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Assessment of $CoCl_2$ Tolerance and Toxic Levels in *Vigna Unguiculata*

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Abstract: **Heavy metal concentrations in soil, water, and air are increasing as a result of anthropogenic activities such as industrial processes, mining, and agriculture. Heavy metals are difficult to eradicate because of their environmental stability, and they can even pose a human health concern by entering the food chain via crop plant uptake. Cobalt (II) chloride is an inorganic compound containing cobalt and chlorine with the formula $CoCl_2$. The study used *Vigna unguiculata,* popularly known as cowpea and black-eyed pea. Cobalt chloride (CoCl2) was employed in this study to assess cobalt tolerance and toxic limits in one cultivar of *V. unguiculata*. The effect of cobalt chloride on *V. unguiculata* was assessed by analyzing germination and growth characteristics.**

Keywords: **Heavy metals, Cobalt chloride, Toxicity, *Vigna unguiculata*, growth parameters.**

1. INTRODUCTION & LITERATURE REVIEW

Pollution of the environment is a worldwide concern. The persistent march of modern civilization towards the twenty-first century carries with it the unbearable pressure of pollution on the environment. Heavy metals, which are hazardous to humans, are among the most dangerous industrial pollutants. For millions of years, the only resource was rocks at the earth's surface, which released heavy metals into water and soil through weathering driven by rain, wind, and other related processes.

According to Sharma and Sobati (1989) heavy metals are the metals, which are several times heavier than water. Blum (1992) emphasized that these heavy metals are usually with densities larger than five grams per cubic centimeter. Heavy metals are environmentally stable and persistent pollutants because they cannot be degraded or destroyed. As a result, they tend to collect in soils, saltwater, freshwater, and sediments. These emissions are mostly caused by combustion activities (such as power generation and road transportation), industrial sources (such as the iron and steel industry and the non-ferrous metal sector), and trash incineration. Mycorrhizal fungi are the only soil microorganisms that provide a direct interface between soil and roots, and so play a significant role in heavy metal availability and toxicity to plants.

Useful guidelines for the interpretation of uptake data in relation to tolerance and toxicity were provided by Backett and Davis (1977) and Barry (1986). The former authors were able to define upper critical levels for elements in plant tissues as the minimum tissue concentrations necessary before the yield of dry matter was reduced by their toxic effects. Barry (1986) combined the yield data with foliar analysis, and defines “tolerance” and “toxic” zones of the uptakes curves for response to external metal concentrations. When the external concentration exceeds tolerable levels, there is a major break in tissue metal concentration.

Metal resistance is a quantitative characteristic which is correlated with the prevailing metal availability levels in the soil (Urquhart, 1971 and Ernst, 1974) and is usually specific to metal (Ernst, 1982). Heavy metal resistance can be achieved in two different ways- By avoidance and tolerance. Avoidance is defined as organism’s ability to prevent excessive metal uptake into its body. Tolerance is an organism's ability to cope with metals that are excessively accumulated within its body. A tolerant plant can be defined as one, which is able to grow and reproduce in soil that is inimical to normal plants of the same species. One of the primary toxic effects of heavy metals is to inhibit root growth; therefore, most methods utilize the relative root growth of plants in a simple growth solution to which the metal under test can be added. The most commonly used parameter is the "Tolerance Index" (Wilkings, 1978). This parameter makes the reasonable assumption that the root growth of a particular genotype in a toxic solution is a function of both its intrinsic rate of root growth, as measured in a control solution, and its degree of tolerance.

Cobalt (II) Chloride

Cobalt (II) chloride is an inorganic compound of cobalt and chlorine, with the formula $CoCl_2$. It is usually supplied as the hexahydrate $CoCl_2.6H_2O$, which is one of the most commonly, used cobalt compounds in the lab. The hexahydrate is deep purple in color, whereas the anhydrous form is sky blue. A blend would be mauve. Because of the ease of the hydration/dehydration reaction, and the resulting color change, cobalt chloride is used as an indicator for water in desiccants. Other names of $CoCl_2$ are cobaltous chloride, Cobalt dichloride, Muriate of Cobalt, etc.

Properties

It dissolves readily in water and alcohol. Concentrated aqueous solutions are red at room temperature but become blue when heated. The molar mass is 129.839 g/mol (anhydrous). Cobalt (Co) is a natural earth element presented in trace amount in soil. Trace elements are necessary for normal metabolic functions in plants, but at higher concentration these metals are toxic and may severely interfere with physiological and biochemical functions (Jaya kumar and Vijaya rengan, 2006). Similarly, Co also affords both beneficial as well as harmful effects to plants. It has long been applied to plants to raise crops yield (Young, 1979). However, in excess concentration it caused a marked reduction in growth together with chlorosis and necrosis (Vanselow, 1966), inhibits photosynthesis (Van Asshe and Clijsters, 1990), seed germination and seedling growth (Dubey and Dwivedi, 1987). The activities of several enzymes are also disturbed by excessive amount of Co present within the plant (Shalygo *et al*., 1990) and thus finally reduced the quality of produce (Chatterjee *et al*., 2006). In general the average level of Co in the soil ranges 30-40 ppm (Kabata-Pendias and Pendias, 1991) and above that it generates toxicity. However, the concentration of Co in the soil is not the only factors that determine toxicity. Plant species vary in their sensitivity to Co, soil type and soil chemistry greatly influence Co toxicity. One of the most important soil properties is soil acidity and the more acidic the soil, the greater the potential for Co toxicity, at any concentration.

Black-eyed pea

The black-eyed pea or black-eyed bean, a legume, is a subspecies of the cowpea, grown around the world for its medium-sized, edible bean. The common commercial variety is called the California Blackeye; it is pale-colored with a prominent black spot. The color of the eye may be black, brown, red, pink or green. All the peas are green when freshly shelled and brown or buff when dried. A popular variation of the black-eyed pea is the purple hull pea; it is usually green with a prominent purple or pink spot. The currently accepted botanical name for the black-eyed pea is *Vigna unguiculata* (L)Walp., subsp. *unguiculata*, although previously it was classified in the genus *Phaseolus*.

Scientific Classification

Kingdom: Plantae

(Unranked): Angiosperms

(Unranked): Eudicots

(Unranked): Rosids

Order: Fabales

Family: Fabaceae

Genus: *Vigna*

Species: *V. unguiculata*

Subspecies: ***V. u. subsp. unguiculata***

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Cultivation

This heat-loving crop should be sown after all danger of frost has passed and the soil is warm. Seeds sown too early will rot before germination. Black-eyed peas are extremely drought tolerant, so excessive watering should be avoided. The crop is relatively free of pests and disease. The blossom produces nectar plentifully.

2. MATERIALS & METHODS

During the present investigation Cobalt Chloride ($CoCl_{2)}$ was used for evaluating the tolerance and toxic limits of cobalt in one cultivar of *V. unguiculata*. The seeds were procured from local market of Rudrapur (Distt. Udham Singh Nagar, Uttarakhand).

The effect of cobalt chloride on *V. unguiculata* was estimated by analyzing the germination and growth related parameters. The seed germination and growth characteristics pattern were estimated with the help of parameters listed below:

(a) Temporal and total (percent) seed germination,

(b) Length of root and shoot of the seedlings, and

(c) R/S ratios of the seedlings.

For estimating temporal pattern (relative frequency of seed germination after every 24 hours) of seed germination per day and total seed germination, 5 replicates of 20 **s**eeds each were analyzed. These seeds were put equidistantly in petriplates lined with filter paper with cotton pads ‘sandwiched’ between them. For estimating the tolerance limit of *V. unguiculata* to $CoCl_2$, ten molar solutions of $CoCl_2$ ranging between **0.01** M to **0.1** M were used for treating the seeds. The control sets were raised in distilled water and treated sets were raised in various molar concentrations of $CoCl_{2.}$ Only a fixed amount (about 50 ml) of distilled water or $CoCl_2$ solutions was added in petriplates. The composition of Hoagland’s solution is as given below:

Chemical	**ml/L**
KNO_3	10
$Ca(NO_3)_2$	10
$MgSO_4.7H_2O$	10
KH_2PO_4	10

A liter of Hoagland’s stock solution was prepared by adding 10ml of each chemical compound (1M) in 960 ml- distilled water. This solution was added at times in petriplates containing treated seeds.

These petriplates were then placed at room temperature in darkness. The seeds were considered germinated as soon as radicles of about 1 mm emerged out of these. This happened next day (24 hours) from start of experiment. Therefore, the relative seed germination frequency was worked out from 1st day after keeping the seeds for germination. Relative seed germination frequency was measured at a regular interval of 24 hours up to 5 days.

The total seed germination and relative seed germination frequency were calculated from the

following formulae:

$$\text{Total seed germination} = \frac{\text{Number of seeds germinated Total}}{\text{The number of seeds taken}} \times 100$$

$$\text{Relative seed germination} = \frac{\text{Number of seeds germinating within 24 hours}}{\text{Total number of seeds taken}} \times 100$$

Proportions between the root and shoot (R/S ratios) were calculated on the basis of formula listed below:

$$R/S = \frac{\text{Mean root length}}{\text{Mean shoot length}}$$

In seed germination experiment as the seeds germinated on the 1st day, the real age of the seedlings at the time of measurement were 5 days. A parameter called Response Coefficient (RC) was also calculated, using the following formula, for estimating stimulating and inhibiting effects of $CoCl_2$ solutions on radical growth.

$$RC = \frac{\text{Value of treated set} - \text{value of control set}}{\text{value of control set}}$$

On the basis of RC tolerant, partially tolerant and non – tolerant response of the cultivars was noted down. These are divided into five categories A to E on the basis of RC. The range of RCs in these five categories is:

Category	A	B	C	D	E
RC Value	>-0.20	-0.2 to -0.39	-0.40 to -0.59	-0.60 to -0.79	< -0.8

The accession whose RC value comes in category A is tolerant, those whose values come in B, C or D are partially tolerant and those whose value comes in category E are non – tolerant.

The entire data was analyzed statistically. Standard error was calculated for estimating the deviation of sample mean from population mean with the help of formula:

$SE = SD/\sqrt{n}$

Where, n = Number of observations

OBSERVATIONS

The seeds of control started germinated after 24 hours from start of the experiment. On first day 40% germination was observed in control and on fifth day maximum germination was found to be 70%. No germination was observed in 0.1M, 0.09M, 0.08M, 0.07M, 0.06M, 0.04M and 0.03M. Very less germination was observed in only 0.05M, 0.02M and 0.01M. In 0.05M and 0.02M was found to be 5%,10% and in 0.01M only 20% maximum germination was observed. The temporal and total germination data is shown in table 1 and graphically represented in fig. 1.

The root shoot ratio was also calculated in control and treated sets. The R/S was found to be highest in control and then 0.01M. R/S decreased with increase in $CoCl_2$ concentration. The data is shown in table 2.

A parameter called response coefficient was calculated to estimate the tolerance and toxic effect of $CoCl_2$ on *V. unguiculata.* In most of the treatments no germination was observed. The germination was observed in only three treatments 0.05M, 0.02M and 0.01M. As already given in materials and methods the RC category was found to be C, C and B showing their partially tolerant habit against $CoCl_2$. The data is shown in table 3.

Table -1. Percent germination in control and treated sets

	Co%	**0.1M**	**0.09M**	**0.08M**	**0.07 M**	**0.06 M**	**0.05M**	**0.04 M**	**0.03M**	**0.02M**	**0.01 M**
1st Day	**40**	0	0	0	0	0	**5**	0	0	**10**	**20**
2nd Day	**50**	0	0	0	0	0	**5**	0	0	**10**	**20**
3rd Day	**60**	0	0	0	0	0	**5**	0	0	**10**	**20**
4th Day	**70**	0	0	0	0	0	**10**	0	0	**10**	**20**
5th Day	**70**	0	0	0	0	0	**10**	0	0	**10**	**20**
TOTAL	**70**	**0**	**0**	**0**	**0**	**0**	**10**	**0**	**0**	**10**	**20**

Table-2: Root hoot ratios in control and treated sets

	R/S
Co	1.38
0.05M	0.78
0.02M	1.11
0.01M	1.31

Table-3: RC values, category and the tolerance limit in treated sets

	RC	Category	Tolerance
0.05M	**-0.56**	C	**PT**
0.02M	**-0.46**	C	**PT**
0.01M	**-0.23**	B	**PT**

Table-4 Showing Percent germination in control and treated sets only in germinated seeds

Days	Co%	0.05M	0.02M	0.01 M
1st Day	40	5	10	20
2nd Day	50	5	10	20
3rd Day	60	5	10	20
4th Day	70	10	10	20
5th Day	70	10	10	20
TOTAL	70	10	10	20

Fig-1: Graphical representation of Table-1

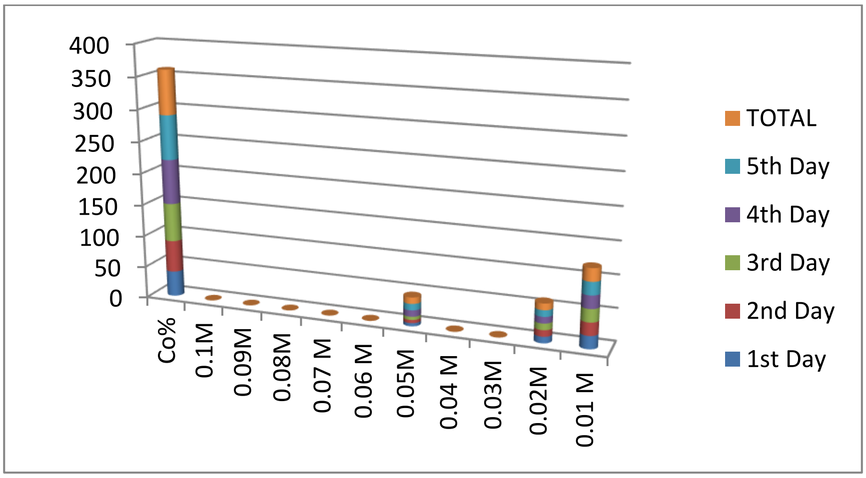

Fig-2: Graphical representation of Table-4

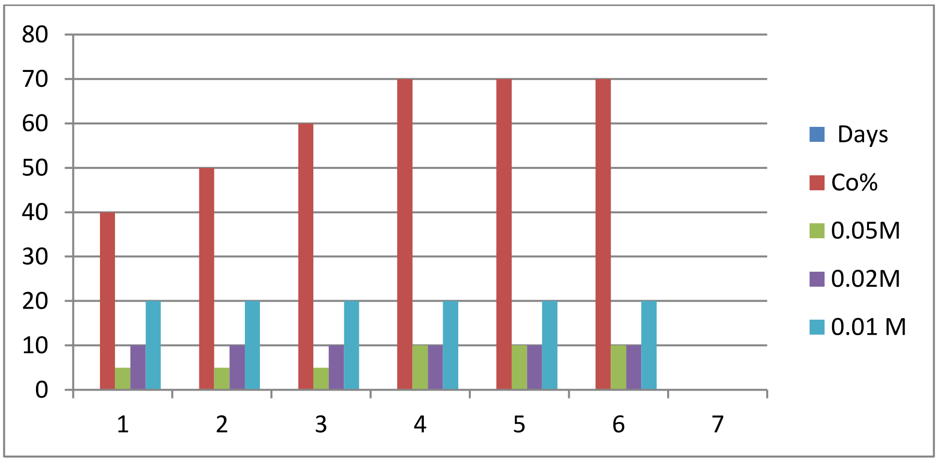

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Table-5: Data showing temporal growth patterns in control and treated sets

I Day	*Co*					
	Mean	**±**	**SE**	**Range**		
R	**1.70**	±	**0.34**	**0.70**	**-**	**2.20**
C	**1.35**	±	**0.05**	**1.30**	**-**	**1.50**
S	**0.50**	±	**0.00**	**0.50**	**-**	**0.50**
II Day						
R	**2.04**	±	**0.34**	**1.40**	**-**	**4.30**
C	**1.29**	±	**0.03**	**1.20**	**-**	**1.40**
S	**1.07**	±	**0.04**	**1.00**	**-**	**1.30**
III Day						
R	**2.19**	±	**0.41**	**1.40**	**-**	**5.10**
C	**1.33**	±	**0.04**	**1.20**	**-**	**1.50**
S	**1.42**	±	**0.05**	**1.20**	**-**	**1.70**
IV Day						
R	**3.52**	±	**0.48**	**2.00**	**-**	**6.00**
C	**1.29**	±	**0.03**	**1.20**	**-**	**1.50**
S	**1.51**	±	**0.10**	**1.20**	**-**	**2.00**
V Day						
R	**3.89**	±	**0.45**	**2.00**	**-**	**6.00**
C	**1.28**	±	**0.04**	**1.20**	**-**	**1.50**
S	**1.54**	±	**0.07**	**1.30**	**-**	**2.00**

I Day	*0.5 M*					
	Mean	**±**	**SE**	**Range**		
R	**0.50**	±	**0.34**	**0.20**	**-**	**0.70**
C	**1.00**	±	**0.01**	**0.80**	**-**	**1.10**
S	**0.20**	±	**0.00**	**0.10**	**-**	**0.30**
II Day						
R	**0.70**	±	**0.34**	**0.50**	**-**	**0.90**
C	**1.20**	±	**0.03**	**1.00**	**-**	**1.40**
S	**0.40**	±	**0.03**	**0.10**	**-**	**0.50**
III Day						
R	**0.78**	±	**0.41**	**0.50**	**-**	**0.90**
C	**1.30**	±	**0.04**	**1.20**	**-**	**1.50**
S	**0.60**	±	**0.05**	**0.40**	**-**	**0.70**
IV Day						
R	**0.90**	±	**0.48**	**0.80**	**-**	**1.00**
C	**1.29**	±	**0.03**	**1.20**	**-**	**1.40**
S	**0.67**	±	**0.10**	**0.30**	**-**	**0.80**
V Day						
R	**1.70**	±	**0.45**	**1.30**	**-**	**2.00**
C	**1.28**	±	**0.04**	**1.20**	**-**	**1.40**
S	**0.90**	±	**0.07**	**0.50**	**-**	**1.00**

I Day	*0.2 M*					
	Mean	**±**	**SE**	**Range**		
R	**1.00**	±	**0.20**	**0.80**	**-**	**1.10**
C	**1.20**	±	**0.05**	**1.10**	**-**	**1.30**
S	**0.10**	±	**0.00**	**0.10**	**-**	**0.10**
II Day						
R	**1.20**	±	**0.21**	**1.00**	**-**	**1.30**
C	**1.20**	±	**0.03**	**1.10**	**-**	**1.20**
S	**0.21**	±	**0.02**	**0.11**	**-**	**0.25**
III Day						
R	**1.50**	±	**0.30**	**1.40**	**-**	**2.00**
C	**1.20**	±	**0.04**	**1.20**	**-**	**1.40**
S	**0.30**	±	**0.05**	**0.20**	**-**	**0.42**
IV Day						
R	**2.10**	±	**0.40**	**2.00**	**-**	**2.30**
C	**1.00**	±	**0.04**	**0.70**	**-**	**1.30**
S	**0.50**	±	**0.10**	**0.40**	**-**	**0.60**
V Day						
R	**2.10**	±	**0.50**	**2.00**	**-**	**2.20**
C	**1.00**	±	**0.04**	**0.60**	**-**	**1.20**
S	**0.90**	±	**0.07**	**0.70**	**-**	**1.00**

I Day	*0.01 M*					
	Mean	**±**	**SE**	**Range**		
R	**1.90**	±	**0.40**	**1.50**	**-**	**2.00**
C	**1.35**	±	**0.05**	**1.30**	**-**	**1.50**
S	**0.20**	±	**0.00**	**0.10**	**-**	**0.30**
II Day						
R	**2.00**	±	**0.31**	**1.70**	**-**	**2.20**
C	**1.30**	±	**0.20**	**1.20**	**-**	**1.40**
S	**0.50**	±	**0.01**	**0.40**	**-**	**0.60**
III Day						
R	**2.10**	±	**0.30**	**1.40**	**-**	**2.50**
C	**1.30**	±	**0.21**	**1.20**	**-**	**1.50**
S	**0.60**	±	**0.02**	**0.50**	**-**	**0.80**
IV Day						
R	**2.40**	±	**0.50**	**2.00**	**-**	**3.00**
C	**1.29**	±	**0.03**	**1.20**	**-**	**1.38**
S	**0.80**	±	**0.20**	**0.70**	**-**	**0.90**
V Day						
R	**3.00**	±	**0.42**	**2.60**	**-**	**3.30**
C	**1.29**	±	**0.03**	**1.20**	**-**	**1.42**
S	**1.00**	±	**0.50**	**0.60**	**-**	**1.60**

3. RESULTS AND DISCUSSIONS

The $CoCl_2$ was found to be highly toxic in concentrations ranging from 0.01M to 0.1M on *V. unguiculata*. Out of ten treatments germination was observed in only three treatments 0.05, 0.02 and 0.01M. The total germination in control set was found to be 70% which decreased to 20% in 0.01M and 10% in 0.02M and 0.05M. The less germination was due to presence of cobalt which was found to be toxic in mostly.

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The root shoot ratio decreased with increase in cobalt concentration showing the negative effect of element on seedling growth.

When RC value was calculated it was found that *Vigna unguiculata* was partially tolerant to 0.05, 0.02 and 0.02M doses of cobalt chloride.

REFERENCES

[1] Backett P.H.T., Davis, R.D., 1977. Upper critical levels of toxic elements in plants. New Phytol. 79: pp.95.

[2] Barry W.L., 1986. Plants factors influencing of plant analysis as a tool for biogeochemical prospecting. In: "*Mineral exploration biological system and organic matter"* (*eds.* D. Carlisle, W.L. Bery, I.R. Kaplan and J.R. Watterson) Prenticemall, Engelwood Uiffs, pp. 13.

[3] Blum E.H., 1992. Treating Toxic Grounds. CERES May- June, 135-136.

[4] Chatterjee C, Gopal R, Dube BK., 2006. Physiological and biochemical responses of French beans to excess cobalt. J. Plant Nutr. 29:127-136.

[5] Dubey R.S., Dwivedi R.S., 1987. Effect of heavy metal on seed germination and seedling growth of soybean. Nat. Acad. Sci. Letter 10:121-124.

[6] Erenst W.H.O. 1974. *Schwernetallvegetation der Erde, crustov Fischer Verlag, stuttgert*.

[7] Erenst W.H.O.1982. *Schwermetallpflanzen. In: Pflazenokologie and mineralstoff weehtel.(eds.*H. Kinzel.).pp.472.

[8] Jayakumar K, Jaleel C.A., Vijayarengan P., 2007. Changes in growth, biochemical constituent and antioxidant potentials in radish (*Raphanus sativus* L.) under cobalt stress. Turkish J. Biol. 31:127-136.

[9] Kabata-Pendias A, Pendias H., 1991. Trace elements in soils and plants. CRC, Boca Raton, FL. pp. 276-285 Lin 1991.

[10] Sharma G.P., Sobti R.C., 1989. Metals and their salts: studies on their carcinogenic and mutagenic potential. In "*Perspectives in cytology and genetics" (eds*. G.K. Manna and U. Sinha) 6: 465-490.

[11] Shalygo N.V. *et al*., 1999. Effect of Mn^{2+}, Fe^{2+}, Co^{2+}, and Ni^{2+} on cholorophyll accumulation and early stages of cholorophyll formation in greening barley seedlings. Russ. J. Plant Physiol.46:496-501.

[12] Urquhart C., 1971. Genetics of lead tolerance in *Festuca ovina*. Heredity 26:pp.19.

[13] Van Assche F, Clijsters H., 1990. Effects of metals on enzyme activity in plant. Plant Cell Environ. 13:195-206.

[14] Vanselow A.P., 1966. Cobalt. In Diadnostic criteria of plants and soils, ed. HD Chapman, 142-156. Riverside, CA: University of California Division of Agricultural Science.

[15] Wilkings D.A., 1978. The measurement of tolerance to edaphic factors by means of root growth. New Phytol.80: pp.623.

[16] Young R.S., 1979. Cobalt in biology and biochemistry. Academic Press. London pp.52-97.