ISSN: 2394-1391

Original Article

Indian Journal of Biology
Volume 10 Number 2/July - December 2023
DOI: http://dx.doi.org/10.21088/ijb.2394.1391.10223.1

Phytotoxic effects of Cobalt, Copper and Cadmium on Seed Germination and Seedling Vigour in Mungbean [*Vigna radiata* (L) Wilczek]

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How to cite this article:
Nand Lal/Phytotoxic effects of Cobalt, Copper and Cadmium on Seed Germination and Seedling Vigour in Mungbean [*Vigna radiata* (L) Wilczek]/Indian J Biol 2023; 10(2):59–76.

Abstract

Investigations were carried to study the influence of 0 (control), 0.5, 1.0, 2.0, 4.0, 6.0 and 8.0 mM of cadmium (Cd++), cobalt (Co++), and copper (Cu++) ions in an aqueous solution (as chloride salts) on germination, root elongation, hypocotyl growth, mobilization efficiency, tolerance index and vigour index of mungbean (*Vigna radiata* (L.) Wilczek) variety K-851 seeds and seedlings. The extent of inhibition of germination was found to depend on, metal concentration, metal type and incubation period. In comparison to control, there was a complete inhibition of root and hypocotyl growth in germinating mung bean seeds at Co and Cu concentration ≥ 6.0 mM. The order of these metals on inhibition of germination, root elongation and hypocotyl growth was Cu > Cd > Co. The phytotoxic effects of tested metal ions were also evident in case of mobilization efficiency, tolerance index and vigour index. All the tested metal concentration could be efficiently tolerated by *V. radiata* seedlings. Variation in these physiological parameters has role in combating the metal chlorides mediated stress, and could be used for measuring the phytotoxicity and degree of tolerance of the test system.

Keywords: Cadmium; Cobalt; Copper; Germination; Mungbean; Phytotoxic; Seedling Vigour; Vigna radiata.

Introduction

Metals are continuously released into the biosphere by volcanoes, natural weathering of rocks, industrial activities, the combustion of fossil fuels and the release of sewage. Though some metals, e.g., Mn, Cu, Zn, Mo, and Ni are essential or beneficial micronutrients for micro-organisms, plants and animals, at high concentrations all metals have toxic effects and pose environmental threat[1] *(Nedelkoska and Doran, 2000)*. This threat is first experienced by plants, the primary producers, mostly through the contamination of soil and water. Like other stresses, plant species differ markedly in their sensitivity to metals. The highest risk for human health occurs when plants develop tolerance against metals and metals are incorporated into the food chain.

During processing of minerals and waste products, plenty of metals in form of finally

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Received date: 05.06.2023

Accepted date: 31.07.2023

powdered stuff are disposed off in the surrounding areas and enter into biogeochemical cycle. Most of metals occur as insoluble organic compound or are bound to organic matter, clays and hydrous oxides of other divalent and trivalent metals in soil. Because of this, only few heavy metals are in available form and show toxicity. The frequent metals are Zn, Cu, Ni and their phytotoxic effects are evident in definite magnitude in different plant systems[2] *(Lal, 2010)*. Cereals are relatively tolerant and in other plants responses are highly genotype specific.

The most characteristic symptoms of metal toxicity appear as stunting and chlorosis. The chlorosis result due to imbalance in Fe nutrition due to interaction of toxic metals with Fe and is affected by climatic and soil conditions, phosphate status of the soil and plants and Fe status of the soil because all these factors regulate the activity of competing metal ions. The stunting observed is generally due to specific toxicity of the metal to the crop which induces antagonism/synergism for the efforts of essential ions or due to inhibition of root penetration. In the second case, the effect is specific to the root tip cells which ultimately restricts the root length and supply of essential ions via diffusion. The seedling stage is generally more sensitive to metal toxicity than other stages where in the toxic symptoms are modified by metal interaction and external factors. Metal ions also enter into cell cytoplasm where they interfere with several essential life processes. Such processes are not clearly known as they require study of ion effects on isolated organelles. However, cumulatively metals disturb the organic acid pool, amino acid pool, cation exchange across membrane and pH of cellular compartments[2] (*Lal, 2010)*. Certain organelles like dictyosome show deposition of metals at preferential level than other organelles and thus seem to be an important metal excretion processes[3] *(Malone et al., 1974)*.

Seed coat is the main barriers to metals and prevent contamination of embryos until the seed coat is torn apart by the germinating embryonic root. The effects of metals on germination of seeds depend on interspecies differences in seed structure, particularly seed coats, because seed coats have a wide range of anatomic forms that exist in no other plant organ or tissue[4] (*Wierzbicka and Obidziniska, 1998)*. Leguminous plants are very sensitive test system for heavy metal toxicity. There are reports on toxic effects of Cu, Cd, Co and other heavy metals in several legumes including lentil, Soybean[5] (*Bazzaz et al., 1974)*, gram[6] *(Lal and Mishra, 2004)*, pea[7] *(Hernandez et al., 1996, 1997)*, Rajamas[9] *(Van Assche and Clijsters, 1990)*, cowpea[10] *(Lal and Mishra, 2006)* etc.

Considering this, the present investigations were carried with mungbean (*Vigna radiata* (L.) Wilczek) variety K-851 to study the phytotoxic effects of Co, Cu and Cd with special references to seed germination, root elongation, hypocotyl growth, mobilization efficiency, tolerance index and vigour index therein. Seed germination stage constitutes the first and foremost important stage of plant life and lays the foundation for future crop health and yield. So the present investigation at germination would be of pivotal importance for future studies on heavy metal effects on plants in general and mungbean in particular.

Materials and Methods

Mung (*Vigna radiata* (L.) Wilczek) variety seeds, procured from authorized shop of Natural Seed Corporation Limited, New Delhi, were used as experimental material. The chloride salt formulations of cadmium (Cd^{++}), cobalt (Co^{++}), and copper (Cu^{++}) metals used as heavy metal source were from Merck Chemicals and comprised of $CdCl_2.½H_2O$, $CuCl_2.2H_2O$ and $CoCl_2.6H_2O$.

Seeds were sown in a series of 7 glass petridishes each containing 20 ml distilled water (dH_2O) or metal chloride solution of different concentrations [0.0 (control), 0.5, 1.0, 2.0, 4.0, 6.0 & 8.0 mM, respectively] and each treatment was replicated to five. The seeds were imbibed by immersing them into 20 ml metal solutions in plant growth cabinet in dark at 25ºC for 4 hours, the swelling period. Swollen seeds were then sown in petridishes lined with double layer of filter paper wetted with 20 ml metal chloride solutions. The petridishes were kept at 25ºC in the dark in growth cabinets for 96 hours, for determining the germination rate for every 24 hours. A 1-mm radicle emergence from seeds was considered seed germination. The root, shoot and hypocotyl lengths, root and shoot fresh weights, and dry weight of seedling and cotyledons, however, were measured only after 96 hours of incubation. At 96 hours, observations were recorded for germination and seedlings growth. Different parameters (root length, shoot length, hypocotyl length, root fresh weight, shoot fresh weight, dry weight of seedling and cotyledons, Mobilization Efficiency, Tolerance Index and Vigour Index) were recorded at specified stages. The time taken for initiation of germination and % germination data were recorded for each metal with each treatment after every 24 hours.

Five randomly selected seedlings were harvested separately from each treatment at 96 hours' stage, respectively, washed with dH_2O and dried on a blotting sheet. The root and shoot portions were separated and observations were recorded on Root length (cm), Shoot length (cm), Hypocotyl length (cm), Root fresh weight (gm) and Shoot fresh weight (gm). For evaluating the dry weight of cotyledons and seedling, the cotyledons and seedling portions were separated and these portions were placed on aluminium foils. All the aluminum foils containing portions of cotyledons and seedling for each treatment were placed in the hot air oven at 72°C temperature for 48 hours in order to achieve constant dry weight.

Mobilization efficiency[11] *(Mohan et al., 1996)* in germinating seedlings for each treatment was calculated by following formula:

$$\textbf{Mobilization Efficiency} = \frac{\textbf{Dry weight of seedling}}{\textbf{Dry weight of cotyledon}} \times 100$$

Tolerance index[12] *(Mishra and Choudhury, 1998)* of seedlings obtained from each treatment was calculated by following formula:

$$\text{Tolerance Index (TI)} = \frac{\text{Mean length of longest root in a treatment}}{\text{Mean length of largest root in a control}} \times 100$$

Vigour index[13] *(Abdul-Baki and Anderson, 1973)* of resulting seedlings from each treatment was calculated by the following formula:

$$\textbf{Vigour Index (VI)} = \textbf{\% germination} \times \textbf{average hypocotyl length.}$$

Data are based on five replications for each parameter, randomly selected from 50 seeds in each treatment. The data were subjected to appropriate statistical analysis and all values are expressed as mean±SD.

Results and Discussion

The results for the effect of selected metals (Co, Cu, Cd) on germination of mungbean seeds are summarized in table 1. The extent of inhibition of germination was found to depend on metal concentration, metal type and incubation period. When the concentration of metals exceeded certain levels, (e.g., at concentrations between 6.0-8.0 mM for both Co and Cu, 0.5-8.0 mM for Cd), an abnormal germination was resulted (e.g. testa torn by radicle, but no development of root at further incubation periods).

In control, at 24 hours' incubation period, 62% germination was found which increased at 48 hours to 98% and at 72 hours and 96 hours to 100% (maximum). The germination percentage (%) gradually increased from 24 hours to 96 hours in control and same pattern was followed by other treatments as well.

In case of $CoCl_2$, germination rate of mungbean at 24 hours' incubation period was maximum (80%) at 1.0 mM. As $CoCl_2$ concentration increased further, there was decrease in germination. At 2.0 mM, it decreased from 80% to 72% and the trend continued till 4.0 mM and reached to minimum i.e. 42%.

In general, the effective concentrations of metals required for inhibition of seed germination to a certain degree increased with increasing incubation period. The effect of metals investigated for germination inhibition was in the following sequence: Cu > Cd > Co. A study by *Munzuroglu and Geckil*[14] *(2002)*, using six metals (Hg, Cd, Co, Cu, Pb and Zn) showed, effect of metals for germination inhibition was in the following sequence: Hg > Cd > Cu > Pb > Co > Zn in wheat grains (monocot) and Hg > Cu > Cd > Pb > Zn > Co in cucumber seeds (dicot). These findings with mungbean largely confirm to above observations with cucumber seeds.

At 6.0 mM concentration, germination again increased up to 66% and further decreased at 8.0

Table 1: Percent germination of *V. radiata* cv. K-851 in the presence of varying concentrations of Cobalt, Copper and Cadmium.

Metal Chloride (mM)		**% germination**			
		24 hours	**48 hours**	**72 hours**	**96 hours**
0.0 (control)		62	98	100	100
CoCl2	0.5	76	96	98	98
	1.0	80	100	100	100
	2.0	72	96	98	98
	4.0	42	98	100	100
	6.0	66	92	98	100
	8.0	56	92	94	100

table cont...

CuCl2	0.5	68	98	98	98
	1.0	64	94	98	100
	2.0	70	96	100	100
	4.0	66	88	94	100
	6.0	12	22	22	48
	8.0	02	02	06	26
CdCl2	0.5	64	98	98	98
	1.0	46	74	96	98
	2.0	42	42	46	98
	4.0	30	40	40	74
	6.0	28	44	48	62
	8.0	32	44	48	60

mM (56%). At 48 hours, maximum germination was found at 1.0 mM (100%) and minimum (92%) at 6.0 mM and 8.0 mM (Table 1). At 72 hours, maximum germination was found with control, 1.0 and 4.0 mM $CuCl_2$ (100%) followed by 0.5, 2.0 and 6.0 mM (98%) and minimum at 8.0 mM (94%). At 96 hours, germination at 0.0, 1.0, 4.0, 6.0, 8.0 mM reached 100% except at 0.5 mM and 2.0 mM (98%). It is known that Co is an essential element only for some plants, and it affords both beneficial effects and toxicity to plants[15] (Liu *et al.*, 2000). That is why this metal caused a lesser inhibition of germination in mungbean seed.

In case of $CuCl_2$, at 24 hours there was alternate increase and decrease in germination % till 4.0 mM. Germination was maximum (70%) at 2.0 mM, at minimum (2%) at 8.0 mM. Germination at 48 hours was maximum at control and 0.5 mM (98%). Germination at 72 hours was maximum at 2.0 mM (100%). Germination at 96 hours was maximum and equal at 1.0, 2.0, 4.0 mM (100%). Minimum germination was found at 8.0 mM for all the incubation periods. Except for mungbean seeds at 24 hours' incubation with 0.5-4.0 mM, all concentrations of this metal reduced the germination substantially. Copper has been rated as one of the most unfavourable metal for plant growth by other workers as well[16] (Quarity et al., 1997). In case of $CdCl_2$, at 24 hours, maximum germination was found at 0.5 mM (64%). Upon further increase in $CdCl_2$ concentration, germination decreased gradually till 6.0 mM and reached to minimum 28% (Table 1). At 8.0 mM concentration germination rate increased slightly (by 4%) in comparison to 6.0 mM. The germination at 48 hours was maximum at control and 0.5 mM (98%) and minimum at 4.0 mM (40%). It decreased from 0.5 mM to 4.0 mM and then slightly increased at 6.0 mM (44%). At 72 hours, maximum germination was 100% (at control). As the concentration increased, the germination decreased till 4.0 mM and increased further at 6.0 and 8.0 mM up to 48%. At 96 hours, germination was maximum at control in comparison to other $CdCl_2$ concentrations. The 0.5, 1.0 & 2.0 mM concentrations showed 98% germination. Upon further increase in $CdCl_2$ concentration, germination reduced reaching minimum at 8.0 mM (60%).

In general, $CoCl_2$ treatment did not inhibit the germination response. In case of $CuCl_2$, germination response remained unaffected till 4.0 mM after which there was a drastic reduction. In case of $CdCl_2$ there was a general inhibition/ reduction in germination response of mungbean seeds. Overall it appears from the results that Cu was most inhibitory metal for germination at higher concentration (6.0 and 8.0 mM). Cd was less inhibitory for germination (magnitude wise) than Cu but at all the levels. Co metal caused least/ no inhibition of germination in mung bean and showed promotory effect on seed germination at lower incubation periods. The order of these metals on inhibition of germination was Cu > Cd > Co.

Effect of different $CoCl_2$, $CuCl_2$ and $CdCl_2$ concentrations on length of root, shoot and hypocotyls and biomass (fresh weight) of root and shoot portion of V. radiata seedlings are shown in table 2. In control the root and shoot length were 1.92±0.13 cm and 2.92±0.17 cm, respectively. At 0.5 mM CoCl2, the root length increased to 2.10±0.17 cm but there was no change in FW (Table 2). At 1.0 mM concentration, root length and biomass increased to 2.14±0.76 cm and 0.01±0.00 gm. Upon further increase in $CoCl_2$, the root length and fresh weight decreased to 0.633±0.047 cm and 0.002±0.001 gm, respectively (Table 2). At 6.0 mM and 8.0 mM $CoCl_2$, no measurable roots and hypocotyls were available. Shoot length was promoted at 0.5 mM and 1.0 mM $CoCl_2$ but further levels of $CoCl_2$ caused a significant reduction in shoot length.

Root length, root FW, shoot length, shoot FW and hypocotyl length were maximum at 1.0 mM Cocl$_2$ concentration. Shoot length and biomass were maximum at 1.0 mM and minimum at 8.0 mM. Hypocotyl length was maximum (1.58±0.23 cm), at 1.0 mM and it was reached to 0.66±0.29 cm (minimum) at 4.0 mM. It appears that root is more sensitive than shoot to Co metal.

Table 2: Growth response of root, shoot and hypocotyl of germinating seeds of *V. radiata* cv. K-851 in the presence of varying concentrations of Cobalt, Copper and Cadmium.

Metal Chloride(mM)		**Root**		**Shoot**		**Hypocotyl length (cm)**
		Length (cm)	**FW (gm)**	**Length (cm)**	**FW (gm)**	
CoCl2	0.0	1.92 ± 0.13	0.008 ± 0.002	2.92 ± 0.17	0.150 ± 0.013	1.00 ± 0.24
	0.5	2.10 ± 0.17	0.008 ± 0.003	3.18 ± 0.37	0.141 ± 0.022	1.08 ± 0.38
	1.0	2.14 ± 0.76	0.011 ± 0.003	3.72 ± 0.77	0.172 ± 0.020	1.58 ± 0.23
	2.0	1.52 ± 0.18	0.006 ± 0.003	2.72 ± 0.34	0.160 ± 0.029	1.20 ± 0.19
	4.0	0.63 ± 0.05	0.002 ± 0.001	1.22 ± 0.22	0.131 ± 0.028	0.66 ± 0.29
	6.0	*	*	0.76 ± 0.19	0.132 ± 0.022	*
	8.0	*	*	0.54 ± 0.05	0.122 ± 0.023	*
CuCl2	0.5	1.56 ± 0.23	0.012 ± 0.004	3.20 ± 0.65	0.154 ± 0.026	1.54 ± 0.65
	1.0	1.92 ± 0.29	0.011 ± 0.002	3.76 ± 1.07	0.154 ± 0.010	1.84 ± 0.95
	2.0	1.72 ± 0.23	0.008 ± 0.004	2.86 ± 0.41	0.146 ± 0.010	1.14 ± 0.48
	4.0	0.40 ± 0.10	0.0021	0.90 ± 0.43	0.126 ± 0.019	1.00 ± 0.20
	6.0	*	*	0.32 ± 0.07	0.093 ± 0.025	*
	8.0	*	*	0.26 ± 0.05	0.092 ± 0.018	*
CdCl2	0.5	*	*	0.48 ± 0.07	0.146 ± 0.017	*
	1.0	*	*	0.36 ± 0.05	0.121 ± 0.012	*
	2.0	*	*	0.32 ± 0.07	0.125 ± 0.009	*
	4.0	*	*	0.28 ± 0.07	0.102 ± 0.016	*
	6.0	*	*	0.36 ± 0.05	0.133 ± 0.036	*
	8.0	*	*	0.36± 0.08	0.129 ± 0.013	*

* No measurable root and hypocotyl available.

1 S.d. not shown due to lack of replicated data.

In case of 0.5 mM CuCl$_2$, root length was decreased to 1.56±0.23 cm but there was increase in FW. At 1.0 mM concentration, root length increased but FW slightly decreased from 0.012±0.004 gm to 0.011±0.002 gm (Table 2). Upon further increase in CuCl$_2$, the root length, root FW, shoot length, shoot FW and hypocotyl length showed a decreasing trend. Shoot length and hypocotyl length were maximum at 1.0 mM CuCl$_2$. Root length was maximum at control and at 1.0 mM CuCl$_2$. Root fresh weight was maximum at 0.5 mM but shoot fresh weight maximum and equal at 0.5 mM and 1.0 mM. At 6.0 and 8.0 mM, no measurable root and hypocotyl lengths were available but shoot length was evident reaching to minimum at 8.0 mM (0.26±0.05) and same was true for shoot biomass (Table 2).

In case of CdCl$_2$ ranging 0.5-8.0 mM, no measurable roots and hypocotyls were available, however, shoot growth was clearly evident. Between 0.5-4.0 mM CdCl$_2$, shoot length showed decreasing trend, but shoot length increased further to 0.36±0.08 at 8.0 mM. The maximum shoot length and shoot FW were at control and these reached to minimum at 4.0 mM (Table 2). Comparative metal toxicity studies in wheat revealed that Cd had toxic effect on plants at concentrations as low as 50 mgL-1, whereas lead did not show the same effect at 500 mgL^{-1} concentrations[17] *(Oncel et al., 2000).*

In comparison to control, there was a complete inhibition of root and hypocotyl growth in germinating mungbean seeds at Co and Cu concentration ≥ 6.0 mM. The 0.5 and 1.0 mM concentration of Co had promotory effect on root, shoot and hypocotyl growth (Table 2). In case of Cu, maximum root length was available at control and 1.0 mM. Shoot length and hypocotyl length were maximum at 1.0 mM. At 0.5 mM maximum root fresh weight was found but shoot fresh weight was maximum at 0.5 and 1.0 mM. However, other concentrations of Cu showed inhibitory effect on growth of seedlings/seedling parts. In case of Cd, no root length & hypocotyl length was available because of complete inhibition of root and hypocotyl growth, however, shoot growth was well marked which was maximum at control and decreased upon increase in $CdCl_2$ concentration. The order of these metals on inhibition of root elongation, shoot elongation and hypocotyl growth was Cu > Cd > Co.

It appeared that above certain concentrations of these metals, the germination of seeds was not normal in the sense that roots and hypocotyls were not properly developed, although they appeared to process a distinct radicle. This might be an indicator of sensitivity of plant's early development stage to these metals. The order of these metals on inhibition of root elongation and hypocotyl growth was Cu > Cd > Co for mungbean. The order of these metals on inhibition of root elongation and hypocotyl growth, reported in the present study show resemblance with results obtained earlier[14] by *Munzuroglu and Geckil (2002)*. Root which is the site of entry of metal ions was much affected in comparison to the shoot which receives metal ions quite late due to metal translocation.

Table 3 summarizes the data on variation in mobilization efficiency (ME), tolerance index (TI) and vigour index (VI) of seedlings of *V. radiata* obtained in the presence of varying concentrations of Cobalt, copper and Cadmium respectively. At control, ME was 120.79±2.83. In case of Co, ME was maximum at 0.5 mM i.e. 121.326±7.41 marginally higher than control. ME showed a decreasing trend due to increase in $CoCl_2$ concentration. In case of Cu, ME was maximum at 1.0 mM but at all other levels it was less than control, and decreased to 101.92± 0.76 at 8.0 mM. In case of Cd, maximum ME was evident at control and minimum ME was recorded at 2.0 mM. Highest level of tested metals showed ME value in close proximity. More growth of seedlings was recorded with Co which shows correspondence with mobilization efficiency. Effect of metals investigated on ME in *V. Radiata* was in the following sequence: Co > Cd > Cu.

At control, TI was 100. In case of Co, TI was maximum at 1.0 mM (111.46). At concentrations between 0.0 to 1.0 mM, TI was increased indicating that the metal was beneficial to root growth rather than being inhibitory/toxic. However, further increase in $CoCl_2$ decreased TI to 32.97. TI could not be determined due to lack of measurable root length

Table 3: Variation in Mobilization efficiency, Tolerance index and Vigour index of seedlings of *V. radiata* cv. K-851 in the presence of varying concentrations of Cobalt, Copper and Cadmium.

(mM)	Mobilization Efficiency (ME)	Tolerance Index (TI)	Vigour Index (VI) (at 96 hours)
0.0 (control)	120.79 ± 2.83	100.00	100.00
CoCl2 0.5	121.33 ± 7.41	109.38	105.84
1.0	113.56 ± 3.51	111.46	158.00
2.0	110.75 ± 5.58	79.17	117.60
4.0	104.98 ± 3.10	32.97	66.000
6.0	102.96 ± 0.94	*	*
8.0	102.12 ± 0.89	*	*
CuCl2 0.5	120.73 ± 5.21	81.25	150.92
1.0	124.12 ± 8.56	100.00	184.00
2.0	117.74 ± 6.45	89.58	114.60
4.0	110.93 ± 5.67	20.83	100.00
6.0	101.60 ± 1.00	*	*
8.0	101.92 ± 0.76	*	*
CdCl2 0.5	102.44 ± 1.54	*	*
1.0	102.05 ± 0.96	*	*
2.0	101.43 ± 1.04	*	*
4.0	101.96 ± 1.55	*	*
6.0	102.28 ± 0.81	*	*
8.0	102.04 ± 0.81	*	*

*Value of TI could not be determined due to lack of measurable root length and value of VI could not be determined because no differentiated/ well marked hypocotyl present.

at 6.0 and 8.0 mM. In case of Cu, TI was maximum at control and 1.0 mM i.e. 100. TI decreased at 0.5 mM to 81.25. Upon increase in $CuCl_2$ beyond, 1.0 mM, TI decreased to 20.83. At 6.0 and 8.0 mM, value of TI could not be determined due to lack of measurable root length.

In comparison to control, Co treated seeds/ seedlings were more tolerant to metal toxicity as evident from TI data. Cd treated seeds were more susceptible to metal toxicity as evident from failure of radicle emergence and root growth. The order of these metals for TI was Co > Cu > Cd.

At control, VI was 100. In case of Co, VI was maximum at 1.0 mM (158.00) and reached to minimum at 4.0 mM. The 0-2.0 mM range of Co metal appears to have a positive effect on growth and health of seedlings (Table 3). At 6.0 and 8.0 mM, value of VI could not be determined as no differentiated/well marked hypocotyls were present.

In case of Cu, VI was maximum at 1.0 mM (184.00) and minimum at 4.0 mM (100.00, equal to control). Like Co, this metal also caused a complete inhibition of VI due to absence of differentiated/ well marked hypocotyl length at 6.0 and 8.0 mM. In case of Cd, value of VI could not be determined at either of $CdCl_2$ concentration as no differentiated/ well marked hypocotyl present. In comparison to control, Co and Cu treated seeds showed same pattern for VI (Table 3). The order of these metals for VI was Cu > Co > Cd.

The selection range of metal chloride concentrations is based on metal concentrations commonly used by other workers, and encountered in environment to their several fold higher concentrations. The type of application (incubation of seeds on filter paper soaked with metal) is a common method for testing metal toxicity effects in seed germination. This method reduces the effect of counter anions associated with other metals/ metal cations normally present in soil. In soil, the effect of a metal is determined synergistically or antagonistically due to interaction with other metal cations and their associated anions. In the present study, the effect of counter anion (Cl^-) associated with these metals has not been further considered as all the metals were used as chloride salt. The effect of metals on development and reproduction of plants can first be quantified by determining the germination characteristics of seeds. The metal treatment used here is simple (no need for highly skilled personnel, plant seeds are simply allowed to germinate in metal and control solutions), rapid (requires a few days), and easy to perform (does not need physical methods using expensive apparatus). All three metals used in this study caused a decrease/ delay in germination of mungbean seeds, though to different extents. In general, the germination inhibition increased with increasing concentrations of metals and each metal at certain concentration level lowered germinability. These three metals also caused complete inhibition of hypocotyls and roots in germinated seeds at certain concentrations. Inhibition of root elongation is considered to be the first evident effect of metal toxicity in plants. Cell division at the root tip and cell elongation in the extension zone are two different mechanisms in root growth, both of which are affected by the presence of metals *(Arduini et al., 1994)* and cause inhibition of root elongation.[18]

Conclusions

The study concludes that 96 hours' exposure to different metal chlorides shows differential phytotoxic effects on different physiological parameters. The shoot length and biomass are little affected due to metal chlorides treatments in comparison to root length and biomass.The phytotoxic effects of metal chlorides are also evident in case of mobilization efficiency, tolerance index and vigour index. All the tested metal chlorides concentration could be efficiently tolerated by *V. radiata* seedlings. Variation in these physiological parameters has an important role in combating the metal chlorides mediated stress, and could be used for measuring the phytotoxicity and degree of tolerance of the test system.

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