nottingham) was used. The effect of each concentration of selected cadmium salt was studied on 30 seeds at each time. The imbibitions of seeds were carried out by immersing them into 27 ml solutions in the presence of various cadmium concentrations and seeds were left in the dark growth cabinets at 24 °C 4 hr for swelling.



**Fig. 3:** Germination rate and root and coleoptile growth of barley seeds imbibed in cadmium and germinated in kinetin containing solution. Values are averages of four trials on 30 seeds in each. Error bars are  $\pm$  SDEVs. Where no error bars are visible, they are smaller than the diameter of the points.

\*Root length is given as the sum of primary and secondary root lengths divided by number of roots.

Swollen seeds were then sown on petri dishes with double layer of filter paper wetted with 9 ml of germination media. Germination media contained either of each CdCl<sub>2</sub>. H<sub>2</sub>O, kinetin, GA<sub>3</sub> or kinetin + GA<sub>3</sub>. The seeds coming from a certain concentration of cadmium containing imbibition medium were sown on to the petri dishes with the same concentration of this metal. The kinetin and GA<sub>3</sub> concentration in germination medium, however, was 100 ppm for each. The control group seeds were also treated in the same way using cadmium for imbibition, but using ddH<sub>2</sub>O for germination instead of cadmium or growth substances. Also, a control group seeds (serving as negative controls) were included where both imbibition and germination were in ddH<sub>2</sub>O only. The covers of the petri dishes were closed and they were kept at 24 °C in the dark in growth cabinets for 72 hr. Germination rate was evaluated for every 24 hr. A 1-mm radicle emergence from seeds was considered as seed germination. However, the root elongation and coleoptile growth of germinated seeds were analyzed only at 72 hr of incubation, a duration required for the observation of these parameters and the inhibitory effects of cadmium used. Each test was carried out in four replicates. Data are the results from four separate analyses with 30 seeds in each. Statistical analysis was performed based on SPSS (version 10.0) program. In order to detect the significance of differences ( $p < 0.01$  or  $p < 0.05$ ) of variables, a multiple comparison (LSD) test was performed. All values are expressed as mean  $\pm$  SEs.

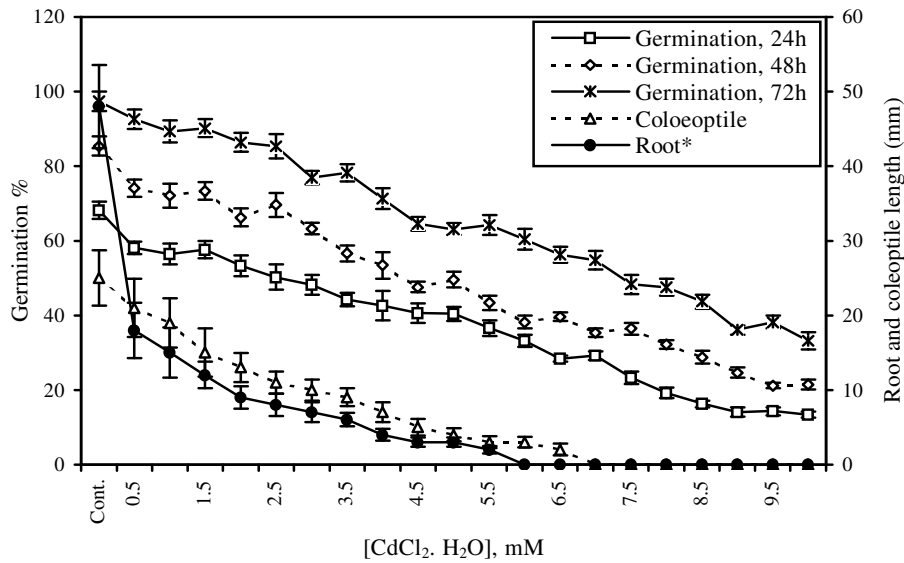
### Results and Discussion

The germination rates along with coleoptile and root growth of barley seeds treated with cadmium and plant growth

substances (kinetin and gibberellic acid) are given in Fig. 1-5. With the exception of control, imbibitions of all seeds were first done in cadmium containing solutions. Then, germination was noted in solutions containing cadmium, ddH<sub>2</sub>O, gibberellic acid, kinetin or gibberellic acid+kinetin. The imbibition and germination of control seeds were both in ddH<sub>2</sub>O.

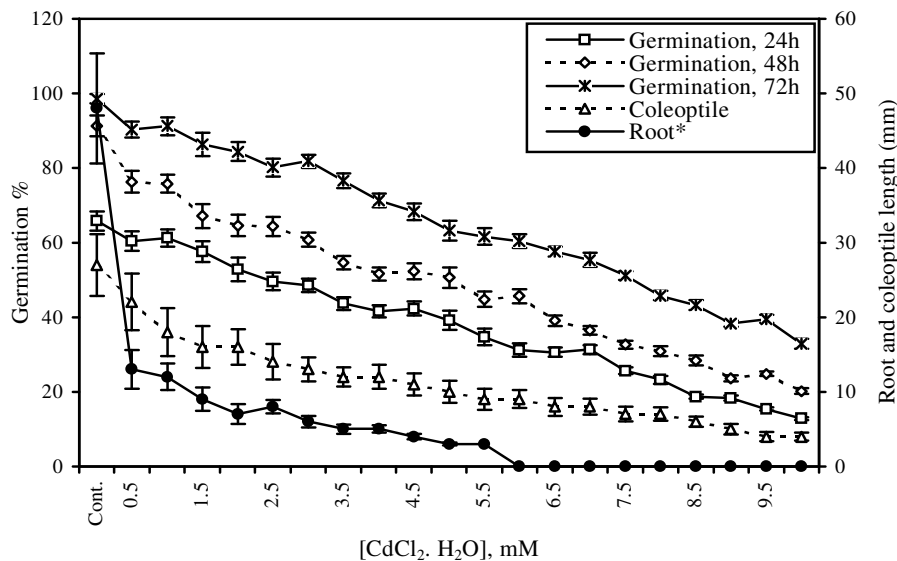
All concentrations of cadmium inhibited seed germination, coleoptile and root growth, though to a different extent. Plant growth substance (GA<sub>3</sub> and kinetin) application did not eliminate the hindering effect, which cadmium showed on germination. Seed germination rate decreased proportionately with increasing concentration of cadmium (Fig. 1). For example, at the end 72 hr of incubation period, the 0.5 mM CdCl<sub>2</sub>.H<sub>2</sub>O has decreased the germination rate of seeds to 25% ( $p < 0.01$ ) compared to the control. These values were determined to be 39%, 66%, 87% and 97% for cadmium concentrations of 2.5, 4.5, 6.5 and 8.5 mM respectively (Fig. 1). Cadmium concentration of 9.5 mM (CdCl<sub>2</sub>.H<sub>2</sub>O) inhibited the germination completely.

Cadmium has inhibitory effects on the root and coleoptile growth, as well. Low concentration of cadmium effected root growth more than it did on the coleoptile growth. The 0.5 mM CdCl<sub>2</sub>.H<sub>2</sub>O caused 81% root growth decrement ( $p < 0.01$ ) and 78% coleoptile growth decrement ( $p < 0.01$ ) compared to the control (Fig. 1). On the other hand, the root growth of germinated seeds stopped completely at 3.0 mM CdCl<sub>2</sub>.H<sub>2</sub>O. Similarly, at 3.5 mM CdCl<sub>2</sub>.H<sub>2</sub>O, coleoptile growth stopped completely (Fig. 1). Both the germination rates, and the root and coleoptile growth of the seeds for which CdCl<sub>2</sub>.H<sub>2</sub>O was used only in the imbibition medium, were greater than those of



**Fig. 4:** Germination rate and root and coleoptile growth of barley seeds imbibed in cadmium and germinated in  $GA_3$  containing solution. Values are averages of four trials on 30 seeds in each. Error bars are  $\pm$  SDEVs. Where no error bars are visible, they are smaller than the diameter of the points.

\*Root length is given as the sum of primary and secondary root lengths divided by number of roots.

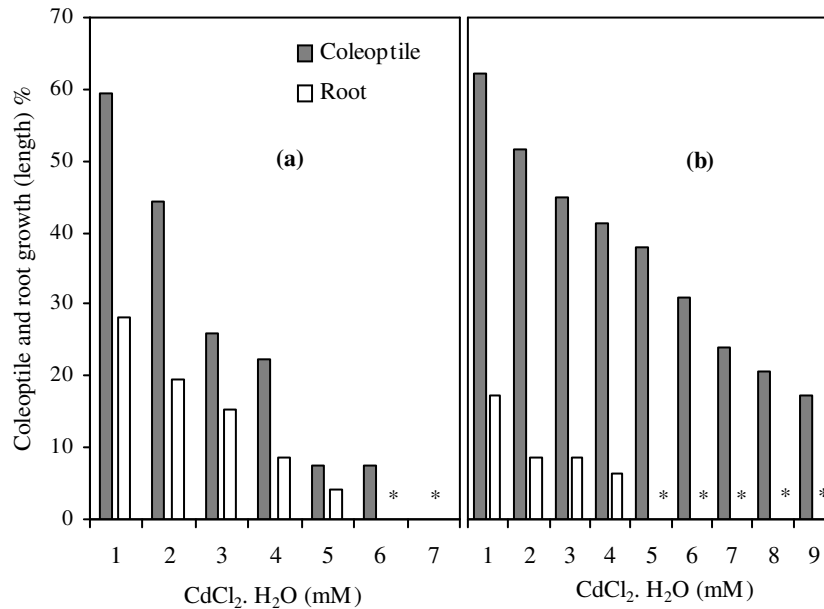


**Fig. 5:** Germination rate and root and coleoptile growth of barley seeds imbibed in cadmium and germinated in kinetin +  $GA_3$  containing solution. Values are averages of four trials on 30 seeds in each. Error bars are  $\pm$  SDEVs. Where no error bars are visible, they are smaller than the diameter of the points.

\*Root length is given as the sum of primary and secondary root lengths divided by number of roots.

of the seeds for which cadmium was used both in the imbibition and germination medium (Fig. 2). For example, the germination rates of the seeds for which only in the imbibition medium was 3.5 mM  $CdCl_2 \cdot H_2O$  and resulted in 22% percent decreased ( $p < 0.01$ ) compared to the control (Fig. 2). The value was determined to be 59% 3.5 mM  $CdCl_2 \cdot H_2O$  of the seeds for which cadmium was used both in the imbibition and germination media (Fig. 1).

There seems to be no important difference ( $p > 0.05$ ) among the germination rates (Figs. 2-5) at the end of 72 hr incubation period of the seeds for which in the imbibition medium  $CdCl_2 \cdot H_2O$  and in the germination medium  $ddH_2O$  or kinetin or  $GA_3$  or kinetin +  $GA_3$  were used. For instance, the germination rates of the seeds for which 1.0, 3.0, 5.0, 8.0 or 10.0 mM  $CdCl_2 \cdot H_2O$  in the imbibition medium and  $ddH_2O$  in the germination medium were used, decreased by 7%, 22%, 32%,



**Fig. 6:** The root and coleoptile growth rates of barley seeds imbibed in cadmium but germinated in ddH<sub>2</sub>O (a) or kinetin (b) containing solution as compared to control group seeds imbibed and germinated in ddH<sub>2</sub>O only.  
\*No root or coleoptile growth.

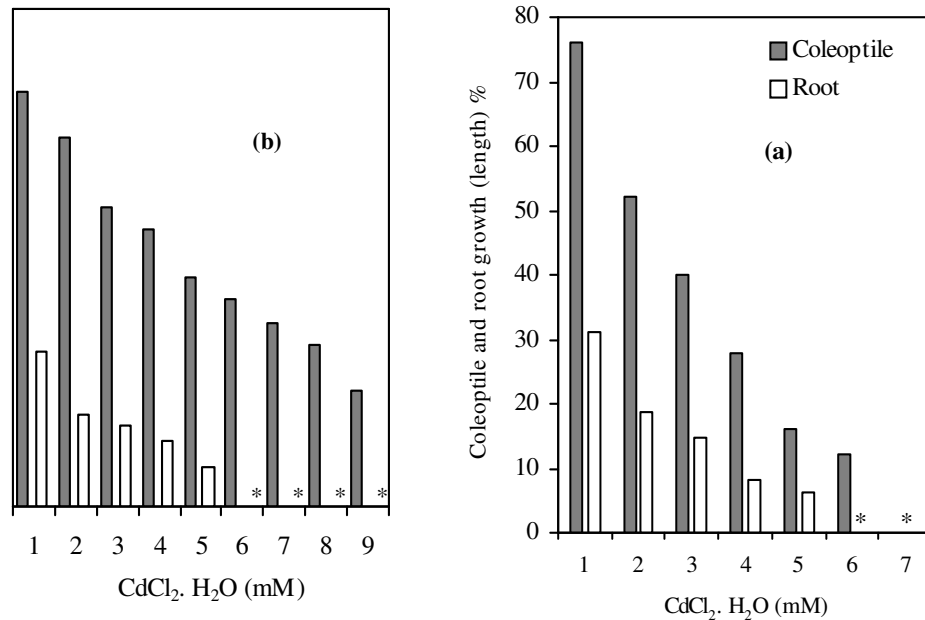
53% and 63% respectively, compared to the control (Fig. 2). In the experiments where same concentrations of CdCl<sub>2</sub>.H<sub>2</sub>O were used in the imbibition media, when 100 ppm kinetin was used in the germination medium, the values were 5%, 22%, 33%, 51% and 66% (Fig. 3); and for 100 ppm GA<sub>3</sub>, they were 8%, 21%, 35%, 51% and 66% (Fig. 4); and for 100 ppm kinetin + 100 ppm GA<sub>3</sub> (v/v), they were 8%, 17%, 36%, 54% and 67% respectively (Fig. 5). These results clearly demonstrate that the inhibitory effect of CdCl<sub>2</sub>.H<sub>2</sub>O on germination cannot be eliminated by external application of hormones.

Contrary to the findings on germination, inhibitory effect of cadmium on coleoptile growth decreased at significant proportions ( $p < 0.05$ ,  $p < 0.01$ ) after the application of kinetin. A clear difference could be noticed when the coleoptile lengths of the controlling plants were compared with that of seeds for which in the imbibition medium CdCl<sub>2</sub>.H<sub>2</sub>O, and in the germination medium ddH<sub>2</sub>O, or kinetin, or gibberellin, or kinetin + gibberellin were added (Figs. 2-5). For example, when compared with the control plants' elongation, the coleoptile lengths of the seeds, for which in the imbibition medium 2.0, 3.0, 4.0, 5.0 or 6.0 mM CdCl<sub>2</sub>.H<sub>2</sub>O and in the germination medium ddH<sub>2</sub>O were used, have decreased by 55%, 74%, 78%, 93% and 93%, respectively (Fig. 6a). These values of the seeds for which in the germination medium 100 ppm kinetin per seed was used are 48%, 55%, 59%, 62% and 69%, respectively (Fig. 6b); when GA<sub>3</sub> was used rather than kinetin these values are detected to be 48%, 60%, 72%, 84% and 88% (Fig. 7a). And when kinetin + GA<sub>3</sub> (v/v) combination was used in the germination medium, these values were 41%, 52%, 56%, 63% and 67%, respectively (Fig. 7b). In the germination medium where ddH<sub>2</sub>O was used 6.5 mM CdCl<sub>2</sub>.H<sub>2</sub>O (Fig. 2),

7.0 mM CdCl<sub>2</sub>. H<sub>2</sub>O where GA<sub>3</sub> was used in the germination medium (Fig. 4) caused the coleoptile growth to stop completely.

The inhibitory effect of cadmium on root growth has been increased ( $p < 0.01$ ) by the application of kinetin. For instance, the root lengths of the seeds for which in the imbibition medium 0.5, 1.5, 2.5 or 3.5 mM CdCl<sub>2</sub>.H<sub>2</sub>O and in the germination medium 100 ppm kinetin were used, have decreased by 82%, 91%, 91% and 93%, respectively, compared with the control plants (Fig. 6b). These values were 72%, 80%, 86% and 91% (Fig. 6a) when ddH<sub>2</sub>O was used instead of kinetin and 69%, 81%, 85% and 92% (Fig. 7b) when GA<sub>3</sub> was used instead of kinetin.

Seed germination begins with water uptake (imbibition) and ends with the start of elongation by the embryonic axis, usually the radicle. The seed germination dynamics are altered under various physical and chemical conditions. There have been various reports on the effect of heavy metals on germination dynamics, root and shoot growth of various plant species: lead, cadmium, arsenic, and chromium inhibition to germination of *Sinapsis alba* seeds (Fargasova, 1994); decrease in fresh weight of seedlings of mung beans exposed to cobalt (Liu *et al.*, 2000); decreased stem length of *Oryza sativa* L. cv. Safari plant with copper (Lidon and Henriques, 1998); decreased root and stem length of *Oryza sativa* L. cv. Bahia by cadmium and nickel (Moya, *et al.* 1993); cadmium inhibition of root growth of *Pinus pinea* and *P. pinaster* seedlings (Arduini, *et al.* 1994); stem length decrease in *Brassica juncea* seedlings exposed to zinc (Prasad, *et al.*, 1999) and root and stem growth inhibition of *Zea mays* L. Dekalp cv. Sponsor plant treated with cadmium (Rascio, *et al.* 1993).



**Fig. 7:** The root and coleoptile growth rates of barley seeds imbibed in cadmium but germinated in GA<sub>3</sub> (a) or kinetin + GA<sub>3</sub> (b) containing solution as compared to control group seeds imbibed and germinated in ddH<sub>2</sub>O only.  
\*No root or coleoptile growth.

It has been reported that plants develop various mechanisms of tolerance and resistance in order to alleviate the heavy metal stress, and one of these is the absorption of metal in the roots and inhibition of its transport to the stem (Fernandes and Henriques, 1991). Application of plant growth substances (GA<sub>3</sub> and kinetin) could not eliminate the inhibitory effects of heavy metals on germination. Very little is known about effect of plant hormones on heavy metal stress. To the best of our knowledge there are no studies on the effect of heavy metals on the level of hormone in seed germination. It is only lately reported that 100 and 200 μM concentrations of salicylic acid postulated to be a new regulator (Raskin, 1992), which alleviates the inhibitory effect of lead and mercury on the germination of *Oryza sativa* seeds (Mishra and Choudhuri, 1997).

The fact that the application of plant growth substances could not eliminate the inhibitory effect of heavy metals on germination indicates that heavy metals achieve this by interruption with other mechanisms. Probably, first of these is enzymatic activity. Enzymes have enormous function in all metabolic activities. Cadmium has been found to inhibit the activities of alcohol dehydrogenase, hexokinase, and glucose-6-phosphate dehydrogenase of the germinated *Pisum sativum* seeds (Chugh and Sawhney, 1999). In the *Lemna minor* L. plant, cadmium and lead have decreased the activities of catalase and protease but caused an increase in the activity of peroxidase (Mohan and Hosetti, 1997). Cadmium was reported to cause a decrease in the activity of nitrate reductase of *Phaseolus vulgaris* L. cv. Morgane, (Gouia *et al.*, 2000). Some of the heavy metal ions have been detected to affect the

electron transport system of respiration and to inactivate the enzymes. For instance, during the germination of *Pinus resinosa* pollen, cadmium did not affect respiration but at higher concentrations, it decreased the exit of CO<sub>2</sub> and entry of O<sub>2</sub> (Strickland and Chaney, 1979). Cadmium has caused a decrease in the respiration of *Beta vulgaris* L. cv. Monohill seedlings (Greger *et al.*, 1991).

The stimulatory effect of kinetin application on seed viability and seedling vigour could be attributed to its favorable effects on synthesis of RNA, proteins and enzymes causing a general metabolic advancing or priming to occur (Moore, 1989). The stimulative effect of kinetin on germination and seedling vigour, observed in the present study agrees with the findings of Bozcuk (1990) and Kabar (1990) with cotton and Singh and Amritphale (1993) with soybean. The present study is that the growth inhibitory effect of cadmium on coleoptile has been reduced significantly ( $p < 0.01$ ) by the application of kinetin. Also, the inhibitory effect of cadmium on coleoptile growth has been significantly ( $p < 0.05$ ) reduced with GA<sub>3</sub> application. The above findings indicate that GA<sub>3</sub> has partially ( $p < 0.05$ ) alleviated the inhibitory effects of cadmium on coleoptile growth. Yet, it was smaller when compared with the effects of kinetin. It has been documented that cytokinins are synthesized at greater rates in the roots and are carried to the stem. These increase the coleoptile growth, block root growth at higher concentrations, increase the activities of many enzymes and cause the mobilization of nutritional substance (Nielsen and Orcutt, 1996; Hopkins, 1995; Wilkins, 1989).

It can be concluded that cadmium inhibited seed germination barley and it had important inhibitory effect on the

growth of root and coleoptile formed at the stage after germination. The application of kinetin and GA<sub>3</sub> did not eliminate the inhibitory effect of cadmium on germination. Kinetin reduced the inhibitory effect of cadmium on coleoptile growth at high rates. When compared with kinetin, similar effect was shown by gibberellin but at very low rates. Inhibitory effect of cadmium on root growth increased greatly with the application of kinetin. Possible explanation(s) for this might be that kinetin is carried to the stem in order to mobilize the nutritional substances to this area; it increases the activation of various enzymes; and it blocks the transportation of cadmium from roots to the stem.

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