Indian Journal of Plant Sciences ISSN: 2319–3824(Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jps.htm
2017 Vol.6 (3) July-September, pp.50-62/Monalisa et al.
Research Article

EFFECTS OF HEAVY METALS ON SEED GERMINATION AND SEEDLING GROWTH OF *PHASEOLOUS ACONITIFOLIUS* JACQ. CV. RMO 40.

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ABSTRACT
Heavy metals namely copper, lead, cadmium, zinc and nickle toxicity were assessed at varying concentration (10 -1000 ppm) on seed germination and seedling growth (root/ shoot length and fresh weight) of *Phaseolous aconitifolius* Jacq.cv. RMO 40. It was observed that seed germination and seedling growth was almost not affected at lower concentrations of Cu, Zn, Pb and Ni but affected at higher concentrations of all the heavy metals. Germination percentage was 100% in all the concentrations of Zn. Toxicity followed the pattern Cd > Pb > Cu > Ni > Zn. It was observed that cadmium was the most toxic on seed germination, shoot length, root length and fresh weight in comparison to other heavy metals.

Key words: *Heavy metals, seed germination, seedling growth, fresh weight*

INTRODUCTION

Heavy metals are a member of an ill-defined subset of elements that exhibit metallic properties. These include the transition metals, some metalloids, lanthanides, and actinides. One source defines heavy metal as one of the common transition metals, such as copper, lead, and zinc. These metals are a cause of environmental pollution from sources such as leaded petrol, industrial effluents, and leaching of metal ions from the soil into lakes and rivers by acid rain (Duffus, 2002). These metals are also considered as trace elements because of their presence in trace concentrations in various environmental matrices (Kabata- Pendia, 2001). Their bioavailability is influenced by physical factors such as temperature, phase association and adsorption. It is also affected by chemical factors that influence speciation at thermodynamic equilibrium, complexation kinetics, lipid solubility and octanol and water partition coefficients (Hamelink *et al.*, 1994). Biological factors such as species characteristics, trophic interactions, and biochemical/physiological adaptation, also play an important role (Verkleji, 1993).

In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by these metals. Also, human exposure has risen dramatically as a result of an exponential increase of their use in several industrial, agricultural, domestic and technological applications (Bradl, 2002). Reported sources of heavy metals in the environment include geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources (He *et al.*, 2005). Environmental pollution is very prominent in point source areas such as mining, foundries and smelters, and other metal-based industrial operations (Fergusson, 1990). In biological systems, heavy metals have been reported to affect cellular organelles and components such as cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair (Wang and Shi, 2001). Metal ions have been found to interact with cell components such as DNA and nuclear proteins, causing DNA damage and conformational changes that may lead to cell cycle modulation and apoptosis.(Chang *et al.*, 1996, Wang and Shi, 2001 and Beyersmann and Hartwig, 2008).

Different workers have studied the effect of heavy metals on different pulse crops of our state but our native crop *Phaseolus aconitifolius* has not been studied. *Phaseolus aconitifolius* is the most drought resistant crop of the kharif (summer crop) pulse and is largely grown in arid and semi arid regions of the country. Economically, it is very important because mature dried seeds contain approximately 23.0% protein and 59.0% carbohydrates. In India its young and tender pods are eaten as a vegetable while the ripe seeds are eaten cooked either whole or split (dhal). In south-western USA, it is grown for pasture, fodder and green manure. The green vine provides a palatable and nutritious pasture and hay for dairy cattle. The crop also constitutes an excellent means of controlling soil erosion. Therefore, increasing world population and concomitant increasing incidences of drought in crop production areas and decreasing amount of available irrigation water necessitates development of crop *Phaseolus aconitifolius* that could produce food and feed by consuming lesser amount of water.

MATERIALS AND METHODS

Certified seeds of RMO 40 of *Phaseolus aconitifolius* Jacq. were obtained from Research Station Beechwal, Rajasthan Agriculture University, Bikaner. Seeds were stored in glass stoppered bottles. After a preliminary selection for uniformity criteria (size and colour of seeds), the seeds were surface sterilized with 0.1% $HgCl_2$ for two minutes (Misra, 1968), then washed with distilled water three times and then soaked for two hours in respective solutions of different concentrations (10, 50, 100, 200, 500 and 1000 ppm) of copper sulphate, cadmium sulphate, lead sulphate, nickel sulphate and zinc sulphate. Seeds soaked in distilled water for two hours constituted the control. After the above treatments, seeds were removed and allowed to germinate in petri plates on filter paper soaked in each of the above metallic solution. Three replicates each of 10 seeds were kept for each concentration of every heavy metal. The filter paper was moistened with metallic solutions. The experiments were carried out for ten days under laboratory conditions of temperature (25±2°C) and diffuse light.

On the day of termination of experiment, (10^{th} day) germinated seeds were counted, seedling growth parameters viz., shoot length, root length, fresh weight of seedling, were recorded.

(i) Seed Germination Percentage

The daily progresses in germination of seeds were recorded for a period of 10 days (I.S.T.A., 1976). The criterion used for seed germination was taken as emergence of stub through the seed coat.

(ii) Root and Shoot Length

The root and shoot length of 10 days old seedlings were measured in centimeters using a meter scale (mean of 3 replicates). Five seedlings were randomly selected from petri plates, growth of these seedlings, both under control and treatments were determined after 10 days by measuring the length of root and plumule (longer leaf).

(iii) Fresh Weight

For fresh weight determination, five seedlings from each treatment were weighed on an electric balance. Average value was calculated in gram/seedling.

RESULTS AND DISCUSSION

Result

The data regarding the effect of heavy metals on seed germination percentage, shoot length, root length and fresh weight of ten days old seedling of *Phaseolus aconitifolius* cv. RMO-40 are recorded in Tables 1 to 4, Fig.1 to 4 and Plates 1-2.

(i) Effect on Seed Germination

All the treatments of heavy metals were found to affect seed germination . However, effects were related to nature of substance and its concentration used. There is gradual decrease in germination percentage with increase in concentration of heavy metals except in zinc. Among germination percentage studies most toxic heavy metals were found to be cadmium and lead(Table-1,Fig.1,Plate5.1,5.2). A significant reduction occurs in seed germination at 100 to 1000 ppm of Cd, Pb and Ni, 500 ppm to 1000 ppm of Cu in comparison to control. Surprisingly, germination percentage was 100% in all the concentrations of Zn.

Maximum depression in seed germination percentage was observed at 1000 ppm concentration of all the metals except Zn. It decreased up to 85% in Cd and Pb, 90% in Ni and 95% in Cu , whereas it was found to be 100% in the treatment of Zn and control. The statistical analysis of data showed highly significant difference between the control and treatments and among various concentrations but among chemicals no such differences were observed.

(ii) Effect on Shoot Length

Shoot length of seedlings were highly affected by treatment of heavy metals (Table-2, Fig. 2 and Plate-5.1 and 5.2). Reduction in shoot length was observed particularly at higher concentration of all the heavy metals studied. It was observed that after seven to eight days of treatment shoot length of seedling decreased with increasing concentration of heavy metals. There was gradual increase in shoot length of seedling in all the heavy metal at 10 ppm, 50 ppm and 100 ppm except Cd and Cu where it was at 100 ppm. Above 100 ppm concentration gradual decrease in shoot length was observed with the increase in concentrations in all the heavy metals. Shoot length was 2.15 cm in Cd, 4.2 cm in Cu, 5.0 cm in Ni, 5.09 cm in Pb and 5.6 cm in Zn at 1000 ppm concentration which was very less as compared to control. Reduction in shoot length starts in 10 ppm of Cd, 100 ppm of Cu, 500 ppm of Pb and Ni and 1000 ppm of Zn.

Most toxic effect among heavy metals was shown by Cd, where shoot length was drastically reduced. Data were statistically analyzed and found significant among control *versus* treatment and highly significant among concentrations and chemicals except among replicates where they were insignificant.

(iii) Effect on Root Length

Root length show decline at different concentrations of all the five heavy metals(Table 3,Fig.3). Root length slightly increased at 10 ppm, 50 ppm and 100 ppm of concentration of Cu, Zn, and Pb, after which it gradually decreased with increasing concentration of heavy metals. Root length showed decline at almost all the concentrations of Cd except 10 ppm. Ni also showed slight decrease in root length at very lower concentration. All heavy metals particularly at higher concentration showed adverse effect on root length. Cadmium proved lethal at higher concentration i.e. 1000 ppm. Root length was 4.6 cm in Cu, 4.8 in Ni, 4.9 cm in lead and 5.0 cm in Zn at 1000 ppm of heavy metals and in control it was 5.6 cm (Table 3; Fig. 3 and Plate 5.1to 5.2). Data were statistically analysed and found not significant among control *versus* treatment and highly significant among concentrations and chemicals (Table 3).

(iv) Effect on Fresh Weight of Seedling

The heavy metals showed inhibitory effect on fresh weight of the seedling at higher concentrations. Fresh weight of seedling increases at 10 ppm and 50 ppm concentrations of Cu, Zn, Pb and Ni except Cd. At the lowest concentration (10 ppm) of heavy metals, fresh weight of seedling was 0.6 g/seedling (Cu), 0.95 g/seedling (Zn), 0.6 g/seedling (Pb), 0.79 g/seedling (Ni) and 0.46 g/seedling (Cd) (Table.4,Fig.4,plate5.1-5.2).

It declined to 0.39 g/seedling (Cu), 0.24 g/seedling (Pb) and 0.47 g/seedling (Ni) under 1000 ppm concentration of different heavy metals when compared with the control condition where it was 0.52 g/seedling. Only in Zn fresh weight did not decline at higher concentration. Statistically, highly significant results were obtained for control *versus* treatments, among concentrations and among chemicals themselves.

Discussion

In this study, we have examined the toxicity of selected heavy metals, namely, Cu, Cd, Zn, Pb and Ni on seed germination and seedling growth in the *Phaseolus aconitifolius cv.*RMO 40. There are several reports regarding the effects of responsive heavy metals. Both inhibitory, as well as, promotory effects were observed by several workers in various studies carried out in different crop plants. In the present study seeds were treated with different concentrations of cadmium, lead, nickel, zinc and copper in the form of their salt viz., Cadmium sulphate, lead sulphate, nickel sulphate, copper sulphate and zinc sulphate, individually under laboratory conditions. The present investigation was made to widen the knowledge of the effect of different concentrations of five metals, namely Cd, Pb, Ni, Zn and Cu on the cultivar of *P. aconitifolius* .

(I) Role of Heavy Metals on Seed Germination

In the present study, an attempt has been made to assess the effect of different concentrations of all the five heavy metals on seed germination. The results indicate that all the heavy metals showed decline in percentage germination with increase in concentration particularly at higher concentration of Cd, Pb and Cu. Cd was found to be the most toxic heavy metal among all the five metals studied. The cultivars

RMO 40 was found to be the most resistant to the heavy metal toxicity. Toxicity followed the pattern Cd > Pb > Cu > Ni > Zn.

Both inhibitory, as well as, promotory effects were observed by Bae *et al*., 2016 ,they studied effect of heavy metals on common ragweed and roadside ground cover legume and concluded similar kind of result, that All metals inhibited *Trifolium arvenses* germination, but the effect was least on *Ambrosia artemisiifolia*. Low levels of Pb and Ni promoted germination initiation of *A. artemisiifolia*. Germination of *Lotus corniculatus* was not affected by Zn, Pb, and Ni, but inhibited by Cu and Cd. Germination of *Coronilla varia* was decreased by Ni, Cu, and Cd and delayed by Zn and Pb. Metal additions hindered seedling growth of all test species, and the inhibitory effect on the belowground growth was greater than on the aboveground growth. Seedling mortality was lowest in *A. artemisiifolia* but highest in *T. arvense* when exposed to the metal treatments.The successful establishment of *A. artemisiifolia* along roadside edges can be associated with its greater tolerance of heavy metals. The findings also revealed that *L. corniculatus* is a potential candidate for supplement ground cover in metal-contaminated roadside edges.

Effect of Heavy metals (Cd, Pb, Cu)on seed germination of *Arachis hypogaea* was studied by Abrahim *et al*.,2013 Every part of cadmium, Lead, and copper showed significantly decreased on seed germination of *Arachis hypogaea*. as compare to control. Increasing concentration of Cd at 75 and 100 mg/L extremely ($p < 0.05$) affected the groundnut seed germination compared with control. Lead treatment at 75 and 100 mg/L significantly ($p < 0.05$) reduced seed germination of groundnut as compared with control. Copper treatment at 100 mg/L also condensed seed germination of *Arachis hypogeae* compared with control. Cadmium produced more significant effect on seed germination of *Arachis hypogeae*.L than lead and copper.

Heavy metals released during industrial activity form a major portion of the contaminants that accumulate in water bodies. Chromium is particularly, being highly soluble and bioavailable, exert toxic effect on biological system arising from possibility of free diffusion across the cell membrane and its strong oxidative potential (Shankar *et al*., 2005) whereas Zn induced damage to living system mainly include impairment in functioning of several essential enzymes involved in the metabolism (Rout and Das, 2003). Several workers (Li *et al*., 2007 and Chen *et al*., 2002) have observed that toxic metals are capable of causing suppression of certain enzymes which are essential for seed germination and in mobilization of reserve food. Gupta *et al*. (2001) observed copper and nickel adversely affected the seed germination at higher concentration (200 ppm and 500 ppm).

Seed germination inhibition was reported with lead and copper in tomato seedling (Jaja and Odoemena, 2004) in *Spinacea oleracea* and *Lycopersicon esculentum* (Hameed *et al*., 2001) in *Triticum aestivum* and *Cucumis sativus* (Munzuroglu and Geckil, 2002). Bhansali (2007) reported inhibition of seed germination in *Cyamopsis tetragonoloba* by heavy metals treatment. Saravanan *et al*. (2001) investigated percentage germination in *Glycine max* (L.) Merr. at increasing concentration of copper sulphate and showed that 100% germination occured at control and progressive decline in germination percentage with increasing copper sulphate concentration. These reports were in favour of inhibitory effects of heavy metals on seed germination of *Phaseolus aconitifolius*. Plant showed 100% germination even at higher concentration of Zn. Toxic heavy metals are mostly absorbed and get accumulated in various plant parts as free metals. However, excessive accumulation of these heavy metals can be toxic to most plants. In the present work data analysis showed that *Phaseolus aconitifolius* cv. RMO 40 was the most resistant to heavy metal toxicity.

(II) Role of Heavy Metals on Shoot Length and Root Length

Observation of data concluded that root length and shoot length responded differently to heavy metals in the RMO 40 cultivar of *Phaseolus* Heavy metal Cd was proved to be most toxic to cultivar. The response of Zn was very good at lower concentrations (10 ppm to 200 ppm cultivar of *P. aconitifolius*. Lower concentrations of Pb enhance shoot length but shoot length decline at higher concentrations. In the same manner Ni showed positive response on shoot length and at higher concentrations shoot length suppressed. Tao *etal*.2015studied effect of Cd on seed germination,coleoptiles growth,root elongation of six pulse. Their results indicated that root and coleoptile growth of six pulse plants were more sensitive than seed germination for measurement of toxic Cd2+ pollution Kopittke *et al*. (2007) studied toxic effect of Ni on growth of cow pea (*Vigna unguiculata* (L.) Walp. cv. Caloona) and observed 10% reduction in shoot and root growth. The primary site of Ni^{+2} toxicity was the shoot, with the younger leaves displaying an interveinal chlorosis. Soni (2003) reported that higher concentration of Zn inhibited both radicle and hypocotyl length in *Tecomella undulata* and *Tecoma stans*. Gupta (2002) reported Zn and Cu at 100, 200 and 500 ppm concentrations decreased seed germination in four cultivars of *Rhaphanus sativus*. Bhansali (2007) observed that Zn and Cu at 500 and 1000 ppm concentrations decreased seed germination in all the cultivars of *Cyamopsis tetragonoloba* studied except in RGC 936. In the present study it was observed that root length decreased in RMO 40 at 500 ppm and 1000 ppm concentrations of all heavy metal studied except in case of Zn, where root growth was not affected even at 500 ppm. In RMO 40, root length was not affected in Zn and Pb at 500 ppm concentration.

In the present study data revealed that root length is more affected by different heavy metals in comparison to shoot . Root length increased at lower concentration in Cu in cv. RMO 40.

It is very interesting to consider some possible mechanism of heavy metal effect and the nature of tolerance to them. It has been observed that the metals including Cu, Ni, Pb and Zn have practically identical speed inside seeds. Thus, the germination delay can be caused by a negative action of these metals on the metabolism of germ axial organs. The delay in seed germination and slow formation of seedling in the presence of heavy metals are probably connected with the processes of cell stretching brake and fission (Kumar *et al*., 1995).

Data of shoot length growth revealed that in cultivar of *Phaseolus aconitifolius* cv. RMO 40 root length was not affected at higher concentration at (500 ppm) in Zn and Pb .

(III) Role of Heavy Metal on Fresh Weight of Seedling

It was observed that fresh weight of treated seedling increased at lower concentrations and decreased at higher concentrations as compared to control. Cadmium, significantly reduce the fresh weight of seedling at all the concentrations whereas Zn promote fresh weight of seedling in cvs. RMO 40 at 10 ppm to 1000 ppm.

Seedling fresh weight decreased significantly with increasing concentrations of different heavy metals. This might be associated with the reason that higher concentrations of heavy metals decrease the number of cells and suppress the elongation of developing root and shoot which in turn reduce the fresh weight of seedling or may be the result of inhibition of wall thickening of cells of root and shoot due to heavy metal toxicity. Previously, several workers have observed the reduction in fresh weight of seedling due to heavy metals at higher concentration. Bashmakov *et al.* (2005) concluded that in contrast to nickel ions, low concentration of copper (10 pm) stimulated an increase of root fresh weight and high concentration of copper checked fresh and dry weight and Zn in higher, as well as, lower concentrations (1000 ppm to 10 pm) resulted in unexpected increase of root fresh weight.

In the present work data analysis showed that *Phaseolus aconitifolius* cv. RMO 40 was the most resistant to heavy metal toxicity The mechanism of plant tolerance to heavy metals is manifested as a spatial isolation of toxic substance from the metabolically relevant centers, followed by the rendering of metals within the plant. As a result, heavy metals are stored predominantly in roots. Consequently, root cell metabolism disorders occur due to increased heavy metal content and therefore, it is evidenced by a more intense depression of root growth. However, the existing heavy metal transport barriers protect shoot from high concentration of phytotoxins. Therefore, a less intensive shoot growth inhibition is observed.

Therefore, we can conclude that growth characteristic of RMO-40 cultivar might be due to variation in their morphological and physiological status and due to inhibition of enzyme activity or replacing of macronutrient from their place or of genetic and environmental variation leading to the cellular alteration and morphological modification in plants.

Among the toxic metals Cd has received considerable attention because it is easily absorbed by plants, thereby interfering with its functioning. In the present work, among all the heavy metals studied Cd was found to be the most toxic to fresh weight of the seedling.

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Table 1: Showing the Effect of Heavy Metals on Seed Germination (%) in *Phaseolus aconitifolius* cv. RMO 40. (Values are mean of three replicates each)

Name of chemical	Control (%)	Concentration (ppm)					
		10	50	100	200	500	1000
Copper sulphate	100	100	98.3	100	100	96.6	95
Cadmium sulphate	100	100	100	95	95	96.6	85
Zinc sulphate	100	100	100	100	100	100	100
Lead sulphate	100	100	100	96.6	93.3	86.6	85
Nickel sulphate	100	100	100	98.3	91.6	90	90

Source	D.F.	S.S.	M.S.S.	'F' ratio
Replication	2	1.73	0.86	2.38 NS
Control vs Treatment	1	6.51	6.51	18.08**
Among Concentrations	6	36.75	6.12	17.0**
Among chemicals	4	10.58	2.64	7.33**
Interaction	2	27.30	13.65	37.91**
Error	89	32.25	0.36	

NS = Not significant, ** = Highly significant

Table 2: Showing the Effect of Heavy Metals on Shoot Length (cm) of Seedling in *Phaseolus aconitifolius* cv. RMO 40. (Values are mean of three replicates each)

Name of chemical	Control (cm)	Concentration (ppm)					
		10	50	100	200	500	1000
Copper sulphate	6.59	6.66	6.9	6.40	5.42	4.94	4.2
Cadmium sulphate	6.59	4.90	4.96	4.54	2.93	2.82	2.15
Zinc sulphate	6.59	8.27	8.33	7.97	7.2	6.76	5.6
Lead sulphate	6.59	7.2	7.59	7.04	6.82	6.20	5.09
Nickel sulphate	6.59	7.21	7.28	7.42	6.71	5.7	5.00

Source	D.F.	S.S.	M.S.S.	'F' ratio
Replication	2	0.20	0.10	0.32 NS
Control vs Treatment	1	4.14	4.14	6.74*
Among Concentrations	6	78.55	13.09	42.22**
Among chemicals	4	117.19	29.30	94.51**
Interaction	2	24.11	12.06	38.90**
Error	89	27.73	0.31	

*NS = Not significant, * = Significant, ** = Highly significant*

Table 3: Showing the Effect of Heavy Metals on Root Length (cm) of Seedling in *Phaseolus aconitifolius* cv. RMO 40. (Values are mean of three replicates each)

Name of chemical	Control (cm)	Concentration (ppm)					
		10	50	100	200	500	1000
Copper sulphate	5.60	5.7	5.8	6.0	5.4	4.8	4.6
Cadmium sulphate	5.60	5.8	5.6	4.4	4.0	3.0	-
Zinc sulphate	5.60	6.0	6.6	6.8	6.3	5.9	5.0
Lead sulphate	5.60	5.7	5.9	7.1	6.8	6.5	4.9
Nickel sulphate	5.60	5.2	5.5	6.2	6.6	5.3	4.8

Source	D.F.	S.S.	M.S.S.	'F' ratio
Replication	2	4.63	2.32	11.60**
Control vs Treatment	1	0.57	0.57	2.85 ^{NS}
Among Concentrations	6	51.96	8.66	43.30**
Among chemicals	4	55.59	13.902	69.50**
Interaction	2	54.10	27.05	135.25***
Error	89	17.54	0.20	

*NS = Not significant, ** = Highly significant ,*** = Very highly significant*

Table 4: Showing the Effect of Heavy Metals on Fresh Weight (g) of Seedling in *Phaseolus aconitifolius* cv. RMO 40. (Values are mean of three replicates each)

Name of chemical	Control (g)	Concentration (ppm)					
		10	50	100	200	500	1000
Copper sulphate	0.52	0.6	0.61	0.55	0.53	0.44	0.39
Cadmium sulphate	0.52	0.46	0.52	0.46	0.37	0.33	-
Zinc sulphate	0.52	0.95	0.97	0.85	0.79	0.72	0.68
Lead sulphate	0.52	0.6	0.71	1.14	0.82	0.59	0.24
Nickel sulphate	0.52	0.79	0.80	0.73	0.71	0.59	0.47

Source	D.F.	S.S.	M.S.S.	'F' ratio
Replication	2	0.0124	0.0062	0.92 ^{NS}
Control vs Treatment	1	0.13	0.1260	18.80**
Among Concentrations	6	1.64	0.2734	40.80**
Among chemicals	4	1.91	0.4768	71.16**
Interaction	2	1.19	0.5940	88.65**
Error	89	0.5937	0.0067	

*NS = Not significant, ** = Highly significant*

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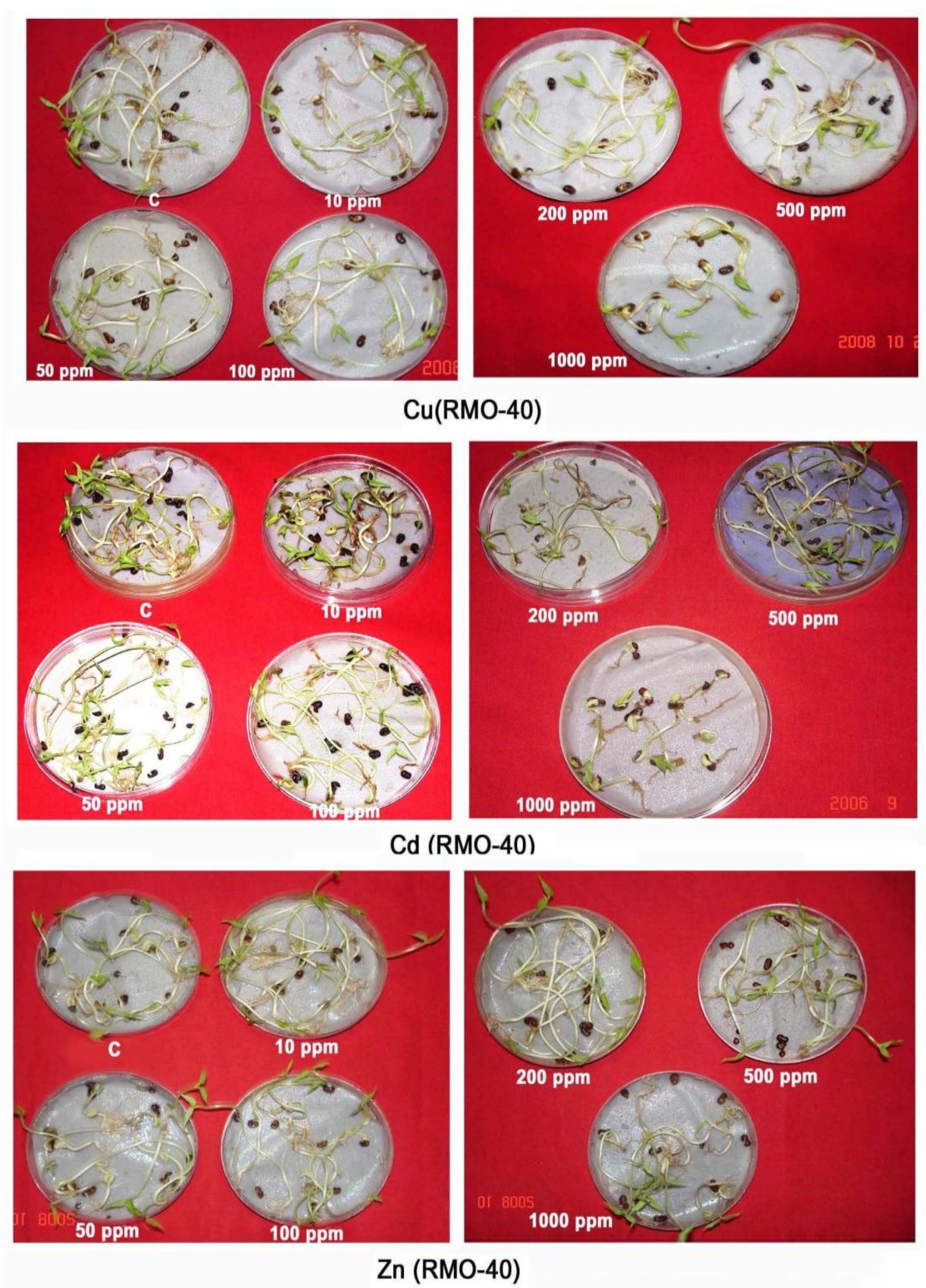
PLATE 5.1



Showing the effect of different concentrations of Copper, Cadmium and Zinc on seed germination and seedling growth in *P. aconitifolius* cv. RMO 40

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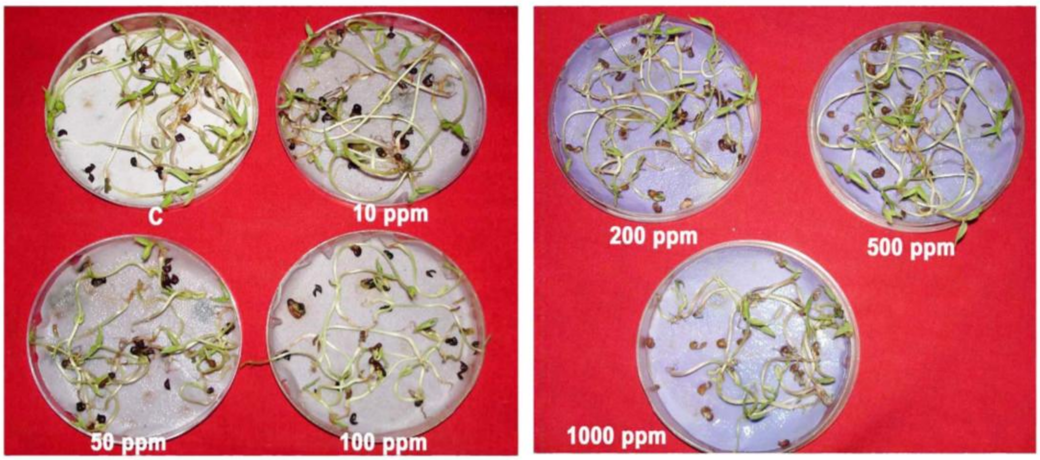
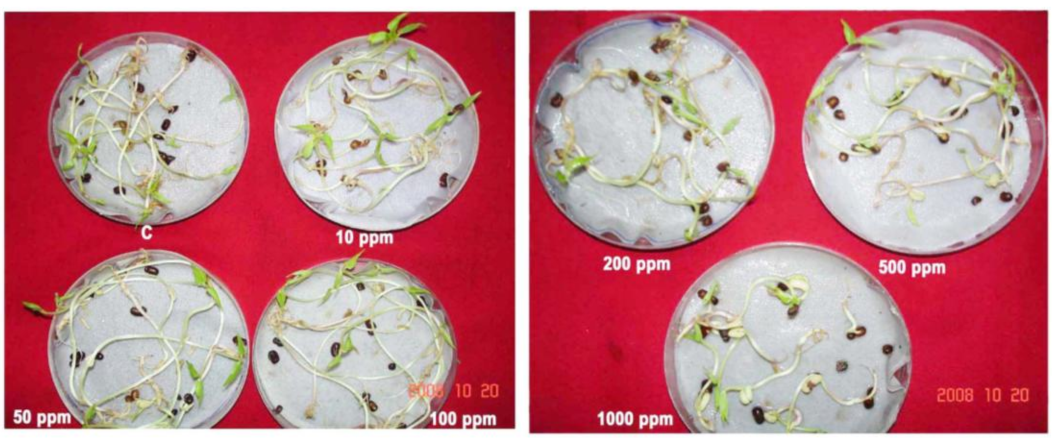
PLATE 5.2



Pb (RMO - 40)



Ni (RMO-40)

Showing the effect of different concentrations of Lead and Nickel on seed germination and seedling growth in *P. aconitifolius* cv. RMO 40

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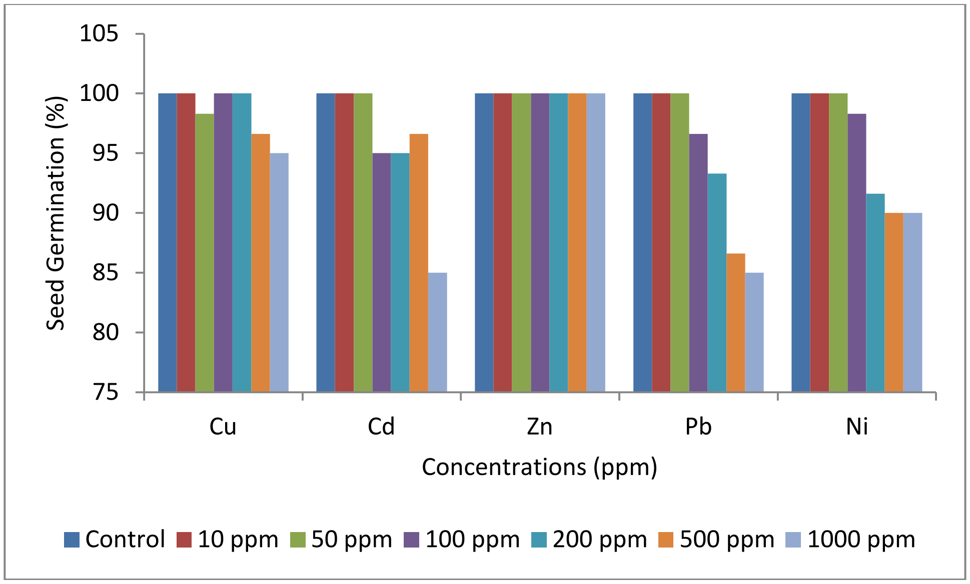

Figure 1: Showing the Effect of Heavy Metals on Seed Germination (%) in *Phaseolus aconitifolius* cv. RMO 40.

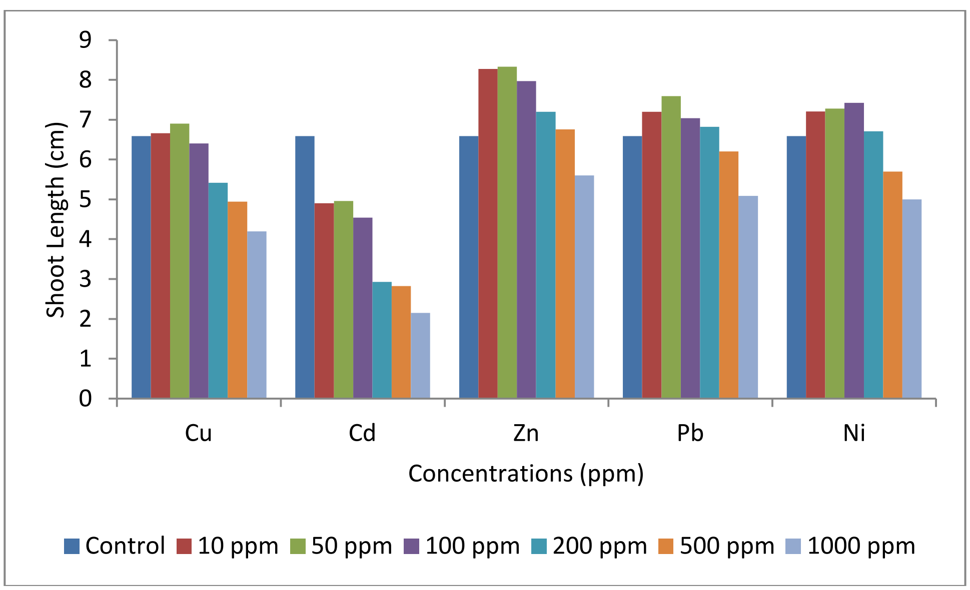

Figure 2: Showing the Effect of Heavy Metals on Shoot Length (cm) of Seedling in *Phaseolus aconitifolius* cv. RMO 40.

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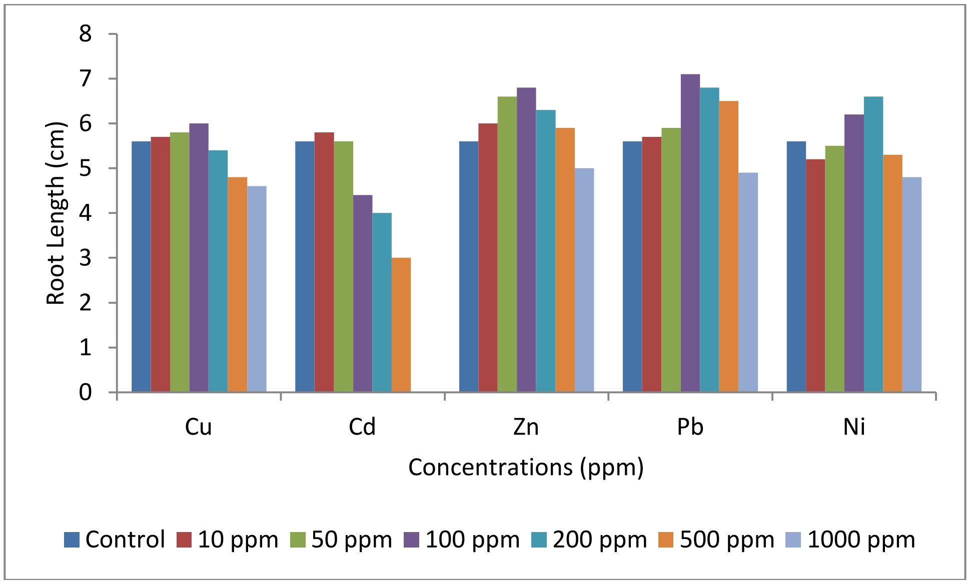

Figure 3: Showing the Effect of Heavy Metals on Root Length (cm) of Seedling in *Phaseolus aconitifolius* cv. RMO 40.

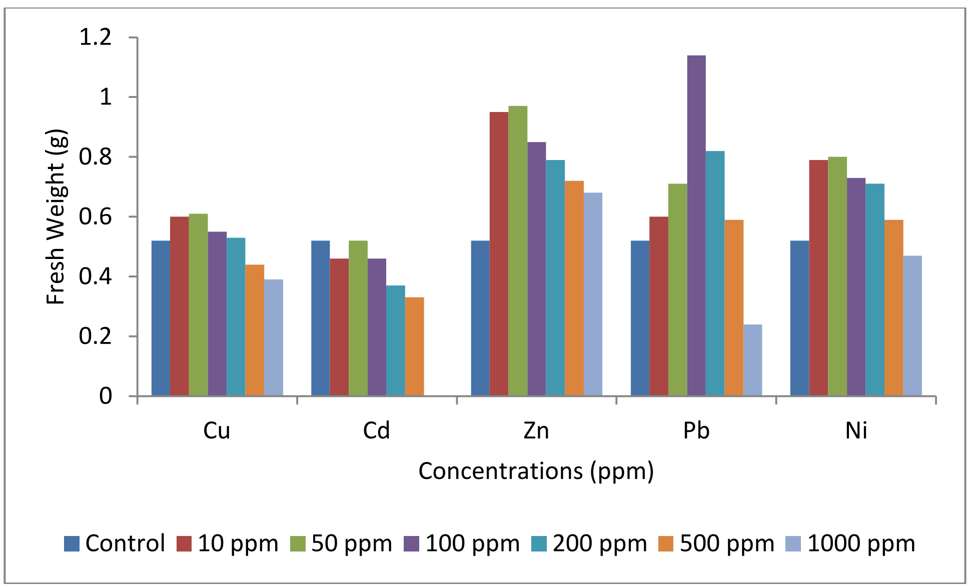

Figure 4: Showing the Effect of Heavy Metals on Fresh Weight (g) of Seedling in *Phaseolus aconitifolius* cv. RMO 40.

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