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EFFECT OF CADMIUM ON SEED GERMINATION AND SEEDLING GROWTH OF FOUR WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS

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Abstract

Cadmium (Cd) is extremely toxic metal and reduces plant growth. Therefore, study was conducted to evaluate the effect of various Cd levels 0, 5, 20, 50 and 80 mg L^{-1} on seed germination and seedling growth of four wheat cultivars (Sehar-06, Fareed-06, Inqlab-91, Chakwal-50). Cadmium showed toxicity at 5 mg L^{-1} in case of root and shoot growth whereas 20 mg L^{-1} in case of seed germination and germination energy which is aggravated on further addition of Cd from 50 to 80 mg L^{-1}. Sehar-06 performed better under Cd stress while Inqlab-91 had poor performance. Later had maximum decrease in root length (70.4%), shoot length (81.2%), percent germination (68%) and germination index (76.8%) at 80 mg L^{-1} Cd compared to control. Tolerance index varied among different cultivars, maximum tolerance was observed in Sehar-06 followed by Fareed-06, Chakwal-50 and Inqlab-91. From these results it is evident that cv. Sehar-06 could germinate well on soils contaminated with Cd, however, more studies are required to signify its effects on growth and yield of wheat.

Introduction

Industry proliferates parallel to urbanization but increased industrialization produced industrial effluent which is hazardous for the environment if not treated. Pakistan is an agricultural country but now it faces acute water shortage therefore farmers look for alternative source of irrigation; in this regard industrial effluent is an attractive option being a cheap and richest source of nutrients. Besides of nutrients, industrial effluents also bring various types of pollutants along themselves like organic, inorganic, radio-active elements and micro-organisms which are becoming potential soil contaminants. Among these contaminants heavy metals (Pb, Cd, Hg, As, Ni, Se) are emerging problem all over the world (Bååth, 1989; Zhang *et al*., 2005a; Ahmad & Ashraf, 2011; Ahmad *et al.*, 2011a). Heavy metals from industries and other sources are deposited in the environment and pose threat to plants, animals and human beings (Jarup, 2003; Azevedo & Lea, 2005). In Pakistan, an area of 0.033 m ha receives industrial effluent (Ensink *et al*., 2004) and its repeated application may cause metal problem in soil (Ahmad *et al.*, 2007; Hussain *et al*., 2010). Among these heavy metals Cd is a potential pollutant which can pollute the soil resulting in its accumulation in different parts of plants because it is a very mobile element and can be easily taken up by plants. It is recycled by anthropogenic activities (Kabata-Pendias & Dudka, 1990). In a study, Hussain *et al*., (2010) reported the presence of ample amount of Cd in the industrial effluent of different locations of Faisalabad and observed more than 200 % increase in Cd contents in soil irrigated with industrial effluent compared to canal water. The Cd accumulated in plants is ultimately taken up by animals and humans. Cheng and his Co-workers have also observed in 2006 that plants are the main pathway for entering toxic elements from soil to humans.

Owing to high solubility and toxicity of Cd; it has deleterious effects on plant growth (Pinto *et al.,* 2004). Gouia *et al.*, (2004) explained Cd toxicity on plants owing to its reaction with SH-group leading to enzyme inactivation. It also affects mineral availability in soil and hinder nutrient uptake by plants (Ramon *et al.*, 2003). It is reported that Cd inhibits plumule and radicle growth in rice (Jun-yu *et al*., 2008) and in case of *Triticum aestivum*, *Zea mays*, *Sorghum biocolor* and *Cucumis sativus*, root growth is more sensitive to Cd than shoot growth (An, 2004). For Cd- phyto-toxicity determination several tests like seed germination, root elongation and seedling growth are used (Correa *et al.,* 2006). Although several studies about Cd toxicity to wheat (Shafi *et al*., 2010; Khan *et al*., 2006; Peralta-Videa *et al*., 2002; Jiang *et al*., 2001) have been reported but still it is needed to explore Cd toxicity on different cultivars of wheat in Pakistan. Therefore, study was planned to investigate the effect of various Cd concentrations on germination and seedling growth of different cultivars of wheat (*Triticum aestivum* L.). This study will help to understand sensitivities of various wheat cultivars under Cd stress conditions and help the grower to manage wheat crop in Cd contaminated soils.

Material and Methods

Effect of Cd on seed germination and seedling growth of four wheat cultivars was investigated in growth room under axenic conditions at the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan.

Seed material: Seeds of four wheat cultivars (Sehar-06, Fareed-06, Inqlab-91, Chakwal-50) were provided by the Cereal Section of Ayub Agricultural Research Institute, Faisalabad, Pakistan. The healthy and robust seeds of each cultivar were surface sterilized with sodium hypochlorite (5%) for ten minutes followed by five washings with de-ionized water.

Cadmium treatment: Cadmium chloride ($CdCl_2.H_2O$) salt of high purity (98%) was purchased from Merck chemicals, Germany and used to prepare desired cadmium concentrations. Four levels of Cd 5, 20, 50, 80 mg L^{-1} were used in the experiment along with control (without Cd).

Experimental conditions: Sand was sieved through 2 mm sieve before filled in thermophore plates which has dimensions 4″x4″x2″ length, width and depth, respectively. Each thermophore plate contained 200 g sand and Cd levels were developed in sand by adding 50 mL of each concentration before sowing. Seeds of each cultivar were dipped in distilled water for 24 hours at 28ºC in incubator. Ten imbibed seeds of each cultivar were placed in the sand at uniform depth. These plates were placed in growth room at 25ºC under 14 h photoperiod. The trial was arranged in CRD-factorial and replicated three times.

Determination of plant parameters: Seed germination rate was determined by counting number of germinated seeds after seven days of treatment exposure. Data regarding shoot and root length were measured with the help of a meter rod (Khan *et al*., 2006). Germination index was calculated according to Zhang *et al*., (2005b). Tolerance indices (T.I) were determined with the formula given by Iqbal & Rahmati (1992).

$$\text{Tolerance indices (T.I)} = \frac{\text{Mean root length in metal solution}}{\text{Mean root length in control}} \times 100$$

Statistical analysis: Statistical analysis was performed using the USA STATISTIX software (version 8.1). A two-way analysis of variance (ANOVA) was performed (Steel *et al*., 1997) and Tukey's HSD test was used to compare treatment means at $p<0.05$.

Results

Effect of cadmium on seed germination: Cadmium had drastic effects on seed germination of wheat but its inhibitory effects varied among cultivars. Inhibition of seed germination, germination energy and germination index were observed at 20, 20 and 5 mg L^{-1} Cd, respectively (Table 1). Among different cultivars, seed germination, germination energy and index were improved significantly ($p<0.05$) in case of Sehar-06 whereas these parameters were reduced in Inqlab-91 in Cd polluted soil. Inhibitory effect of Cd was more prominent at higher concentration (80 mg L^{-1} Cd) but it showed a significant ($p<0.05$) reduction at 20 mg L^{-1} Cd and germination index was found more sensitive in case of main effect while interaction effects were significant at 50 mg L^{-1} Cd compared to control. Final germination, germination energy and index showed stimulation at 5 mg L^{-1} Cd, whereas severe inhibition was observed at 20 to 80 mg L^{-1} Cd (Fig. 3).

Table 1. Effect of different levels of Cd on final seed germination (%), germination index, germination energy (%) and mean germination time (days), average of three repeats.

	Seed Germination					Germination Index				
	Seh06	**Far06**	**Inq91**	**Chk50**	***Mean**	**Seh06**	**Far06**	**Inq91**	**Chk50**	***Mean**
Cd-0	93.3 a-b	83.3 a-c	83.3 a-c	93.3 a-b	88.3 A	7.6 c-d	8.3 b-c	7.5 c-d	10.9 a	8.6 A
Cd-5	96.7 a	76.7 a-e	66.7 c-g	83.3 a-c	80.8 A	7.7 c-d	7.6 c-d	7.7 c-d	9.1 b	8.0 B
Cd-20	80.0 a-d	73.3 b-f	60.0 d-h	63.3 c-h	69.2 B	6.9 d-f	7.2 d-e	6.0 f-g	6.3 e-g	6.6 C
Cd-50	66.7 c-g	60.0 d-h	50.0 g-h	56.7 e-h	58.3 C	6.7 d-g	5.7 g	4.5 h	4.6 h	5.4 D
Cd-80	66.7 c-g	53.3 f-h	26.7 i	43.3 h-i	47.5 D	6.1 f-g	4.3 h	1.7 i	2.3 i	3.6 E
**Mean	80.7 A	69.3 B	57.3 C	68 B		7.0 A	6.6 B	5.5 C	6.6 B	
LSD	21.4***					1.05***				
	Germination Energy					**Mean Germination Time**				
Cd-0	76.7 a-c	73.3 b-d	66.7 c-e	90.0 a	76.7 A	5.3 a-b	5.1 a-b	5.1 a-b	4.9 b	5.1 B
Cd-5	76.7 a-c	73.3 b-d	63.3 c-f	83.3 a-b	74.2 A	5.3 a-b	5.1 a-b	4.9 b	5.0 a-b	5.1 B
Cd-20	73.3 b-d	66.7 c-e	50.0 f-j	56.7 e-h	61.7 B	5.3 a-b	5.1 a-b	5.1 a-b	5.1 a-b	5.1 AB
Cd-50	60.0 d-g	53.3 e-i	43.3 h-j	40.0 i-j	49.2 C	5.3 a-b	5.1 a-b	5.1 a-b	5.3 a-b	5.2 AB
Cd-80	50.0 f-j	46.7 g-j	20.0 k	36.7 j	38.3 D	5.4 a-b	5.3 a-b	5.5 a-b	5.7 a	5.4 A
**Mean	67.3 A	62.7 AB	48.7 C	61.3 B		5.2 A	5.1 A	5.2 A	5.2 A	
LSD	16.64***					0.74***				

Different uppercase letters indicate significant differences at $p<0.05$ between main effects while lowercase between interactions
*It indicates main effect of Cd
** It indicates main effect of cultivars
*** It indicates least significance difference of interactions (cadmium × cultivars)

Effect of cadmium on mean germination time: It was recorded that Cd had less drastic effects on mean germination time (MGT) as were noticed in case of seed germination, germination index and seedling growth. It was observed that MGT significantly ($p<0.05$) increased in Chakwal-50 at 80 mg L^{-1} compared to control (Table 1). All cultivars showed non-significant ($p<0.05$) differences regarding MGT but it prolonged by the application of Cd at the rate of 80 mg L^{-1}. Mean germination time of Sehar-06, Fareed-06, Inqlab-91 and Chakwal-50 were increased by 1.5, 4.13, 7.23 and 16.08%, respectively, at 80 mg L^{-1} Cd compared to control. Cadmium stress enhanced MGT of all four cultivars and elaborated its inhibitory rather stimulatory effect on germinating ability of seed. Mean germination time of all the cultivars increased at 5 to 50 mg L^{-1} Cd except Inqlab-91 but at the same time Cd applied at the rate of 80 mg L^{-1} increased MGT more in case of Inqlab-91 than Sehar-06 and Fareed-06.

Effect of cadmium on seedling growth: There was a more pronounced effect of Cd stress on shoot, root and seedling length because its inhibition was significant ($p<0.05$) at 5 mg L^{-1} of Cd application compared to control (Fig. 1). It was also observed that further addition of Cd from 20 to 80 mg L^{-1} remarkably reduced shoot length but maximum inhibition occurred at 80 mg L^{-1} Cd. Shoot length of various wheat cultivars in Cd stress decreases in order; Sehar-06 < Fareed-06 < Chakwal-50 < Inqlab-91. Root length follow similar trend as shoot length against Cd stress. Root length showed a significant reduction at 5 mg L^{-1} Cd which became worse on its additional increments from 20 to 80 mg L^{-1}. Root length of all the cultivars inhibited but Sehar-06 performed better even under Cd stress compared to other cultivars. Cadmium concentrations 5, 20, 50 and 80 mg L^{-1} showed adverse effects on root length by lowering it from 5.10, 4.64, 4.05 and 3.27 cm, respectively. Seedling length varied from 10.7 to 6.4 cm in response to Cd concentrations ranging from 5 to 80 mg L^{-1}.

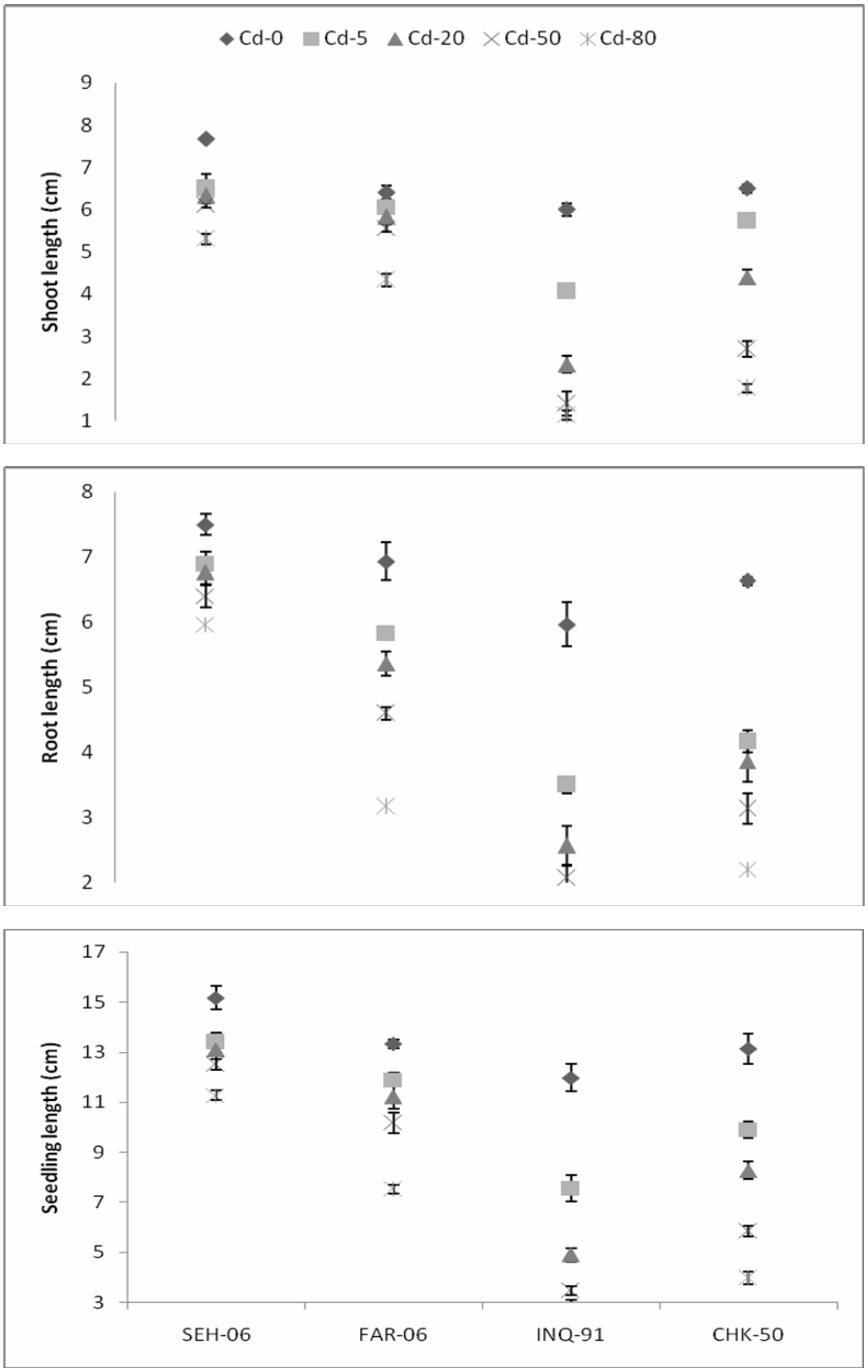

Fig. 1. Effect of Cd on shoot, root and seedling length of different wheat cultivars. SEH-06 = Sehar-2006, FAR-06 = Fareed-2006, INQ-91= Inqlab-1991, CHK-50 = Chakwal-50.

Fig. 3 shows stimulatory and inhibitory effect of Cd on different traits of wheat cultivars. Amongst all cultivars, Inqlab-91 had poor performance and showed maximum decrease in root, shoot and seedling length under Cd stress.

Effect of cadmium on tolerance indices: Wheat cultivars under test showed varying response to Cd stress. Maximum tolerance was observed in Sehar-06 followed by Fareed-06, Chakwal-50 and Inqlab-91 to various concentrations of Cd (Fig. 2). Tolerance indices of Sehar-06 were 92, 90.2, 85.3, 79.6%, whereas Inqlab-91 had 58.7, 43, 34.6, 29.6% to Cd concentrations 5, 20, 50 and 80 mg L^{-1}, respectively.

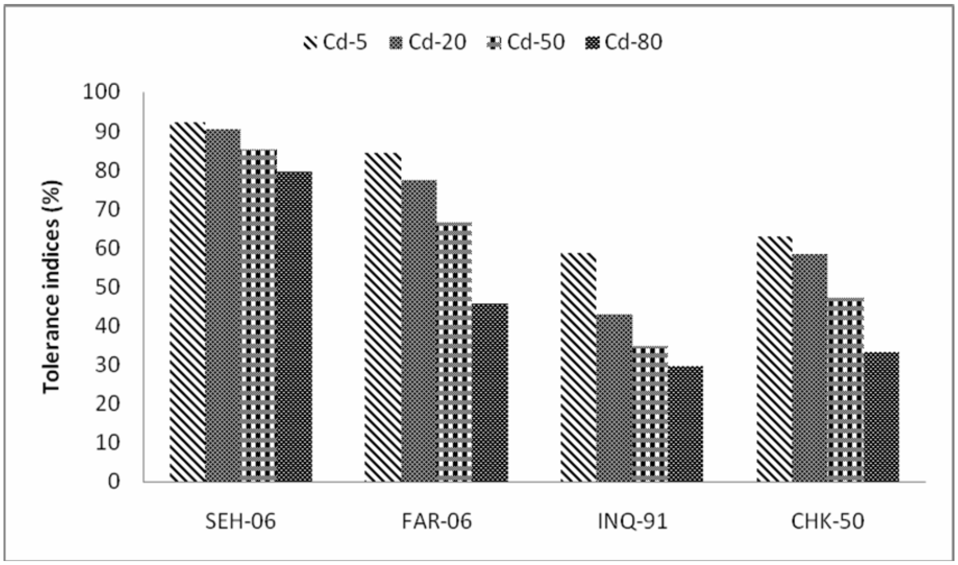

Fig. 2. Tolerance indices of different wheat cultivars to Cd stress.

Discussion

Cadmium is very harmful to cereals and other crops (An, 2004) and it causes many health problems in humans (Anon., 2008). Therefore, it is dire need to investigate the critical toxic level for wheat growth. The significant differences were observed in various wheat cultivars when these were exposed to varying Cd levels, 5, 20, 50 and 80 mg L^{-1} (Fig. 3). In the present study, seed germination and germination energy found less sensitive than germination index. Similar results were reported earlier by (An, 2004). Adverse effects of Cd on germination index showed its phytotoxicity to wheat seedlings. Variation in seed germination ability of different cultivars against Cd stress might have been due to genetic diversity of the cultivars. Such kind of response has been reported by Zhang *et al*., (2005b) while investigating the effect of lead on seed germination and vigour of three rice cultivars that performed differently under Pb stress due to genetic diversity. Seed germination under Cd stress could be decreased owing to accelerated breakdown of reserved food material in seed embryo. These findings are in line with those reported earlier (Raziuddin *et al*., 2011; Aydinalp & Marinova, 2009; Jun-yu *et al*., 2008; Titov *et al*., 1996) in which it was reported that Cd stress decreased seed germination, germination index and vigour index of different crops.

Wheat cultivars differed non-significantly (p<0.05) in MGT while Cd showed significant (p<0.05) difference at 80 mg L^{-1}. Prolonged MGT may be due to inhibitory effect of Cd on germination ability of wheat seed. Our results show that Cd had more drastic effect on seedling growth. Root, shoot and seedling length are most sensitive end-points and considered good indicators for metal toxicity (An, 2004; Correa *et al.,* 2006; Ahmad *et al*., 2008; Jun-yu *et al*., 2008; Ahmad *et al*., 2011b). In this study, a lower concentration of Cd (5 mg L^{-1}) significantly reduced these sensitive parameters. The range of Cd toxicity to root, shoot and seedling length were 20.5, 30.9 and 25.6%, respectively for Sehar-06, while for Inqlab-91 were 70.4, 81.2 and 75.8%, respectively, at 80 mg L^{-1} Cd compared to control. Similar findings have been observed by Bhardwaj *et al*., (2009) while working with green gram. Seedling growth was affected because metal contamination disturbs the plant metabolism due to interactions with enzymes and biochemical reactions take place inside the plant body (Ashraf *et al*., 2011). Different researchers (Shafi *et al*., 2010; Aydinalp & Marinova, 2009; Oncel *et al*., 2000) reported adverse effects of Cd on plant growth. Tolerance indices for Sehar-06, Fareed-06, Inqlab-91 and Chakwal-50 were 80, 46, 30 and 33% respectively, at 80 mg L^{-1} Cd compared to control. When various wheat cultivars were exposed to Cd stress, their response to Cd varied. This may have been due to the reason that some cultivars had inherent ability to withstand against Cd stress, while others could not. The low tolerance indices might have been due to the changes in physiological mechanisms of wheat plant during growth stages (Khan *et al*., 2006).

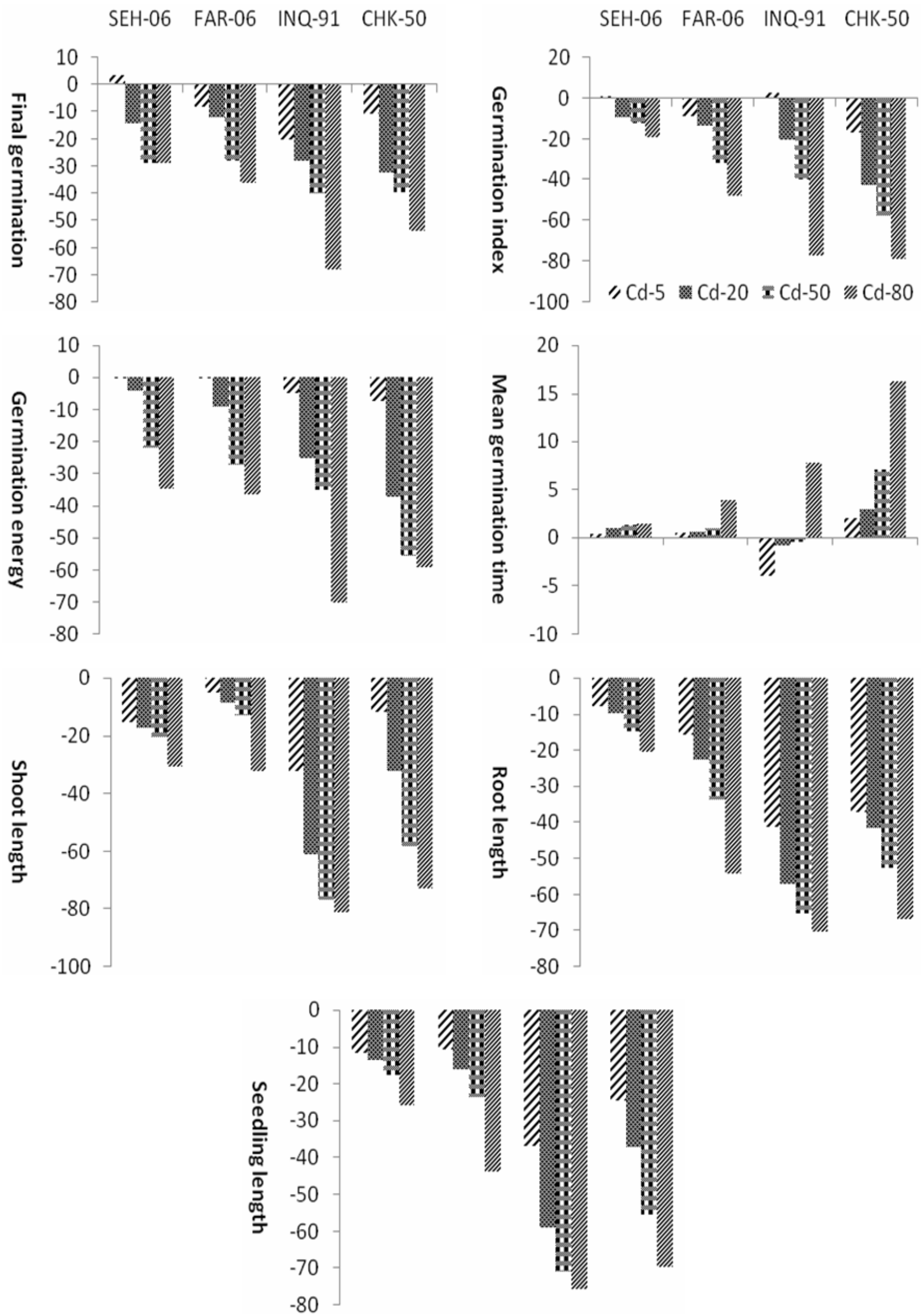

Fig. 3. Percent increase or decrease in various parameters of wheat in Cd stressed conditions compared to non-stressed conditions (control).

Conclusion

In conclusion, germination index, and root, shoot and seedling length were found to be good indicators of Cd toxicity in wheat. Cadmium showed adverse effects on wheat growth indices at low concentration, but maximum inhibition occurred at 80 mg L^{-1}. Tolerance indices for Sehar-06, Fareed-06, Inqlab-91 and Chakwal-50 were 80, 46, 30 and 33%, respectively. Being a most tolerant cultivar, Sehar-06 could be successfully grown on Cd contaminated soils.

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