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Effect of cadmium and chromium stress on the germination, seedling growth and enzyme activities of black gram (*Vigna mungo*)

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Abstract

Heavy metal pollution is a growing concern worldwide, and cadmium (Cd) and chromium (Cr) are two of the most toxic metals affecting plant growth. This study investigated The effect of cadmium and chromium on germination and seedling growth of black gram (*Vigna mungo*) seeds. The germination test was done in petriplates and seeds were exposed to varying concentrations of cadmium and chromium (50, 100, 150, 200, 250, and 300 μ M) and studied for germination potential, germination rate, seedling length, and biomass production. For seedling growth essay *Vigna mungo* seedlings grown in Hoagland's solution were exposed to cadmium and chromium stress with dosages (10, 30, 50 μ M). The seedling growth, biomass, chlorophyll, proline levels and antioxidant enzyme levels were studied. Results showed that both cadmium and chromium significantly reduced germination percentage, seedling length, biomass production, photosynthetic pigments, proline levels and antioxidant enzyme content in a concentration dependent manner. The exposure to higher dosages of cadmium and chromium resulted in more pronounced adverse effects on seedling growth. The cadmium showed more toxic damage than chromium. The study highlights the sensitivity of black gram to cadmium and chromium stress and the potential risks of heavy metal pollution on agricultural productivity.

Keywords: Cadmium, chromium, stress, *Vigna mungo*

Introduction

Environment contamination by heavy metals has become a serious problem worldwide as it has negative effect on the ecosystem and human being. The use of fertilizers, pesticides, mining and industrial activities are the main anthropogenic activities that increase the pollution of environment. Uptake of these toxic pollutants by plants incorporates those in the food chain causing hazardous health effects. According to World health Organization (WHO), presence of heavy metal in the drinking water is one of the major causes for degradation of the ecosystem¹. Heavy metals such as lead, arsenic, cadmium, chromium and mercury are extremely toxic causing acute and chronic poisoning in humans and animals and may even lead to cellular damage. Not only animals and humans, plants are also sensitive to such toxic chemicals as heavy metal toxicity can effect the seed germination and seedling growth, photosynthesis and other physiological events of plants. Heavy metals, particularly cadmium and chromium are persistent environmental pollutants that pose significant threats to plant growth and development. It was noticed that heavy metal like cadmium and chromium concentration levels between 10 to 200 ppm have significant effect in seed germination, viability of the seeds and their further development. In the present experiment the effect of cadmium and chromium toxicity on germination of black gram seeds (*Vigna mungo*), seedling growth, development and the effect on enzymatic parameters were studied.

Materials and Methods

Determination of germination rate and germination potential

The experiment was performed in the Plant Physiology and Biochemistry Laboratory of the Department of Botany, University of Science and Technology Meghalaya. The tested black gram seeds or *Vigna mungo* seeds (LOT NO: PU31) were purchased from NRI Agritech Pvt Ltd. the seeds were surfaced sterilized with 0.1% mercuric chloride (HgCl₂) solution for 3 min and then washed 6-7 times with distilled water.

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The seeds were blotted dried. Cadmium and chromium stress solutions were prepared by diluting 0.1N solutions of $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $\text{K}_2\text{Cr}_2\text{O}_7$ to get concentrations of (50, 100, 150, 200, 250, and 300 μM) for each. 9-cm diameter petridish lined with two layers of filter paper were taken for germination testing experiment. Petriplates with filter paper were saturated with distilled water (Control), cadmium (Cd) and chromium (Cr) solutions of given concentrations. Twenty seeds were placed in each petriplates (n=3 replicates per treatment) and incubated at 24°C darkness. The number of germination seeds was recorded each day for 7 days and germination was defined as radicle emergence $\geq 2\text{mm}$. The germination potential (GP), germination rate (GR), radical and plumule length, fresh and dry weigh of roots and shoots were collected. The germination potential (GP) was calculated using the following equation ^[21].

$$\text{GP}(\%) = (\text{G3}/\text{Gn}) \times 100$$

where GP is the 3-day germination count (G3) divided by the tested count (Gn). And the formula for the germination rate (GR) is given by the following equation,

$$\text{GR}(\%) = (\text{G7}/\text{Gn}) \times 100$$

where GR is the 7-day germination count (G7) divided by Gn.

Seedling growth assessment

Seeds were allowed to germinate in plastic containers (depth 12 cm, width 12 cm, height 12 cm) containing cocopeat. Water was added everyday to keep the cocopeat moist. The 7-day old seedlings were transferred to 1-litre flasks filled with ½ strength Hoagland's nutrient solution, 10 plants in each container. After 3 days, the Hoagland's solution in each pots was replaced with a fresh solution containing (10, 30, 50 μM of Cd and Cr each). Plants growth and treatment continued for 20 days (maintaining $15 \pm 2^\circ\text{C}$, 70-80% relative humidity). After 10 days of Cd and Cr treatment seedlings were observed and the length of root and shoot was recorded. Moisture content was recorded following the method of Wang *et al.*, 2025 ^[21].

Chlorophyll pigment analysis

The whole seedlings from each pot per group were harvested separately 10 days after Cd and Cr treatment and were subjected to chlorophyll and other assays. Chlorophyll analysis was done as per methods described by Arnon (1949) ^[2]. 1g of leaf samples were cut into small pieces and immersed in 10 ml of 80% acetone in a glass tube. The open end of the tubes was wrapped with aluminum foil to avoid the loss of acetone solution. All the tubes were labeled and kept in a big beaker and then kept in the refrigerator at 4°C for three days (72 hrs.) after covering the beaker with carbon paper to avoid light penetration through the beaker. After 72 hrs, the supernatant was decanted, and absorbency for each sample was recorded at 645 nm, 480 nm, and 663 nm in a spectrophotometer taking acetone as a blank.

Estimation of Proline

Proline content was estimated according to Bates *et al.* (1973) method ^[3]. 0.5 g plant tissue was homogenized in 5 ml of 3% sulphosalicylic acid. The homogenate was centrifuged at 1000 rpm. The supernatant was treated with 2

ml of acid ninhydrin and 2 ml of glacial acetic acid for 1 hour in a boiling water bath. After cooling the solution in an ice bath, free proline was extracted in 4 ml of toluene by shaking the tubes vigorously for a min. The Chromophore phase containing toluene was separated from the aqueous phase, and the optical density of the solution was measured at 520 nm. The proline concentration was determined from a standard curve.

Antioxidant enzyme activity

Antioxidant enzymes such as peroxidase (POX), and superoxide dismutase (SOD) activity were determined by spectrophotometer. For the extraction of the enzyme, 0.5g leaf samples were homogenized in ice-cold 0.1 M phosphate buffer (pH: 7.5) containing 0.5 mM EDTA with pre-chilled mortar and pestle. Each homogenate was transferred to centrifuge tubes and centrifuged at 4°C for 15 min at $15,000 \times g$. The supernatant was taken for the enzyme activity assay ^[8, 9]. The nitro blue tetrazolium (NBT) photoreduction method measured superoxide dismutase activity. The reaction mixture (3ml) contains 1.7 ml of 50 mM phosphate buffer saline (PBS) (pH 7.8), 0.3 ml of 130 mM methionine, 0.3 ml of 0.75 mM nitroblue tetrazolium (NBT), 0.3 ml of 1 mM EDTA, 0.3 ml of 20 μM riboflavin and 0.1 ml of enzyme extract. The reaction was conducted for 20 min under 4000 lx fluorescent lamp. One unit of SOD activity was defined as the amount of enzyme needed to inhibit NBT photoreduction by 50%. It is expressed as $\text{U g}^{-1} \text{FW}$. Total peroxidase activity (POX) was determined with the guaiacol method with modifications ^[16, 25]. The reaction mixture (3 ml) was as follows: 2.75 ml of 50 mM potassium phosphate buffer (pH 6.1), 0.1 ml of 0.4% (v/v) H_2O_2 , 0.1 ml of 1% (w/v) guaiacol and 0.05 ml of enzyme extract. One unit of POX activity was defined as the amount of enzyme causing the change in absorbance per minute. It is expressed as $\text{U g}^{-1} \text{FW}$.

Statistical Analysis

Data analysis was done using one-way ANOVA. Intergroup differences were measured using Turkey's post hoc test. All the data were analyzed and expressed as Mean $\pm$ SD (standard deviation). p value ≤ 0.05 was considered to be significant. Microsoft Excel statistical software 365 was employed for this purpose.

Results

Effect on germination

germination potential (GP), germination rate (GR), radical and plumule length, fresh and dry weigh of roots and shoots of cadmium and chromium treated *Vigna mungo* seedlings at different dosages (50, 100, 150, 200, 250, and 300 μM) was shown in Table 1 and Fig 1. The cadmium and chromium treatment markedly reduced the morphological parameters of germinated seedlings in comparison to the control group ($p \leq 0.05$). It was seen that the lower dosages of Cd treatment has shown similar effect on morphological parameters but with the increase of dosages the toxic effects became more prominent. In case of Cr toxicity also similar effects was seen in lower dosages but in plumule length and biomass the differences are not significant but in higher dosages the toxic effects became more prominent. It was also seen that the Cd treatment effects more than Cr with respect to the toxicity. It was evident that Cr at higher concentrations can harm the roots and causing wilting and

plasmolysis in root cells [6]. Moreover it was also proved by other scientists that Cd prevents root elongation [10]. Both heavy metals effect on the shoot length and biomass of germinating seedlings as reduced growth and chlorosis are easily identifiable symptoms caused by toxicity of Cd and

chromium [5, 13, 20]. The Fig 1 shows that the Cd and Cr has reduced the germination percentage and germination potential of *Vigna mungo* seeds in a dose dependent manner. Similar results also proved by other scientists [4, 17, 21].

Table 1: Effect of Cadmium (Cd) and Chromium (Cr) treatment on the morphological traits of germinating *Vigna mungo* seedlings.

Heavy metal treatment	Concentration (μM)	Radicle length (cm)	Plumule length (cm)	Root fresh wt (g)	Shoot fresh wt (g)	Root dry wt (g)	Shoot dry wt (g)
Control	-	13.03±0.45 ^{aA}	16.5±0.5 ^{aA}	0.12±0.02 ^a	0.219±0.02 ^{aA}	0.036±0.003 ^{aA}	0.084±0.004 ^{aA}
Cadmium	50	8.7±0.1 ^b	13.2±0.25 ^b	0.089±0.001 ^b	0.145±0.03 ^b	0.03±0.004 ^b	0.078±0.002 ^b
	100	8.2±0.06 ^b	12.7±0.1 ^c	0.088±0.003 ^b	0.142±0.023 ^b	0.026±0.002 ^c	0.072±0.003 ^c
	150	7.9±0.1 ^c	12.2±0.2 ^c	0.084±0.002 ^b	0.136±0.024 ^c	0.02±0.003 ^d	0.066±0.002 ^d
	200	6.67±0.15 ^d	11.7±0.12 ^d	0.077±0.002 ^c	0.129±0.04 ^d	0.016±0.004 ^e	0.058±0.002 ^e
	250	6.4±0.1 ^e	10.5±0.2 ^e	0.075±0.002 ^c	0.125±0.04 ^d	0.013±0.003 ^e	0.054±0.004 ^f
	300	5.63±0.15 ^f	10.2±0.2 ^e	0.069±0.001 ^d	0.120±0.02 ^e	0.011±0.004 ^f	0.048±0.002 ^g
Chromium	50	9.1±0.46 ^B	14.3±0.3 ^B	0.09±0.002 ^b	0.164±0.032 ^B	0.031±0.002 ^B	0.086±0.003 ^B
	100	8.7±0.2 ^b	13.6±0.1 ^C	0.092±0.003 ^b	0.158±0.03 ^C	0.029±0.002 ^B	0.082±0.004 ^C
	150	8.16±0.15 ^B	12.6±0.2 ^D	0.085±0.001 ^e	0.152±0.024 ^C	0.026±0.003 ^C	0.078±0.001
	200	7.43±0.06 ^C	12.2±0.06 ^D	0.08±0.001 ^f	0.147±0.04 ^D	0.02±0.002 ^D	0.071±0.003 ^D
	250	6.97±0.15 ^D	11.3±0.13	0.077±0.001 ^c	0.143 ^D	0.016±0.002 ^E	0.063±0.004 ^E
	300	6.46±0.11 ^E	10.6±0.15 ^E	0.072±0.002 ^g	0.135±0.032 ^E	0.015±0.004 ^E	0.052±0.005 ^F

Data represented as Mean ± SD (n=3), Superscripts indicates significant difference among treatments using Turkey's post hoc test at $\alpha=0.05$ level ($p\leq 0.05$)

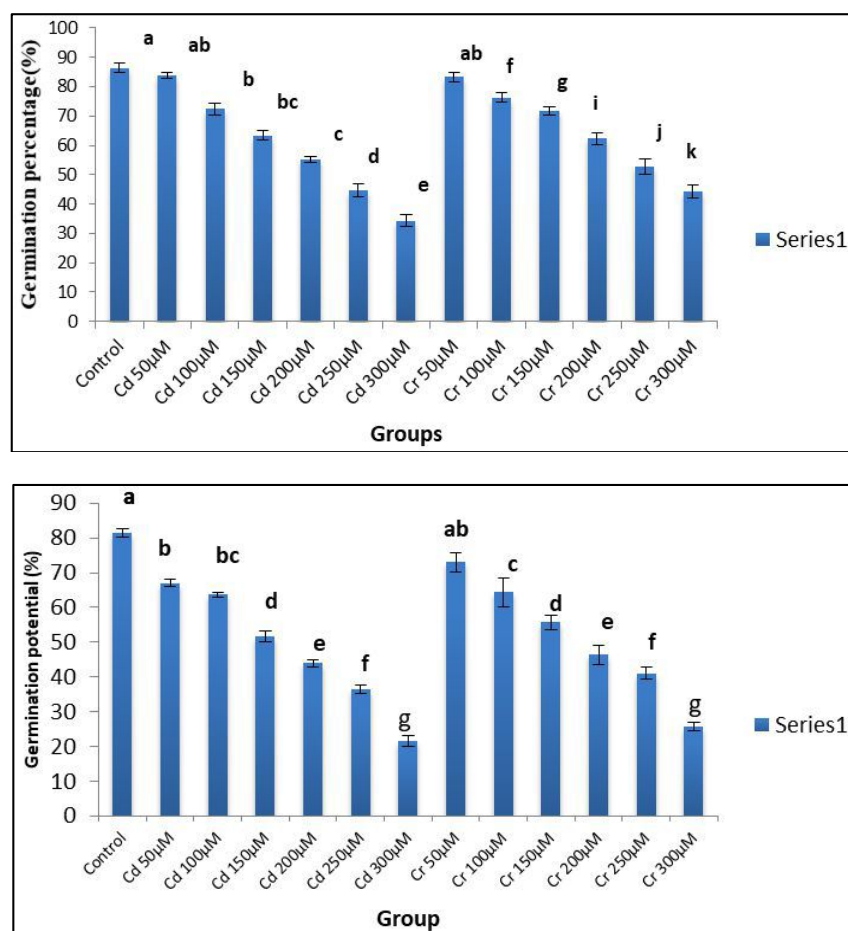


Fig 1: Effect of cadmium (Cd) and Chromium (Cr) treatment on the germination percentage and germination potential of *Vigna mungo* seeds. Data represented as Mean ± SD (n=3), Superscripts indicates significant difference among treatments using Turkey's post hoc test at $\alpha=0.05$ level ($P\leq 0.05$)

Effect on seedling growth

The Cd and Cr stress significantly reduced the root and shoot length of the *Vigna mungo* seedlings ($p\leq 0.05$). The effects of Cd and Cr was found as dose dependent and Cd (50μM) concentration was found more toxic than Cr

treatment for the same dose. The adverse effect on root, shoot growth and moisture content (%) was more pronounced at the higher dosages of Cd and Cr. Cd effects the seedlings growth more adversely than Cr (table 2).

Table 2: Effect of cadmium and chromium exposure on seedling growth:

Heavy metal treatment	Concentration (μM)	Root length (cm)	Shoot length (cm)	Moisture content (%)
Control	-	20.5 $\pm$ 0.62 ^a	15.6 $\pm$ 0.54 ^a	85.4 $\pm$ 0.23 [*]
Cadmium	10	17 $\pm$ 0.12 ^b	12.2 $\pm$ 0.65 ^b	82.4 $\pm$ 0.33 ^{**}
	30	14.6 $\pm$ 0.3 ^c	10.7 $\pm$ 0.35 ^c	80.5 $\pm$ 0.43 ^t
	50	11.5 $\pm$ 0.23 ^d	9.6 $\pm$ 0.4 ^d	78.6 $\pm$ 0.32 ^a
Chromium	10	17.4 $\pm$ 0.5 ^b	12.5 $\pm$ 0.5 ^b	82.7 $\pm$ 0.24 ^{**}
	30	17.8 $\pm$ 0.32 ^b	11.3 $\pm$ 0.8 ^e	81.3 $\pm$ 0.34 ^{***}
	50	15.6 $\pm$ 0.5 ^e	10.8 $\pm$ 0.87	80 $\pm$ 0.5 ^t

Data represented as Mean $\pm$ SD (n=3), Superscripts indicates significant difference among treatments using Turkey's post hoc test at $\alpha=0.05$ level ($p \leq 0.05$)

Impact of Cd and Cr treatment on Chlorophyll, carotenoid pigments and proline levels

Leaf Chlorophyll (a, b), carotenoid and proline levels significantly decreased with Cd and Cr treatment, Table 3

($p \leq 0.05$) when compared with control group. The heavy metals effected the pigment levels and proline level in a dose dependent manner and the higher doses of Cd and Cr has more adverse effect.

Table 3: Effect of cadmium and chromium exposure on Chlorophyll, carotenoid pigments and proline levels

Heavy metal treatment	Concentration (μM)	Chlorophyll a (mg/g.F.W.)	Chlorophyll b (mg/g.F.W.)	Carotenoids (mg/g.F.W.)	Proline (μ Moles/g)
Control	-	0.95 $\pm$ 0.04 ^{aA}	0.48 $\pm$ 0.03 ^{aA}	0.65 $\pm$ 0.03 ^{aA}	2.54 $\pm$ 0.04 ^{aA}
Cadmium	10	0.59 $\pm$ 0.03 ^b	0.25 $\pm$ 0.02 ^b	0.42 $\pm$ 0.02 ^b	2.86 $\pm$ 0.01 ^b
	30	0.42 $\pm$ 0.01 ^c	0.17 $\pm$ 0.03 ^c	0.36 $\pm$ 0.01 ^c	2.96 $\pm$ 0.02 ^c
	50	0.32 $\pm$ 0.02 ^d	0.15 $\pm$ 0.02 ^d	0.34 $\pm$ 0.02 ^c	3.52 $\pm$ 0.01 ^d
Chromium	10	0.64 $\pm$ 0.2 ^B	0.31 $\pm$ 0.02 ^B	0.46 $\pm$ 0.01 ^B	2.73 $\pm$ 0.02 ^B
	30	0.45 $\pm$ 0.01 ^C	0.19 $\pm$ 0.01 ^C	0.397 $\pm$ 0.02 ^C	2.89 $\pm$ 0.02 ^C
	50	0.38 $\pm$ 0.03 ^D	0.17 $\pm$ 0.02 ^D	0.37 $\pm$ 0.02 ^C	3.13 $\pm$ 0.05 ^D

Data represented as Mean $\pm$ SD (n=3), Superscripts indicates significant difference among treatments using Turkey's post hoc test at $\alpha=0.05$ level ($P \leq 0.05$)

Effect of Cd and Cr treatment on antioxidant enzyme levels

The antioxidant enzyme levels were also effected by Cd and

Cr toxicity as shown in Figure 2. The heavy metals treatment increased the peroxidase and SOD levels to many fold and maximum increase can be seen in Cd (50 μM) dose.

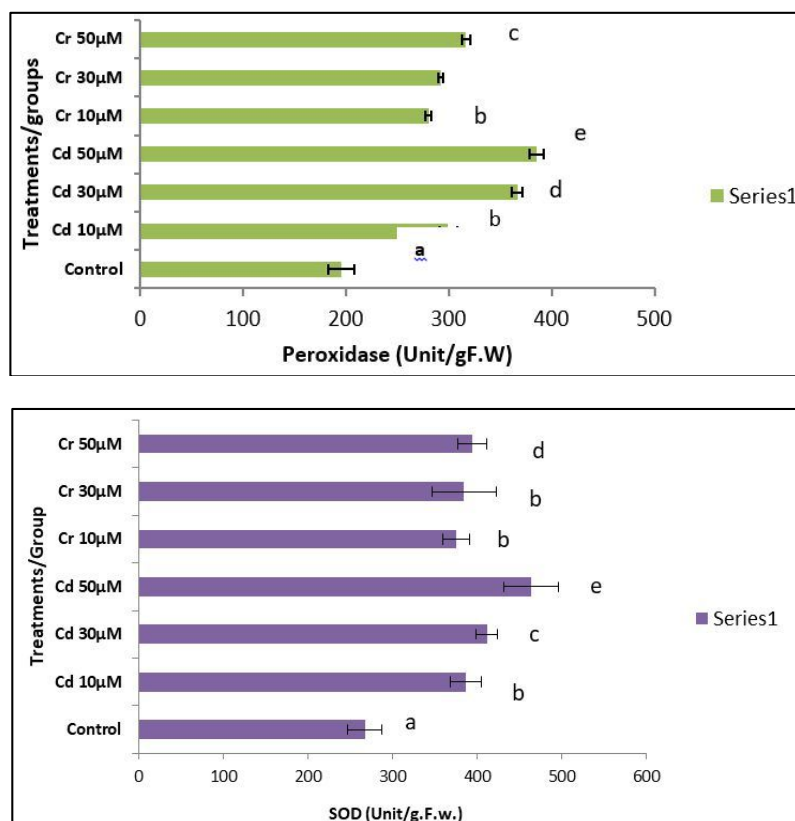


Fig 2: Effect of cadmium (Cd) and Chromium (Cr) treatment on the peroxidase and SOD levels of *Vigna mungo* seedlings. Data represented as Mean $\pm$ SD (n=3), Superscripts indicates significant difference among treatments using Turkey's post hoc test at $\alpha=0.05$ level ($P \leq 0.05$)

Discussion

In the present research higher levels of Cd and Cr concentration effects the germination of seedling growth of *Vigna mungo*. The reduction in the germination rate can be described as Cd decreases the metabolic and physiological activities such as movement of water and reserves towards the embryonic axis^[22]. Cd also leads to the membrane damage and solute leakage as on interaction with calmodulin causing inhibition of the transport of reserve substances in the seed and/or interference with the hormonal status of the seed during germination^[15]. Chromium enters the plants through root systems and transported to other parts by nutrient uptake and effects the aerial parts and the roots systems of the plant. Reduction in the plant height is therefore a significant parameter for Cr induced stress^[20]. Cr effects the nutrient and water uptake of the roots and thereby reduce the root and shoot biomass due to disproportion movement of Ca^{2+} through the plasma membrane to the cytoplasm which alters the normal activity of calcium modulated binding protein (CAM)^[7]. Cd and Cr treatment also decreased the moisture content and dry matter content of *Vigna mungo* seedlings. Cd toxicity decreases the root elongation, root formation, suppression of elongation growth rate of cells and effecting ultracellular structure of meristematic cells. It affects the plant-water relationships, loss of cellular turgor, inhibiting the activity of the cell and its enlargement, mineral nutrition, resulting in less moisture content, poor growth and low biomass^[22]. Other scientists also proved that Cr reduce the biomass and growth of plants^[7]. The Cd and Cr stress also elevated the proline and antioxidant enzyme (peroxidase and SOD) levels of the *Vigna mungo* seedlings. These heavy metals reduced the photosynthetic pigment levels of the plants. Proline is an important parameter to indicate the extent of stress in plants. It acts as an osmoprotectant, helping to stabilize cell structures, scavenge ROS, and maintain cellular homeostasis. The Cd and Cr toxicity increased the proline levels of the plants proved the cellular damage of the plants. Cr decreases the photosynthetic rate and stomatal conductance¹¹. It was found by other researchers that Cr negatively affects the light harvesting capacity of the plants as it affects the performance index of photosystem II and lowers the rate of photosynthesis^[18, 19]. Cadmium is involved in chlorophyll reduction by inhibiting its biosynthetic enzymes (i.e., protochlorophyllide reductase and δ -aminolaevulinic acid dehydratase)^[14]. Cadmium substitutes the central Mg^{2+} molecule from chlorophyll due to its binding nature. The Mg^{2+} atom substitution from chlorophyll reduces its absorption capacity^[24]. Under normal condition, the plant systems maintains the dynamic balance of synthesis and decomposition of reactive oxygen species (ROS) but on exposure to biotic or abiotic stresses the balance is disturbed and the ROS level increases significantly which induces hyperoxia affects and the production of antioxidant enzymes such as peroxidase (POX) and superoxide dismutase (SOD) levels increases in plants, which are used to resist the toxic effect of stress. In the present experiment the elevation of enzyme levels proved the toxic effects of the heavy metals as higher activity of antioxidant enzymes was in proportion to the concentration of Cd and Cr. Higher activity of antioxidant enzymes offers a greater detoxification efficiency which provides better resistance against heavy metal induced oxidative stress^[12].

Conclusion

The heavy metals cadmium (Cd) and chromium (Cr) effected the germination, seedling growth and antioxidant enzyme levels of *Vigna mungo* seedlings. The extent of toxicity of the heavy metals is proportional to the dosages. Cadmium shows more inhibitory and toxic effect in seed germination and seedling growth.

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