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Phyto-toxic Effect of Heavy Metal ($CdCl_2$) on Seed Germination, Seedling Growth and Antioxidant Defence Metabolism in Wheat (*Triticum aestivum* L.) Variety HUW-234

Anaytullah Siddique* and Anand Prakash Dubey

Dept. of Plant Physiology, Institute of Agricultural Sciences, RGSC, Banaras Hindu University, Varanasi, U.P. (221 005), India

Corresponding Author

Anaytullah Siddique
e-mail: anaytullahsiddique@gmail.com

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Abstract

The experiments were conducted to assess the phytotoxic effect of heavy metal ($CdCl_2$). The responses of treatment effect comprises the variables germination %, length (cm), fresh weight and dry weight (mg) of shoot and root, root/shoot ratio, seed vigour index (SVI), reducing sugar, non reducing sugar, total sugar (mg g^{-1}), chlorophyll content (mg g^{-1}), cadmium uptake (ppm g^{-1}), proline content (µg g^{-1}), relative water content (RWC%) and superoxide dismutase (SOD, unit×10^2 g^{-1} f. weight min^{-1}). $CdCl_2$ treated sets showed reduction in percent of seed germination, length (cm), fresh weight and dry weight (mg) of shoot and root, root/shoot ratio, seed vigour index (SVI) with increasing the concentrations of $CdCl_2$ in comparison to control set. A reduction in breakdown of reducing sugar to non reducing sugar and total sugar is correlated with decline of amylase activity along with increasing the concentrations of $CdCl_2$ in comparison to control one. The chlorophyll content was found in decreasing trend with increasing the concentrations of $CdCl_2$ in comparison to control. However, the uptake of cadmium was increased with increasing the concentrations up to 100 ppm $CdCl_2$. The proline content and superoxide Dismutase (SOD) was found to be high in higher concentrations of $CdCl_2$ treated set and while the enhancement was started since initial concentration $CdCl_2$ (i.e., 25 ppm). However, the least amount was recorded in control set. On the contrary, RWC% was found to be reduced with increasing the concentrations of $CdCl_2$ up to 100 ppm even though the maximum RWC% was measured in control set.

Keywords: Heavy metal, $CdCl_2$, wheat, SOD, SVI, RWC%, proline

1. Introduction

Wheat (*Triticum aestivum* L.) belongs to family Gramineae and is one of the leading cereals of many countries in respect to growth, production and consumption of the world (Farooq et al., 2011a). India is the world's second largest producer of wheat (Goncalves et al., 2011). Thus, it is important to study the toxicity of heavy metals on this crop. Metal contamination is always increasing by the activities of human which interferes with the environment and makes adverse condition for living organism. Some plant species have capacity to grow in the metal contaminated soil and accumulate high amount of heavy metals (hyper-accumulation) as an eco-physiological adaptation in Metalliferous soil. Among the contaminations heavy metals Pb, Cd, Hg, As, Ni, and Se are emerging problems for all over worlds. (Ahmad and Ashraf, 2011; Ahmad et al., 2011a). Cadmium (Cd) is one of the most common toxic metals present in the environment that induces various toxic effects in plants even at low doses. Contamination of soil in cultivated fields with toxic heavy metals such as cadmium, copper, nickel and zinc has emerged as a new challenge to agriculture (Singh et al., 2007). Initial development of the plant, comprises various processes of seed germination (Dominguez and Cejudo, 2014) that culminates the metabolic transformations that trigger the development of the embryonic axis, consequently, the emergence of radical (Oliveira et al., 2013). On the other hand, heavy metals are abiotic factors that may interfere in the development of seed germination. The presence of cadmium permanently in the soil contaminates water and impedes the absorption of plant nutrients causing morphological, physiological, biochemical and structural changes in plants (Augusto et al., 2014). The immobilization of cadmium outside of the plant or inside of the root may be one of the first barriers of the plant against toxicity to the heavy metal since cadmium levels are highest in roots rather than in leaves, with low transport level from the root to the aerial segment of the plant (Chaves and Souza, 2014). Although, several studies about Cd toxicity to the wheat have been reported but still it is needed to explore Cd toxicity in wheat crop (Shafi et al., 2010; Rizwan et al., 2016).

2. Materials and Methods

A lab experiment was conducted in the Department of Plant

Biotechnology, Institute of Agricultural Sciences, RGSC, BHU, Mirzapur, India. The experiment was repeated thrice during the year 2015–16 to know the influence of cadmium toxicity or stress by imposing different concentrations of $CdCl_2$ (i.e. 25, 50, 75 and 100 ppm) along with three replications and one variety of wheat (i.e. HUW-234). Distilled water was used as a control. Seeds of wheat (*Triticum aestivum* L.) variety HUW-234 were procured from Farm of RGSC, Barkachha, Mirzapur. After sterilization of seeds with 0.1% of mercuric chloride solution for two minutes, the seeds were washed thoroughly with distilled water and 25 seeds were placed in each petridish on filter paper and kept in plant growth chamber (Model No. 193) under controlled temperature, moisture and relative humidity (i.e. 20 °C, 150 Volt and 75%). Different concentrations of $CdCl_2$ solution were used to moist filter paper that maintains the level of stress also. Seeds were considered to be germinated when the radicals became visible. Germination was recorded on every 24 hrs interval up to 240 hrs. The germination percent was calculated on the basis of following formula.

$$\text{Germination percent} = \frac{\text{Total no. of germinated seeds}}{\text{Total no. of seeds in petridish}} \times 100$$

Length, fresh and dry weights of roots and shoots of uniformly growing seedling were measured as an index of growth. The dry weights of plant parts were taken after keeping them at a constant temperature of 70±2 °C for 72 hrs. Lengths of root and shoot were recorded with the help of meter scale. The root/shoot ratio was calculated by using the dry weight of root/dry weight of shoot. The seedling vigour index and RWC% was calculated by using the formula given by (Abdul-Baki and Anderson, 1973) and Weatherly, 1950).

Seedling vigour index=Germination (%)×Total seedling length (cm)

$$\text{RWC (\%)} = \frac{\text{Fresh weight of shoot-Dry weight of shoot}}{\text{Turgid weight of shoot-Dry weight of shoot}} \times 100$$

Spectrophotometric (Model No.: UV-800S) analysis of sugar content, amylase activity, chlorophyll content, proline content and SOD were done by using the following procedure given by (Somogyi, 1952; Bernfield, 1955; Witham, 1971; Bates et al., 1973; Dhindsa et al., 1981 respectively). The AAS (Atomic absorption spectrophotometer, Model No.: AA-6300 Shimadzu) was used to estimate the Cadmium uptake from the roots of wheat seedling according the procedure given by (Intawongse and Dean, 2006). All the experiments were repeated thrice in triplicates. The statistical analyses were performed as per standard. All the observations including physiological as well as biochemical analysis were performed at constant time i.e. 120 hr.

3. Results and Discussion

The data presented in Table 1 showed that as the time of germination increased, the percent of germination was also increased up to last study period i.e. 240 hrs. in each and every treatment. However, percent of germination was started to reduced when the concentration of $CdCl_2$ increased from 25 ppm to 100 ppm during the study period in comparison to control (without treated set). Among the $CdCl_2$ treated set, percent of germination reached at 100% in 25 ppm and 50 ppm up to 168 and 192 hrs. of observation while higher concentrations of $CdCl_2$ (75 ppm and 100 ppm) could not complete up to 240 hrs of observations (i.e. 94.7% and 93.3%) in wheat seed variety HUW-234. Data in Table 2 represent the effect of different concentrations of $CdCl_2$ treatment on shoot and root length. As increasing the concentrations of $CdCl_2$ treatment, the length, fresh weight and dry weight of shoot and root was started to reduce from least concentration to higher concentration of $CdCl_2$ treatment (i.e. 25 to 100 ppm) in comparison to control at the time of observation (i.e. 120 hr). The length and fresh weight of root was affected more severely in comparison to shoot. This can be understood by observing the data given in (Table 2) % decrease over control.

Table 1: Effect of different concentrations of [$CdCl_2$] treatment on seed germination of wheat variety HUW-234

Treatments \ Hours	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs	192 hrs	216 hrs	240 hrs
Control	96.0 [+212.7]	96.0 [+212.7]	100.0 [+4.2]	100.0 [0.0]	100.0 [0.0]	100.0 [0.0]	100.0 [0.0]	100.0 [0.0]	100.0 [0.0]	100.0 [0.0]
25 ppm	18.7 [0.0]	92.0 [+392.0]	96.0 [+4.3]	96.0 [0.0]	97.3 [+1.3]	98.7 [+1.4]	100.0 [+1.3]	100.0 [0.0]	100.0 [0.0]	100.0 [0.0]
50 ppm	12.0 [0.0]	77.3 [+544.2]	85.3 [+10.3]	90.7 [+6.3]	90.7 [0.0]	94.7 [+4.4]	97.3 [+2.6]	100.0 [+2.8]	100.0 [0.0]	100.0 [0.0]
75 ppm	10.7 [0.0]	74.7 [+598.1]	77.3 [+3.5]	81.3 [+5.2]	84.0 [+3.3]	88.0 [+4.8]	90.7 [+3.1]	93.3 [+2.9]	94.7 [+7.2]	94.7 [0.0]
100 ppm	8.0 [0.0]	65.3 [+716.2]	68.0 [+4.1]	74.7 [+9.9]	76.0 [+1.7]	81.3 [+7.0]	86.7 [+6.6]	88.0 [+1.5]	93.3 [+6.0]	93.3 [+7.2]
SEM±	0.63	1.15	0.67	0.77	0.63	0.89	0.52	0.47	0.49	0.49
CD (*p*=0.05)	1.99	3.64	2.10	2.44	1.99	2.82	1.63	1.49	1.49	1.56

Table 2: Effect of different concentrations of [$CdCl_2$] treatment on length and fresh weight of shoot and roots in wheat variety HUW-234

Hours / Treatments	Length of shoot (cm)		Length of root (cm)		Fresh weight of shoot		Fresh weight of root	
	Observation at 120 hrs		Observation at 120 hrs		Observation at 120 hrs		Observation at 120 hrs	
	Length of Shoot (cm.)	% Decrease over control	Length of Root (cm.)	% Decrease over control	Fresh weight of Shoot (mg.)	% Decrease over control	Fresh weight of root	% Decrease over control
Control	5.1	0	5.1	0	40.0	0	36.7	0
25 ppm	3.9	23.53	2.8	45.10	29.3	26.75	19.6	45.56
50 ppm	2.8	45.10	2.2	56.86	28.2	29.50	14.4	61.94
75 ppm	2.6	49.02	1.2	76.47	26.3	34.25	10.3	73.33
100 ppm	2.2	56.86	0.9	82.35	22.1	44.75	5.9	83.61
SEM±	0.19		0.15		0.74		0.74	
CD (p=0.05)	0.61		0.46		2.33		2.34	

The highest reduction in % decrease over control for length and fresh weight of root was recorded at 100 ppm concentration of $CdCl_2$ treatment i.e. (82.35% and 56.86% length of root and shoot) and (83.61% and 44.75% fresh weight of root and shoot). The same trend was not found in case of dry weight of root and shoot (Table 3). The above result is well correlated with the finding of (Guilherme et al., 2015). Seed germination and seedling growth inhibition by heavy metals has also been reported by (Siddiqui et al., 2014; Howladar, 2014). While Cd stress attribute some physiological changes that decrease plant vigour and inhibit plant growth. Inhibition of root elongation is considered to be the first evident effect of metal toxicity in plants (Aiman et al., 2009; He et al., 2010). Since its inhibitory effect on the growth of *T. aestivum* seedlings was higher in the root than in the aerial segment, the dry weight of the seedlings decreased significantly with increase in cadmium concentration. High sensitiveness of roots for cadmium may be due to the fact that the root is the apex region that faces directly heavy metal and be involved in the absorption process (Magna et al., 2013). Cadmium may also affect root metabolism, which shows sensitivity to Cd^{2+} toxicity by a reduction in lateral root size while the main root became brown and rigid (Rascio, and Navari-Izzo, 2011).This is due to reductions in both new cell formation and cell elongation in the apex region of the root (Liu et al., 2004). The toxic effect of cadmium on cell expansion and division might be due to inhibition of growth promoters via blocking enzyme activation. It was depicted from (Table 3) showed that ratio of root/shoot and Seed Vigour Index (SVI) was greatly affected with $CdCl_2$ treatment. Among the $CdCl_2$

Table 3: Effect of different concentrations of [$CdCl_2$] treatment on dry weight of shoot and roots, root/shoot ratio and seedling vigour index in wheat variety HUW-234

Hours / Treatments	Dry weight of shoot (mg)		Dry weight of root (mg)		Root/shoot ratio		Seedling vigour index (SVI)	
	Observation at 120 hrs		Observation at 120 hrs		Observation at 120 hrs		Observation at 120 hrs	
	Dry weight of Shoot (mg.)	% Decrease over control	Dry weight of Root (mg.)	% Decrease over control	Root/ shoot ratio	% Decrease over control	SVI	% Decrease over control
Control	3.5	0	4.9	0	0.71	0	510.8	0
25 ppm	2	42.86	4.0	18.37	0.50	29.58	380.3	25.55
50 ppm	1	71.43	3.3	32.65	0.30	57.75	252.3	50.61
75 ppm	0.8	77.14	2.8	42.86	0.29	59.15	219.5	57.03
100 ppm	0.5	85.71	2.3	53.06	0.22	69.01	167.9	67.13
SEM±	0.04		0.06		0.01		0.79	
CD (p=0.05)	0.13		0.18		0.02		2.48	

treatment, maximum root/shoot ratio and SVI was recorded by the 25 ppm concentration of $CdCl_2$ at 120 hr of observation (i.e. 0.50 and 380.3). The ratio of root/shoot and SVI was also recorded to decrease with increasing the concentration of $CdCl_2$ from onwards of 25 ppm up to 100 ppm. While the minimum ratio of root/shoot and SVI was recorded at 100 ppm (i.e. 0.22 and 167.9). The control set was recorded maximum value of root/shoot ratio and SVI (i.e. 0.71 and 510.8) (Table 3). Severity about the level of toxicity for ratio of root/shoot and SVI was appeared in data % decrease over control in Table 3 showed that maximum reduction was (69.01 and 67.13). The study therefore indicates that the impact of Cadmium is higher on the root growth of wheat seedling compared to shoot growth. This may be due to the fact that roots are the primary plant organs in direct contact with heavy metal pollutants in the growing media. Subin and Steffy (2013) also reported that the higher concentration of cadmium adversely affect the seedling growth, root/shoot ratio and seed vigour index in *Cucurbita maxima.* Heavy metals at toxic level may disturb cell division and RNA replication therefore; suppress the DNA repair process and other vital physiological process also. Recent studies suggested that heavy metals are taken up by the cell through metal transporters (Clemens, 2006). The study regarding the reducing, non reducing sugar and total sugar (Figure 1) showed that among the treated set, reducing sugar was higher in the least concentration of $CdCl_2$ while the highest amount of non reducing sugar and total sugar was recorded in highest concentration of $CdCl_2$ treatment. The trend of amylase activity was also same as reducing sugar. Amylase activity was started to decline onward from the least concentration up to higher concentration of $CdCl_2$ treatment in comparison to control. This positive correlation between the activities of amylase and reducing sugar is helpful for germination of seeds under adverse conditions. Rahman et al. (2008) reported that the during the seed germination, a number of hydrolysing enzymes become active i.e. amylase, proteases and lipase that hydrolyse sugar, protein and lipid in to monomers respectively. The materials reserve in the cotyledons and endosperm are hydrolysed and transferred to the growing region (Rahman et al., 2008). The results are also well correlated with the finding of Bhardwaj et al., (2009) they reported that along with other biochemical parameters, photosynthetic pigments, total soluble sugar, starch content decreased as concentration of heavy metals was increased. (Figure 2) shows that a positive correlation between chlorophyll content and cadmium uptake by roots of wheat seedling. The chlorophyll content was found to be adversely reduced (from 0.362 to 0.263 mg g^{-1}) as the amount of cadmium uptake was increased (from 3.2 to 12.2 ppm g^{-1}) along with the concentrations of cadmium chloride increased (from 25 to 100 ppm) at 120 hr of observation. However, the highest concentration of chlorophyll content was recorded in control set (i.e. 0.385 mg g^{-1}). The result is well correlated with finding of (Liu et al., 2014) they reported that total chlorophyll content declined progressively with increasing concentrations of Cadmium. Ci et al. (2010) reported that as the plant accumulate cadmium, at phytotoxic levels, may cause biochemical and molecular disturbances along with oxidative stress in cells, Although the plants possess antioxidant mechanisms to develop in oxidative stress conditions (Wang et al., 2011), they may not maintain homeostasis of the toxic heavy metal when exposed to high concentrations of the contaminant. Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis (Chakraborty et al., 2015) (Figure 2). Cadmium was reported to affect chlorophyll biosynthesis and inhibit protochlorophyllreductase and aminolevulinic acid (ALA) synthesis (Appenroth et al., 2010). A compound proline in identified that may protect the integrity of cell membrane and protein structure under adverse condition by maintaining osmotic pressure, turgor and driving of water in cell involved in tolerance mechanisms against oxidative stress (Ashraf and Foolad, 2007; Shevyakova et al., 2009). Proline content was increased with increasing

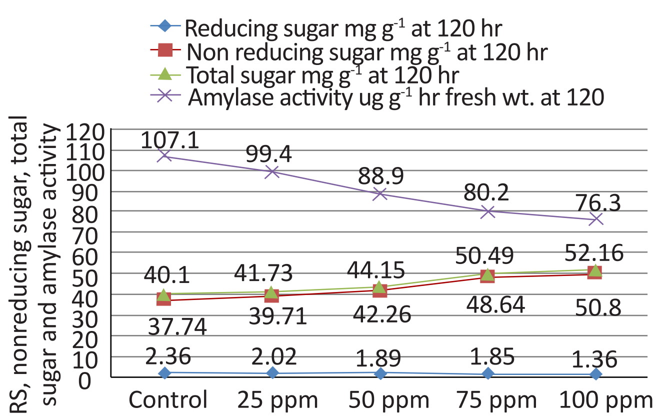

Figure 1: Effect of $CdCl_2$ treatment on RS, Nonreducing total sugar and amylase activity in germinating seed at 120 hrs

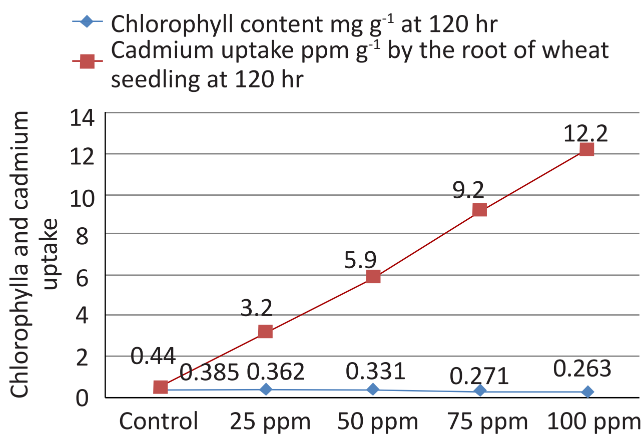

Figure 2: Effect of $CdCl_2$ treatment on chlorophyll content (mg g^{-1}) and cadmium uptake (ppm g^{-1}) by the root of germinating seedling at 120 hrs

the concentration of $CdCl_2$ treatment at 120 hrs of observation while least amount of proline was found in control set. The increment in proline content from its normal level speaks about the presence of stress in the system (Tatar and Gevrek, 2008). Exposure to heavy metals, especially Cadmium is known to dis-balance the plant water relation (De Maria et al., 2013). Proline accumulation in plants under cadmium stress is induced by imposed Cd concentrations followed by decrease of the plant water potential, and the functional significance of this would lie in its contribution to water balance maintenance; proline-mediated alleviation of water deficit stress could partially contribute to Cd tolerance (Ashraf and Foolad, 2007). Proline are able to strengthen metal detoxification capacity of cellular antioxidant enzymes (Emamverdian et al., 2015) and increases the stress tolerance of plants through such mechanisms as osmoregulation (Figure 3). Szabados and Savoure (2009) suggested that proline can act as a signalling molecule to regulate the mitochondrial functions, influence the specific gene expression, which is essential for plant recovery from stress. Relative water content (RWC%) is considering as important parameter to measure the plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance (Lata and Prasad, 2014). Data depicted from (Figure 3) showed that as concentration of cadmium chloride increased from 25 to 100 ppm the RWC% was found to be

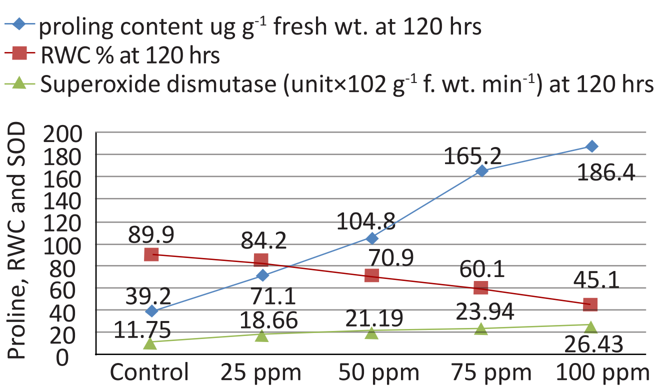

Figure 3: Effect of $CdCl_2$ treatment on Proline content (µg g^{-1} fresh), RWC (%) and SOD (unit×102 g^{-1} f. wt. min^{-1}) in germinating seedlings at 120 hr

decreased (i.e., 84.2% to 45.1%). While the highest amount of RWC% was recorded in Control (i.e. 89.9%). Result is well correlated with the finding of (De Maria et al., 2013) reported that Cd may change water relations by altering water balance through its effects on stomatal conductance, water transport and cell wall elasticity. Therefore, the differences in RWC between plants treated with different concentrations of $CdCl_2$ treated set and control. The results of the present study showed all the tested concentrations of $CdCl_2$ caused oxidative stress in wheat seedlings. The enzyme Superoxide Dismutase (SOD) was also increased with increasing the concentration of $CdCl_2$ treatment at 120 hr of observation while the least amount of SOD was found in control set. It is well known that any type of stress regulate the metabolic activities which enhances the formation of reactive oxygen species (ROS) and during that period the enzyme Superoxide Dismutase (SOD) plays the most defensive role via increasing its activity (Viehweger, 2014) which virtually improves the antioxidant defence mechanism in living system. As $CdCl_2$ concentrations increased, Superoxide Dismutase (SOD) activity enhanced progressively. This result was in agreement with the finding of (Namjooyan et al., 2012). Subin and Steffy (2013) reported that activities of antioxidant enzymes which were found to gradually increase as the concentration of cadmium increased. Mohamed et al. (2012) reported that Cadmium induces oxidative stress by inhibiting ROS detoxifying enzymes.

4. Conclusion

Cadmium toxicity caused an enhancement in the production proline content and activity of antioxidant enzymes (SOD) compared to healthy control seedlings, which may help to survive seedlings under toxic/stress condition via osmoregulatory process and by eliminating the ROS from tissues. However, more work is needed to optimize the knowledge about the exact detoxification mechanism involved in the actions of biological molecules and the level of their interaction in alleviating adverse effects of $CdCl_2$.

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