Physiological and Biochemical Changes in *Vigna Mungo* (l.) Under Heavy Metal Stress

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Abstract. The study was aimed to investigate the effects of copper and zinc in Vigna mungo (L.) growth and metabolism, grown hydroponically for 7 days. Physiological parameters decrease consequently with increasing copper and zinc concentration in nutrient medium. Copper at 0.2mM and zinc at 1.50mM concentration led to 47.82% and 70.56% reduction in dry weight of seedlings with respect to the control. Moreover, a significant reduction was observed in protein content in treated seedlings. The molecular response of the plants was studied by comparing the protein content with that of control seedlings. SDS-PAGE of crude protein extract showed absence of some protein band in the region between 31.0 KD – 66.2 KD due to copper and zinc stress in comparison to control.

Keywords: Heavy metal stress, seedling biomass, protein content and protein banding pattern

1. INTRODUCTION

Metals like zinc and copper are essential micronutrients required for a wide range of physiological processes in all plant organs for the activities of various metal-dependent enzymes and proteins. But the problem arise when cells are confronted with an excess of these vital ions that lead to cellular damage. The molecular and cellular basis of crop plant interactions with the environment has attracted considerable interest in recent years. Being sessile organisms, plants are constantly exposed during their life cycle to adverse environmental conditions that negatively affect growth, development and productivity. With the ongoing technological advancements in industrialization and urbanization process, release of toxic contaminants like heavy metals in the natural resources has become a serious problem worldwide [7]. The presence of toxic compounds, such as heavy metals, is one important factor that can cause damage to plants by altering major physiological and metabolic processes. Use of industrial effluent and sewage sludge on agricultural land has become a common practice in the world, as a result of which these toxic metals can be transferred and concentrated into plant tissues from the soil. The objective of this study was to investigate the toxic effects of copper and zinc on the growth and metabolism of *Vigna mungo* (L.).

2. MATERIALS AND METHODS

Seeds of *Vigna mungo* (L.) Hepper Cv. T-9 (Black Gram) was obtained from National Seed Corporation Unit, I.A.R.I., New Delhi.

Growth Conditions- Healthy seeds of uniform size were sorted and sterilized with 0.1% $HgCl_2$ solution for 5 minutes and washed with distilled water. Then, seeds were germinated in petriplates containing Whatman filter paper No.-1, moistened with Arnon and Hoagland media (Control). Copper metal was added to the nutrient solution at concentration 0.05, 0.1 and 0.2 mM as $CuSO_4.5H_2O$. Zinc metal was added as 0.25, 0.50, 1.00 and 1.50 mM of $ZnSO_4.7H_2O$.

Sterile conditions were maintained by adding 20µg/ml of streptomycin sulphate in the medium to suppress microbial growth. All experiments were carried out for 7 days at 28 ± 2^0C in dark. At regular interval of time required number of seeds were withdrawn and used for analysis of various parameters. On the indicated days, seedlings were taken and washed thoroughly with distilled water and then used.

Extraction and estimation of Protein was done by Bradford method and SDS-PAGE was performed by the method as described by Laemmli.

3. RESULTS AND DISCUSSION

Effect of copper and zinc metal ions on biomass production in *Vigna mungo* (L.)- The effect of copper and zinc metal ions on biomass produced has been expressed in terms of dry weight of the seedlings. Though seed biomass has been increased with time but there was a progressive decrease in seedling biomass with increasing concentration of copper as well as zinc as shown in Fig.-1, 2 and 3. On 7^{th} day copper reduced the dry weight by 25.08%, 30.10% and 47.82% at the concentration of 0.05mM, 0.1mM and 0.2mM respectively.

The effect of zinc ions was severe on seedling growth. It resulted in 48.16%, 55.51%, 62.21% and 70.56% decrease in dry weight at 0.25mM, 0.50mM, 1.0mM and 1.50mM concentration. Growth is the best indices for evaluating plant response to any type of stress. Reduction in seedling biomass in response to various heavy metals has also been reported by several researchers. The reduction in seedling growth during copper and zinc stress may be due to low water potential, hamper nutrient uptake and secondary stress such as oxidative stress [2,5].

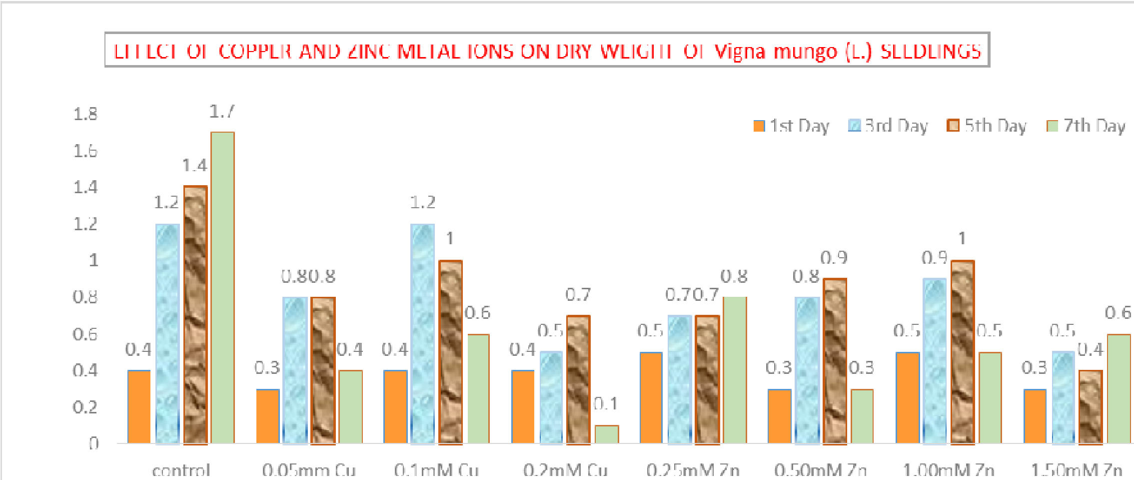
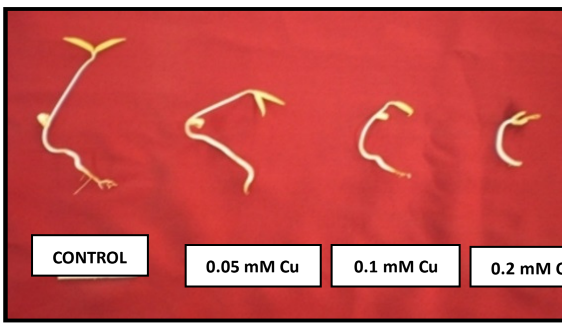


Figure-1) Effect of copper and zinc metal ions on dry weight of Vigna mungo (L.) seedlings



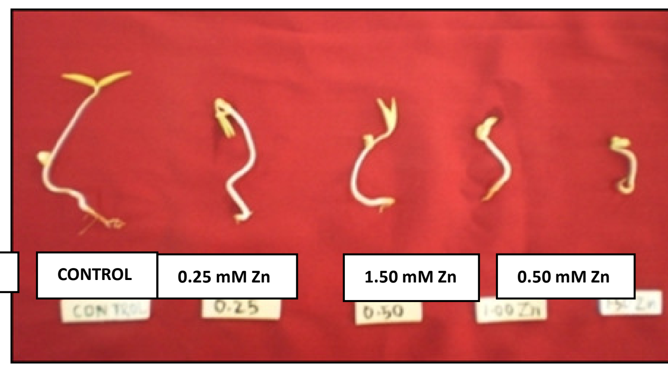


Figure – 1 **Figure – 2**

Figure– 2) Showing the effect of copper metal ions on *Vigna mungo* (L.) seedling growth

Figure –3) Effect of zinc metal ions on *Vigna mungo* (L.) seedling growth

Protein content- The protein content declined in cotyledons and increased in embryonic axis with the onset of germination (1-7th day). The protein content was greater in cotyledons of seedlings exposed to copper and zinc stress in comparison to control seedlings as shown in Table-1. In one day old seedlings, the protein content was found 20.86%, 40.48% and 65.95% greater in the presence of 0.05mM, 0.1mM and 0.2mM concentration of copper ions as compared to control. Whereas, zinc ions had caused 7.02%, 17.85%, 22.83% and 37.47% accumulation of proteins in cotyledons at the concentration of 0.25mM, 0.50mM, 1.00mM and 1.50mM respectively. This trend showed that protein accumulation was more at high metal concentrations. In seven day old seedlings the protein content was 10.70 mg/g FW under control conditions. The protein content was 13.50, 17.30 and 290.33 mg/g FW at 0.05mM, 0.1mM and 0.2mM copper ion concentration. Zinc ion stress also led to accumulation of protein in cotyledons.

Table-1: Effect of zinc and copper metal ions on protein concentration (mg/ g.f.wt.$^{-1}$) in *Vigna mungo* (L.) seedlings

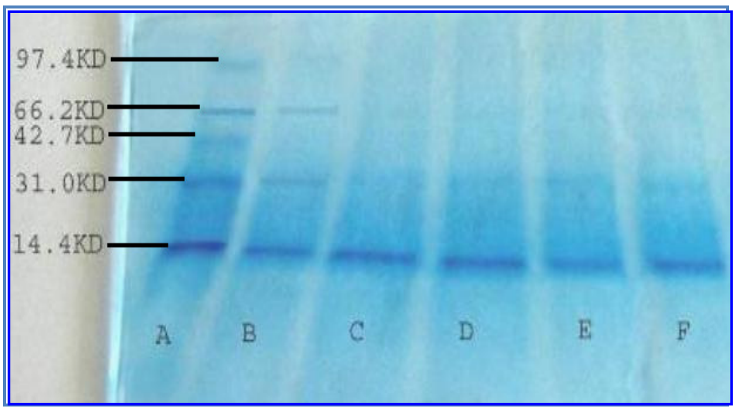
Concentration		In cotyledons				In embryonic axis			
		Days after imbibition				Days after imbibition			
$CuSO_4$.$5H_2O$ [mM]	$ZnSO_4$.$7H_2O$ [mM]	1	3	5	7	1	3	5	7
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.0	0.0	28.29 ± 0.45	25.46 ± 0.65	17.36 ± 1.05	10.70 ± 0.50	13.64 ± 1.73	18.00 ± 0.57	22.96 ± 0.11	29.16 ± 0.57
0.05	0.0	34.93 ± 0.85 [a]	28.63 ± 0.55[a]	21.50 ± 0.60[a]	13.50 ± 0.65[a]	11.33 ± 0.40[b]	14.86 ± 0.72 [a]	15.16 ± 0.36 [a]	20.40 ± 0.36 [a]
0.1	0.0	40.60 ± 0.55 [a]	32.23 ± 0.65[a]	28.50 ± 0.50[a]	17.30 ± 0.65[a]	8.50 ± 0.60 [a]	9.80 ± 0.65 [a]	11.70± 0.50 [a]	15.46 ± 0.55 [a]
0.2	0.0	47.96 ± 0.61 [a]	38.96 ± 0.70[a]	31.46 ± 0.85[a]	20.33 ± 0.85[a]	6.80 ± 0.65 [a]	7.40 ± 0.55 [a]	9.33 ± 0.75 [a]	12.40 ± 0.75 [a]
0.0	0.25	30.93 ± 0.75 [a]	27.00 ± 0.80	20.63 ± 0.55[a]	13.03 ± 0.35[a]	11.66 ± 0.65[c]	15.83 ± 0.70 [a]	17.90 ± 0.70 [a]	22.66 ± 0.55 [a]
0.0	0.50	34.06 ± 0.70 [a]	31.36 ± 0.66[a]	24.86 ± 0.66[a]	17.46 ± 0.35[a]	9.40 ± 0.56 [a]	8.26 ± 0.50 [a]	12.36 ± 0.47 [a]	18.00 ± 0.25 [a]
0.0	1.00	35.50 ± 0.81 [a]	32.46 ± 0.90[a]	28.93 ± 0.61[a]	20.83 ± 0.32[a]	6.13 ± 0.65 [a]	6.83± 0.30 [a]	9.73 ± 0.25 [a]	13.00 ± 0.25 [a]
0.0	1.50	39.73 ± 0.90 [c]	33.13 ± 1.00[a]	30.43 ± 0.55[a]	23.63 ± .035[a]	4.33 ± 0.55 [a]	5.40 ± 0.45 [a]	7.83 ± 0.60 [a]	10.56 ± 0.45 [a]
Source of variation % of total variation Interaction - 04.53 Time - 39.38 Treatment - 55.61						Source of variation % of total variation Interaction - 06.77 Time - 57.08 Treatment - 34.97			

(1) Above values are averages of three replicates ± SD

(2) **a**, **b** and **c** indicates the values that differ significantly from the control at $p\leq 0.05$, $p\leq 0.01$ and $p\leq 0.001$ level respectively.

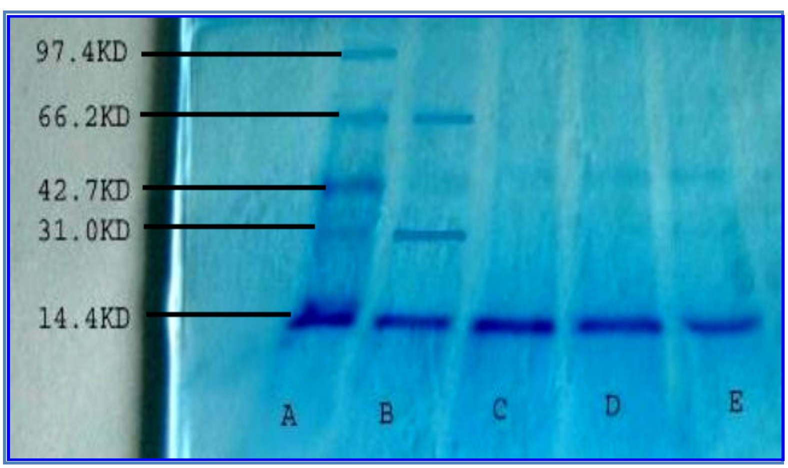
A marked decrease in the protein content was observed in embryonic axis with an increase in zinc and copper concentration. In one day old seedlings, copper treatment at the concentration of 0.05mM, 0.1mm and 0.2mM resulted in 16.93%, 37.68% and 50.14% decrease in protein content. Whereas, zinc ions have caused 14.51%, 31.08%, 55.05% and 68.28% reduction in protein level of embryonic axis at the concentration of 0.25mM, 0.50mM, 1.00mM and 1.50mM respectively. The trend was continued upto seven days. The effect of copper metal ions was found to be more severe than zinc ions.

Using a proteomic approach the effect of zinc and copper ions on the protein profile of *Vigna mungo* (L.) was also investigated as shown in Figure-4 &5. Proteins were extracted from embryonic axis and separated by SDS-PAGE. The electrophoretic patterns of buffer soluble proteins showed that there were three bands having molecular weight-14.4 KD, 31.0 KD and 66.2 KD in control. However in case of copper and zinc treated seedlings pronounced variations were found in the region between 31.0 KD – 66.2 KD. Copper ions at various concentration has caused inhibition of 31.0 KD and 66.2 KD protein bands whereas zinc stress led to the inhibition of 66.2 KD protein band. The decrease in total protein content may be attributed to the-(1) retardation in the assembly of amino acids into proteins which inturn affects the rate of protein synthesis [3]. (2) Enhanced protein degradation process as a result of increased protease activity that has been recorded to get increased under stressed conditions [6].



A - Marker
B - Control
C - 0.25mM Zn
D - 0.50mM Zn
E - 1.00mM Zn
F - 1.50mM Zn

Figure –4: Changes in root protein banding pattern of *Vigna mungo* (L.) grown under zinc stress.



A - Marker
B- Control
C- 0.05 mM Cu
D- 0.1 mM Cu
E- 0.2 mM Cu

Figure – 5: Changes in root protein banding pattern of *Vigna mungo* (L.) grown under copper stress.

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