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Phyto-Toxic Effects of Various Concentrations of Cu and Pb on the Seed Germination and Seedling Survival of V. Radiata

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Abstract**--**The present study is an attempt to explore a possible relationship, the individual and interactive effects of lead and copper on seed germination behavior and seedling growth at seedling stage. All the studies were conducted on control and heavy metal treated seeds. Healthy seeds of uniform size were sorted, sterilized and soaked for 12 hours in the respective heavy metal solution. The seeds were then kept for germination in sterilized petriplates lined with sterilized Whatman No.1 filter paper. 20 seeds were kept in each petriplate. The heavy metal treatments of the seeds were given as 0.05, 1.0, 10.0 and 100 ppm concentrations of Cu and Pb separately as well as in different combinations. The result of this study showed that increasing concentrations of heavy metals (Cu and Pb) inhibited seed germination and limited seedling growth. Both metals have exerted a harmful effect on seedling growth. The combination of copper and lead ions at the low concentration had no effect on seed germination, but higher concentration caused the reduction in seed germination. Moreover, copper has more toxic effect than lead at high concentrations. These findings can be used practically for selection and for application with caution of pesticides with high concentrations of copper and lead. It could be helpful to improve the plant growth security in polluted soil.

Keywords**--***Seed germination, seedling* growth*, vigor index, tolerance index, lead and copper*

I. INTRODUCTION

Heavy metals are largely found in dispersed form in rock formations. Industrialization and urbanization have increased the anthropogenic contribution of heavy metals in biosphere. Some of these heavy metals are bio-accumulative, and they neither break down in the environment nor easily metabolized. Such metals accumulate in ecological food chain through uptake at primary producer level and then through consumption at consumer levels. Plants are stationary, and roots of a plant are the primary contact site for heavy metal ions. Some of the heavy metals (Fe, Cu and Zn) are essential for plants (Table 1) and animals (Wintz et al. 2002)[1]. The availability of heavy metals in medium varies, and metals such as Cu, Zn, Fe, Mn, Mo, Ni and Co are essential micronutrients (Reeves and Baker 2000)[2], whose uptake in excess to the plant requirements result in toxic effects (Monni et al. 2000)[3].

Copper (Cu) is considered as a micronutrient for plants (Thomas et al. 1998)[4], (Kabir et al, 2009)[5] and plays important role in CO2 assimilation and ATP synthesis (Pichhode and Nikhil, 2015)[6]. Cu is also an essential component of various proteins like plastocyanin of photosynthetic system and cytochrome oxidase of respiratory electron transport chain (Demirevska-kepova et al. 2004)[7]. Excess of Cu in soil plays a cytotoxic role, induces stress and causes injury to plants. This leads to plant growth retardation and leaf chlorosis (Lewis et al. 2001)[8], oxidative stress and ROS (Stadtman and Oliver, 1991)[9], damage to macromolecules (Pichhode and Nikhil[6], 2015; Hegedus et al 2001)[10]. Lead (Pb) is one of the ubiquitously distributed most abundant toxic elements in the soil. It exerts adverse effect on morphology, growth and photosynthetic processes of plants and inhibits seed germination (Morzck and Funicclli 1982), (Nakos 1979)[12]. Heavy metals are of great interest for research purpose with respect to toxicological importance to human health, plants and animals (Pandey, 2008[13] Jain et al., 2010[14]). Currently, environmental pollution and plant exposure to heavy metals is a matter of great concern at the global level. It is very important to know which heavy metal and in what concentration, they will be toxic to the plants in order to assess optimal growth on more or less contaminated soils. Thus, the present study is an attempt to explore a possible relationship between lead and copper metal induced changes in germinating seeds of Vigna radiata.

Mung bean [Vigna radiata (L.) Wilczek] also known as green gram, green bean, mash bean, golden gram and green soy is an excellent source of easily digestible proteins with low flatulence which complements the staple rice diet in Asia (Saminathan, 2013)[15].

II. METHODS OF STUDY

Pure and viable tested seeds of Vigna radiata were obtained from IGKVV, Raipur, Chhattisgarh. Heavy metals standards used for the study were of Merck Company. All the glass wares used were of borosil. The parameters considered for the present study are given below:

A. Germination Studies**--**All the studies were conducted in the control as well as in lead and copper metal treated seeds. Healthy seeds of uniform size were sorted and sterilized with 0.1% HgCl2 solution for 5 min. and washed with distilled water, then soaked for 12 hours in the respective heavy metal solution. The seeds were then kept for germination in sterilized petriplates lined with sterilized Whatman No.1 filter paper. 20 seeds were kept in each petriplate. For different concentrations of the two heavy metals separate sets of seeds were kept. The seeds were treated with the respective metal solutions at regular intervals. Same volume of the solutions was taken each time for the treatment. The control seeds were watered with distilled water. Triplicates were maintained for each treatment.

The heavy metal treatments of the seeds were given as 0.05, 1.0, 10.0 and 100 ppm concentrations of Cu and Pb separately as well as in different combinations. The combinations used are shown in table no. 2. The controlled seeds were well prevented from the introduction of any of the heavy metal. They were irrigated with ultrapure water. The interval of the treatment and the volume of water and heavy metal solutions were kept constant. Treatments were given on alternate days. The seeds were kept under aseptic conditions and observed for a period of 3days incubation at 25 ± 2°C. As soon as the radical emerged out, the filter paper lining the upper lid of the petriplates were removed in order to allow enough light to get in.

B. Germination Percentage- After incubation, germinated seeds after 24 hrs were separated and counted to calculate percent germination.

C. Radicle and hypocotyle length- After incubation of 24, 48 and 72 hr the hypocotyle and radicle length were measured for all the seedlings.

D. Vigor index (VI) - The method described by Sharma and Saran (1992)[16] was adopted for determining vigour index. It was calculated using the formula.

Vigor index (VI) = germination percentage x axis length

E. Tolerance indices (T.I.) - Tolerance indices were determined through use of the following formula given by Iqbal and Rahmati (2000)[17]

T.I. = (Mean root length in metal solution / Mean root length in distilled water) x 100

F. Phytotoxicity- The % of phytotoxicity of the metal was calculated by the formulae suggested by Chou et al (1978)[18]

% Phy. of seedling = <u>seedling length of control – seedling length of treatment X 100</u>

Germination **%** = number of seeds germinated /total number of seeds × 100

Seedling length of control

III. RESULT AND DISCUSSION

Table: 1. Seed germination rate (%) compared to the control at different individual concentration of Cu and Pb

SN		**1**	**2**	**3**	**4**	**5**
Metal Conc. (ppm)		0	0.1	1	10	100
Germination rate % of V. radiate	Cu	100	95	90	85	00
	Pb	100	100	90	85	00

Table: 2. Seed germination rate (%) compared to the control at different concentration of Cu and Pb in combination

SN	**1**					**2**				**3**				**4**			
Metal Conc (ppm)	Control	0.1 Cu + 0.1 Pb	0.1 Cu + 1.0 Pb	0.1 Cu + 10.0 Pb	0.1 Cu + 100 Pb	1.0 Cu + 0.1 Pb	1.0 Cu + 1.0 Pb	1.0 Cu + 10.0 Pb	1.0 Cu + 100 Pb	10 Cu + 0.1 Pb	10 Cu + 1.0 Pb	10 Cu + 10 Pb	1.0 Cu + 100 Pb	100 Cu + 0.1 Pb	100 Cu + 1.0 Pb	100 Cu + 10.0 Pb	100 Cu + 100 Pb
Germination %	100	95	95	95	25	90	95	95	20	95	95	95	25	20	30	25	05

Table: 3 % Phytotoxicity, Vigour Index and Tolerance Index of V. radiata in different concentrations of Pb and Cu metal ions

SN	**Metal conc. (ppm)**	**Phytotoxicity**		**Vigour Index**		**Tolerance Index**	
		Cu	Pb	Cu	Pb	Cu	Pb
1.	0	00	00	500	500	500	500
2.	0.1	16	22	399	350	84	78
3.	1	26	20	333	360	74	80
4.	10	6	42	400	230	94	58
5.	100	100	100	00	00	00	00

Copper and lead ions had an inhibitory effect on both the seedling growth. Radical length and hypocotyls length were significantly reduced under copper and lead metal stress and the rate of reduction went on increasing with the increase in metal concentration. In one day old seedlings, hypocotyls length was 1.4 cm which got extended up to 2.0 cm after three days whereas radical length was increased by 1.2 to 3.0 cm during 3 days of the experiment under control conditions. However, the rate of seedling growth reported in 0.1ppm copper and lead treated seedlings was less in comparison to control. On 3^{rd} day after imbibitions, Pb ions at the concentration of 0.01, 1 and 10 ppm caused reduction in hypocotyls length and radical length respectively, whereas, Cu ions at the concentration of 1 and 10 ppm resulted in increase in hypocotyls length and decrease in radical length. Least reduction in hypocotyls length has been noticed in case of 10 ppm Cu, whereas least reduction in radical length has been noticed in 0.1ppm Cu treated seedlings. With Pb ion treatment a continuous reduction of hypocotyls and radical length has been noticed. Moreover, maximum reduction in hypocotyls and radical length has been found in seedlings grown in 10 ppm individual Pb concentration of metal ions. At 100 ppm of Cu and Pb concentration of metal ions there was no hypocotyls and radical observed. The percentage of seedling growth was presented in table 4.

Table: 4 Effect of different concentrations of individual Cu and Pb metal ions on the length of hypocotyls and radical of V. radiata after a period of 24 hr, 48 hr and 72 hrs of control and treatment

SN	Metal Conc. (ppm)	Cu treatment						Pb treatment					
		Length of hypocotyls(cm)			Length of radical (cm)			Length of hypocotyls(cm)			Length of radical (cm)		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
1.	0	1.4	3.5	2.0	1.2	1.9	3.0	1.4	3.5	2.0	1.2	1.9	3.0
		Growth of hypo. (%)			Growth of radical (%)			Growth of hypo. (%)			Growth of radical (%)		
2.	0.1	60	34	85	50	42	83	85	40	95	66	57	66
3.	1	60	31	125	50	31	40	85	45	100	58	57	66
4.	10	50	34	115	41	31	80	85	48	60	58	47	50
5.	100	00	00	00	00	00	00	00	00	0.0	00	00	0.0

Table: 5 Effect (after 24, 48 and 72 hrs of treatment) of different concentrations and combination of Cu and Pb metal ions on the length of radical and hypocotyls of the seeds of V. radiata

SN	Metal Concentration (ppm)	Length hypocotyls (cm)			Length of radical (cm)		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
1.	**Control**	1.40	3.6	2.0	1.20	1.9	3.0
	Combination of Cu & Pb	**Growth of hypocotyls (%)**			**Growth of radical (%)**		
2.	0.1 Cu + 0.1 Pb	60.7	50	150	50	68.4	83.3
3.	0.1 Cu + 1.0 Pb	85.7	52.7	100	70.3	84.2	83.3
4.	0.1 Cu + 10.0 Pb	64.2	52.7	175	62.5	21.0	50
5.	0.1 Cu + 100.0 Pb	00	41.6	150	00	57	33.3
6.	1.0 Cu + 0.1 Pb	135.	50	150	83.3	47.3	83.3
7.	1.0 Cu + 1.0 Pb	67.8	44.4	75	62.5	63.1	110
8.	1.0 Cu + 10.0 Pb	39.2	50	75	33.3	57.8	116
9.	1.0 Cu + 100.0 Pb	21.4	19.4	35	16.6	26.3	16.6
10.	10 Cu + 0.1Pb	78.5	50	75	62.5	73.6	66.6
11.	10 Cu + 1.0 Pb	71.4	55.5	175	58.3	31.5	116
12.	10 Cu + 10.0 Pb	100	50	125	70.8	63.1	73.3
13.	10 Cu + 100 Pb	00	00	50	00	00	43.3
14.	100 Cu + 0.1Pb	00	38.8	100	00	21.0	33.3
15.	100 Cu + 1.0 Pb	00	19.4	100	00	10.5	5
16.	100 Cu + 10.0 Pb	00	38.8	50	00	00	33.3
17.	100 Cu + 100 Pb	00	00	00	00	00	00

1. Control

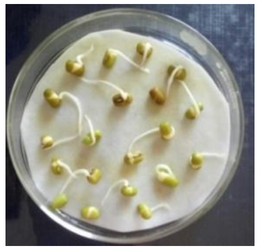

2. Cu 0.1ppm

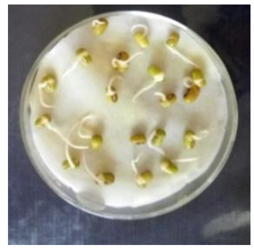

3.Cu1ppm


4.Cu10ppm


5.Cu100ppm


6. Pb 0.1PPM


7. Pb1PPM


8. Pb10PPM


9. Pb100 PPM

Images 1-9: Effect of Cu & Pb on V. radiata after 72 hrs treatment

At higher copper and lead concentration the root tips of all the seedlings had changed their color to dark brown, stunted, thick and brittle as shown in Images 1- 9. The change in root characteristics may be the consequence of direct exposure of the radical to metal toxicity.

The combination of copper and lead ions at the low concentration had no effect on seed germination, but high concentration caused the reduction in seed germination up to 5% in 3rd day treatment condition. Lead and copper metal ions combination at the low concentration (0.1ppm Cu + 0.1ppm Pb) was antagonistic, but beyond this level of interaction ions had caused serious consequences on seedling growth

The results have shown that there was a marked difference in hypocotyl and radical length of seedlings grown under different treatment conditions and control conditions. Reduced hypocotyl and radical length in response to heavy metals has been reported by a number of investigators (Tomulescu et al., 2004[19], Zhang et al., 2009[20] John et al., 2009[21]). The lower sensitivity of the roots to lead and copper could be explained by the capacity to accumulate metal in a non-active form and to the mobility of metal ions within the plants, which facilitates its transport to the aerial parts of the plant (Barceló et al., 1988)[22]. Moreover, as studied by Cohen et al. (1998)[23] roots can accumulate metal ions in the apoplast by ionic interactions with the carboxyl and/or sulphydryl groups from components of the cell wall and part of metal can be complexes by phytochelatins and sequestered in the vacuole.

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