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PHYTO-TOXIC EFFECT OF CHROMIUM OF Cow Pea [*Vigna unguiculata* (L.) Walp.]

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Abstract: ***A laboratory experiment was conducted to determine the phytotoxic effect of [Chromium (Cr) Potassium dichromate ($K_2 Cr_2 O_7$)] on seed germination and seedling growth of cow pea [Vigna unguiculata (L.) Walp.]. The seeds were treated under 5, 25, 50, 75, 100, 125 and 150 ppm of Cr concentration solutions individually. Each treatment was replicated thrice in a randomized block design. Observations were complete on Germination Percentage, Root and Shoot Length, Number of Leaves, Total Leaf Area, Fresh and Dry Weights of seedling. And Phytotoxicity, Tolerance Index, Vigour Index and pigments content such as Chlorophyll 'a', Chlorophyll 'b', Total Chlorophyll and carotenoid content of cow pea seedlings at 14^{th} days of seedlings. Among the results gradual increase in Cr concentration under different treatments significantly leads to inhibition of seed germination and other growth parameters. Percentage of phytotoxicity showed an increasing trend with gradual increase in Cr concentration for the cow pea seedlings. Maximum inhibition in all growth parameters and pigments content were recorded.***

Key words: Potassium dichromate ($K_2 Cr_2 O_7$), Cow pea, Phytotoxicity, Vigour Index Morphological parameters, Pigments content.

1. INTRODUCTION

Environmental pollution has been converted into an explanation focus of distress for all the nations worldwide, as not only the developing countries but developed nations as well are affected by and suffer from it. Pollution has many forms, the air we breathe, the water we drink, the ground where we cultivate our food crops and even the increasing noise we hear everyday-all contribute to health problems and lesser quality of life [1].

Metal pollution of agricultural soils by distinctive declaration or by discarding of industrial and sewage sludge constitutes a risk of each discharge of metals into the groundwater. Metal concentrations in soil range from less than 1 mg/kg to high as 100,000 mg/kg, whether due to the biological origin of the soil or as a consequence of human activity [2]. Excess concentrations of some heavy metals in soils such as Cr, Cd, Cu, Ni, and Zn have caused the distraction of natural aquatic and terrestrial ecosystems [3].

In heavy metal polluted soil, plant growth can be kept back by metal assimilation. However, some plant species are accomplished to accumulate moderately enormous quantity of heavy metals without showing stress, which represents a possible exposure for animal and humans. Heavy metals uptake by crops growing in contaminated soil is a potential exposure to human health since of diffusion in the food chain. There is also anxiety with regard to heavy metal transmission through natural ecosystems [4].

Cr compounds are highly toxic to plants and are injurious to their growth and development. Although some crops are not affected by low Cr concentrations [5], Cr is toxic to higher plants [6]. The Cr is also used in the create of refractory bricks, furnace linings, mortars, and castables and in coating materials to close pores and to join bricks in furnaces. Another primary use of Cr is in chemical applications such as metal finishing (Cr(VI)), corrosion control (Cr(III)), leather tanning (Cr(III)) and finishing, wood treatment (Cr(VI)), and the production of pigments (both Cr(VI) and Cr(III)) [7].

Cr toxicity affects plant growth and metabolism to a significant extent [8]. It has been reported that Cr caused decline in root length, shoot length, decreased the number of branches and induced biochemical and physiological changes in crops [9]. This study has been assigned to phytotoxicity of Cr on some morphological specifications and pigment content changes and evaluate of tolerance index and vigour index of cow pea seedlings.

2. MATERIALS AND METHODS

Plastic cup Experiments

The seeds cow pea (variety Co (cp)-7) was obtained from Tamilnadu Agricultural University (TNAU), Coimbatore and Tamilnadu. The uniform seeds are selected for the experimental purpose. Source of Cr (Potassium dichromate ($K_2Cr_2O_7$)) stock solution prepared by dissolving the molecular weight of (Cr) and different concentrations *viz.*, (Control, 5, 25, 50, 75, 100, 125 and 150 ppm) of (Cr) the solution were prepared freshly at the time of experiments. The plastic cups were filed with 1 Kg of garden soil, selected cow pea seeds were sown in the plastic cup and one set of plastic cup irrigated with normal tap water was maintained as the control.

Shoot length and root length (cm/seedling): Five plants from each plastic cup were randomly selected for 14^{th} days of seedlings recorded the shoot length and root length of experimental plants. They were measured by using centimeter scale (Cm).

Root nodules: Five plants from each plastic cup with intact roots were removed with the help of digging fork. The root nodules were carefully separated from the soil by gently pinching and washing the soil particles. The root nodules were counted and recorded.

Total leaf area: Five plant samples were collected at 14^{th} day sampling seedlings and the length and breadth of the leaf samples were measured and recorded. The total leaf area was calculated by using the Kemps constant [10].

Total leaf area = L × B × K

Where, L - length, B - breadth and K - Kemp's constant (for dicot - 0.66).

Fresh weight and dry weight (g/seedling): Five plant samples were randomly selected at 14^{th} day seedlings. Their fresh weight was taken by using an electrical single pan balance. The fresh plant materials were kept in a hot air over at 80°C for 24 hr and then their dry weight were also determined.

Vigour index

Vigour index of the seedlings were calculated by using the formula proposed by [11].

Vigour index = Germination percentage × seedling length.

Tolerance index

Tolerance index of the seedlings were calculated by using the formula proposed by [12].

$$\text{Tolerance index} = \frac{\text{Mean length of longest root in treatment}}{\text{Mean length of longest root in control}}$$

Phytotoxicity

The percentage of phytotoxicity of effluent was calculated by using the formula proposed by [13].

Percentage of phytotoxicity =

$$\frac{\text{Radicle length of control - Radicle lenght of test}}{\text{Radicle length of control}} \times 100$$

Estimation of Chlorophyll [14]

Hundred milligram of fresh leaf was ground in a Mortar and Pestle with 20 ml of 80 per cent acetone. The homogenate was centrifuged at 3000 rpm for 15 min. The supernatant was saved. The pellet was re-extracted with 5 ml of 80 per cent acetone each time, until it become colourless. All the supernatants were pooled and utilized for chlorophyll determination. Absorbance was measured at 645 and 663 nm in spectrophotometer. The chlorophyll content was determined by using the following formula.

$$\text{Chlorophyll 'a' } (\text{mg g}^{-1}) = \frac{12.7\ A_{663} - 2.69\ A_{645}}{a \times 1000\ \text{x w}} \times V$$

$$\text{Chlorophyll 'b' } (\text{mg g}^{-1}) = \frac{22.9\ A_{645} - 4.68\ A_{663}}{a \times 1000 \times w} \times V$$

$$\text{Total chlorophyll } (\text{mg g}^{-1}) = \frac{20.2\ A_{645} + 8.02\ A_{663}}{a \times 1000 \times w} \times V$$

Where,

a = length of light path in the cell (1 cm)
v = volume of the extract in ml and
w = fresh weight of sample in gram.

Estimation of carotenoid [15]

The same chlorophyll extract was measured at 480 nm, in spectrophotometer to estimate the carotenoid**.**

$$\text{Carotenoid } (\text{mg g}^{-1}) = \frac{(A_{480} + 0.114A_{663}) - .638\ A_{645}}{a \times 1000 \times w} \times V$$

3. RESULTS AND DISCUSSION

Increasing concentrations of Cr caused considerable reduction in germination percentage root length and shoot length, fresh and dry weights, no. of leaves, total leaf area, vigour index, tolerance index, phytotoxicity, were also significantly decreased in all Cr treatment as compared to control (table.1).

The seed germination is the first physiological process affected by Cr, the ability of a seed to germinate in a plastic cups containing Cr would be indicative of its level of tolerance to this metal [16]. Seed germination aptitude and seedling growth is affected by Cr. Several other metabolic activities in plants are also interfered by Cr toxicity which causes reduction in root growth, photosynthesis and plant biomass following chlorosis [17]; [18]. Toxic effects of Cr on plant growth and development include alterations in the germination process as well as the growth of roots, stems and leaves, which may affect total dry mass production [19].

The reduced germination of seeds under Cr stress could be a depressive effect of Cr on the activity of amylases and on the subsequent transport of sugars to the embryo axes. Protease activity, on the other hand, increases with the Cr treatment, which could also contribute to the reduction in germination of Cr-treated seeds [20]. Decrease in root growth is a well-documented effect due to heavy metals stress in crops [21]. Decreased root length in plants was due to Cr stress and inhibited root cell division unable roots to absorb water. Present findings also agreed these documentations following inhibition in plant height comprising root and shoot length [22]. It predicts stressed efficiency of plant growth hormones and abnormal longitudinal cell division due to increased osmotic pressure along Cr accumulation. Similarly at Cr concentration 40-80 ppm, root length and plant growth was affected significantly [20] [23].

[24] Reported that heavy metals could affect the density and distribution of beneficial *Rhizobium leguminosarum* at soil and plant level. These might be reasons for the decrease in the number of root nodules in the plants at all levels of heavy metals in the soil. The most prominent effect of heavy metals in plants is the inhibition of growth because of the direct exposure of roots to heavy metals. Inhibition of growth is considered to be primarily the result of inhibition of cell elongation, at least in early stages of toxicity, while reduced cell division can obviously affect growth and leaf area in older stage [25]. The appearances of the inhibited dry matter production were also observed in all parts of the plant at all growth stages at all levels of Cr. Likewise, Cr toxicity also interferes with the water relation and membrane permeability and causes the break of photosynthesis and resulted in a decrease in the growth of plants effected to metal stress [26].

Chlorophyll is one of the important pigment content which is used as an index of plant production capacity. The pigment content is an indication of photosynthetic and metabolic activity. The chlorophyll is an integral component of plant pigments and plays

an important role in the process of photosynthesis. It is the molecule that absorbs sunlight and uses its energy to synthesis carbohydrates from CO_2 and water. It also plays an important role in ATP synthesis [27].

The results obtained on the effect of different concentration of Cr treated plants were summarized and discussed as follows. The results showed (Table. 2) the pigment content such as chlorophyll a, Chlorophyll b, Total chlorophyll and carotenoid, content were decreased when increasing the Cr concentration (Table. 2). Cr toxicity in plants also leads to leaves chlorosis, tissue necrosis, decreases enzyme activity, causes membrane damage, diminished photosynthesis and changing of chloroplast [28]. Cr inhibits metabolic processes by inhibiting the action of enzymes, and this may be the most important cause of inhibition. Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. Some heavy metals were reported to affect chlorophyll biosynthesis and inhibit protochlorophyll reductase and aminolevulinic acid (ALA) synthesis [29]. Several reports show chlorophyll biosynthesis inhibition by metals in higher plants [30]

The decline in chlorophyll content in plants exposed to heavy metal stress is believed to be due to the inhibition of important enzymes, such as δ-aminolevulinic acid dehydratase (ALA dehydratase) and protochlorophyllide reductase [31], associated with chlorophyll biosynthesis and anther mechanisms impairment in the supply of Mg_2^+ and Fe_2^+ required for the synthesis of chlorophylls. The result of this study showed that increase the concentrations of Cr inhibited seed germination and seedling growth, phytotoxicity, vigour index is more sensitive to Cr stress than seed germination in cow pea seedlings.

4. REFERENCES

[1] Yoon J, CaoX, Zhou Q, Ma LQ. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Sci. Total Environ.* (2006).368(2-3): 456-464.

[2] Devries, W., Lofts, S., Tipping, E., Meili, M., Groenenberg, J.E. and Schutze, G. Impact of soil properties on critical concentrations of cadmium, lead, copper, zinc and mercury in soil and soil solution in view of ecotoxicological effects. *Rev. Env. Cont. Toxicol.* (2002) 191:47–89.

[3] Salt DE, Blaylock M, Kumar NPBA, Dushenkov V, Ensley D, Chet I, Raskin I Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechn.*, (1995) 13:468–474

[4] Ayeni, O. O., Ndakidemi, P. A., Snyman, R. G. and Odendaal, J. P., Metal contamination of soils collected from four different sites along the lower Diep River, Cape Town, South Africa, *Int. J. Phys. Sci.*, (2010) 5(13), 2045-2051

[5] Huffman EWD Jr, Allaway HW Chromium in plants: distribution in tissues, organelles, and extracts and availability of bean leaf Cr to animals. *J Agric Food Chem* (1973) 21:982–986.

[6] Davies FT, Puryear JD, Newton RJ, Egilla JN, Grossi JAS Mycorrhizal fungi increase chromium uptake by sunflower plants: influence on tissue mineral concentration, growth, and gas exchange. *J Plant Nutr* (2002) 25:2389–2407.

[7] Nriagu JO A silent epidemic of environmental metal poisoning? *Environ Pollut* (1988) 50:139–161.

[8] Mohanty, M. and Patra, H. K., Attenuation of Chromium toxicity in mine waste water using water hyacinth, *J. Stress Phy. & Biochem*., **(2011)7(4)**, 335-346.

[9] Virendra Kumar Mishra and Tripathi, B.D., Accumulation of chromium and zinc from aqueous solutions using water hyacinth (Eichhornia crassipes), *J. Haz. Mat.*, **(2009) 164**, 1059-1063.

[10] Kalra, G.S. and S.D. Dhiman,. Determination of leaf area of wheat plants by a rapid methods. *J. Indian Bot. Soc.*, (1977) 56: 261-264.

[11] Abdul-Baki, A.A. and J.O. Anderson, Vigour determination in soybean seed by multiple criteria. *Crop Sci.*, (1973)13: 630-633.

[12] Turner, R.G. and C. Marshal, Accumulation of zinc by subcellular of root of *Agrostis tannis* sibth in relation of zinc tolerance. *New Phytol.*, (1972)71: 671-676.

[13] Chou, C.H., Y.C. Chiang and C.I. Rao, Impact of water pollution on crop growth in Taiwan II. Phytotoxic nature of six rivers and twenty seven industrial wastewaters in Kaohaiung area, Taiwan. *Bot. Bull. Acad. Sinica*, (1978) 19: 107-124.

[14] Arnon, D.I., Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.*, (1949) 24: 1-15.

[15] Kirk, J.T.O. and R.L. Allen,. Dependence of chloroplast pigments synthesis on protein synthetic effects of acitilione. *Biochem. Biophys. Res. Cann.*, (1965) 27: 523-530.

[16] Peralta JR, Gardea Torresdey JL, Tiemann KJ, Gomez E, Arteaga S, Rascon E Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (Medicago sativa) L. B. *Environ Contam Toxicol* (2001) 66:727–734.

[17] Arun, K., Shanker, T., Cervantes, C., Loza-Tavera, H., & Avudainayagam, S.. Chromium toxicity in plants. *Environment International*, (2005) 31, 739-753.

[18] Akinci, I. E & Akinci, S. Effect of chromium toxicity on germination and early seedling growth in melon (*Cucumis melo* L.) African Journal of Biotechnology, (2010) 9(29), 4589 - 4594.

[19] Shanker AK, Sudhagar R, Pathmanabhan G Growth Phytochelatin SH and antioxidative response of Sunflower as affected by chromium speciation. In: 2nd international congress of plant physiology on sustainable plant productivity under changing environment, New Delhi (2003).

[20] Zeid IM Responses of Phaseolus vulgaris to chromium and cobalt treatments. *Biol Plant* (2001) 44:111–115.

[21] Tang SR, Wilke BM, Brooks RR, Tang SR Heavy-metal uptake by metal tolerant Elsholtzia haichowensis and Commelina communis from China. *Commun Soil Sci Plant Anal* (2001) 32:895–905.

[22] Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S Chromium toxicity in plants. *Environ Intern* (2005). 31:739-753.

[23] Kranner, I. and Colville, L. Metals and seeds: Biochemical and molecular implications and their significance for seed germination. *Environmental and Experimental Botany*, Vol. 72, No. 1, (August 2011), pp. 93-105.

[24] Belimov, A.A., N. Hontzeas, V.I. Safronova, S.V. Demchinskaya, G. Piluzza, S. Bullitta and B.R. Glick, Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). *Soil Biol. Biochem.,* (2005) **37:** 241-250.

[25] Arduini, I., L. Ercoli, M. Maritti and A. Masoni, Response of miscanthus to toxic cadmium applications during the period of maximum growth. *Environ. Exp. Bot*., (2006) **55:** 29-40.

[26] Ali, S., F. Zeng, L. Qiu and G. Zhang, The effect of chromium on growth, root morphology, photosynthetic parameters and transpiration of the two barley cultivars. *Biol. Plant.*, (2011) **55:** 291-296.

[27] Kochot, C.K., S.B. Golbale and A. Purohit, *In:* Textbook of pharmacognosy, Nirali Prakashan, Pune, pp. (1998) 17-18.

[28] Orhue, E.R & Ekhomun, A.M. Chromium effects on growth of early water leaf (*Talinumtriangulare*) in an Ulitisol. *American-Eurasian J. Agric. & Environ. Sci*., (2010) 7(5), 586-590.

[29] Stobart, A., W. Gri, I. Ameen-Bukhari and P. Sherwood, The effect of Cd_2^+ on the biosynthesis of chlorophyll in leaves of barley. *Physiologia Plantarum* (1985) 63, 293- 298.

[30] Padmaja, K., D.D.K. Prasad, A.R.K. Prasad, Inhibition of chlorophyll synthesis in *Phaseolus vulgaris* Seedlings by cadmium acetate. *Photosynthetica*., (1990) 24, 399-405.

[31] Van Assche, F., H. Clijsters, Effects of metals on enzyme activity in plants. *Plant Cell Environ.* (1990) 13,195- 206.

Table.1 Effect of Cr on the morphological parameters of Cow pea [*Vigna unguiculata* (L.) Walp.] on 14th Days.

Cr Treatments	G (%)	R L (cm)	S L (cm)	N of L	TLA	F W mg.fr.wt	D W mg.dr.wt	V I
Control	96 ± 4.8	5.0 ± 0.25	13.5 ± 0.675	6 ± 0.3	4.4 ± 0.22	4.682 ± 0.2341	0.586 ± 0.0293	1340 ± 67
05ppm	93 ± 4.65	4.2 ± 0.21	12 ± 0.6	6 ± 0.3	4.4 ± 0.22	3.208 ± 0.1604	0.532 ± 0.0266	1227 ± 61.35
25ppm	89 ± 4.45	3.5 ± 0.175	10.6 ± 0.53	5 ± 0.25	4 ± 0.2	2.746 ± 0.1373	0.487 ± 0.02435	1085 ± 54.25
50ppm	83 ± 4.15	3 .0 ± 0.15	7.9 ± 0.395	5 ± 0.25	3.2 ± 0.16	1.478 ± 0.0739	0.414 ± 0.0207	970 ± 48.5
75ppm	76 ± 3.8	2.8 ± 0.14	5.7 ± 0.285	3 ± 0.15	2.1 ± 0.105	1.995 ± 0.09975	0.308 ± 0.0154	752 ± 37.6
100ppm	51 ± 2.55	1.6 ± 0.08	4.4 ± 0.22	3 ± 0.15	2.1 ± 0.105	1.078 ± 0.0539	0.25 ± 0.0125	504 ± 25.2
125ppm	45 ± 2.25	1.2 ± 0.06	3.8 ± 0.19	3 ± 0.15	1.8 ± 0.09	0.869 ± 0.04345	0.134 ± 0.0067	340 ± 17
150ppm	23 ± 1.15	0.4 ± 0.02	3.2 ± 0.16	3 ± 0.15	1.2 ± 0.06	0.656 ± 0.0328	0.093 ± 0.00465	208 ± 10.4

± Standard deviation

G (%) – Germination percentage R L – Root Length S L – Shoot Length N of L – Number of Leaves
TLA – Total Leaf Area F W – Fresh Weight DW – Dry weightV I – Vigour Index.

Table.2. Effect of Cr on pigment content (mg/g fr. wt.) of Cow pea [*Vigna unguiculata* (L.) Walp.] on 14th Days.

Cr treatments	T I	P T	Chl 'a' mg.fr.wt	Chl 'b' mg.fr.wt	T Chl mg.fr.wt	Carotenoid mg.fr.wt
Control	0 ± 0	0 ± 0	2.87 ± 0.1435	2.05 ± 0.1025	4.92 ± 0.246	2.08 ± 0.104
05ppm	2.65 ± 0.1325	0.764 ± 0.0382	2.79 ± 0.1395	1.8 ± 0.09	4.59 ± 0.2295	1.75 ± 0.0875
25ppm	1.05 ± 0.0525	1.67 ± 0.0835	2.2 ± 0.11	1.32 ± 0.066	3.52 ± 0.176	1.02 ± 0.051
50ppm	0.702 ± 0.0351	2.89 ± 0.1445	1.67 ± 0.0835	0.97 ± 0.0485	2.64 ± 0.132	0.87 ± 0.0435
75ppm	0.586 ± 0.0293	4.23 ± 0.2115	1.02 ± 0.051	0.58 ± 0.029	1.6 ± 0.08	0.75 ± 0.0375
100ppm	0.367 ± 0.01835	5.7 ± 0.285	0.87 ± 0.0435	0.43 ± 0.0215	1.3 ± 0.065	0.68 ± 0.034
125ppm	0.258 ± 0.0129	6.58 ± 0.329	0.68 ± 0.034	0.28 ± 0.014	0.96 ± 0.048	0.51 ± 0.0255
150ppm	0.120 ± 0.006	7.84 ± 0.392	0.49 ± 0.0245	0.15 ± 0.0075	0.64 ± 0.032	0.22 ± 0.011

± Standard deviation

T I – Tolerance Index P T – Phytotoxicity Chl 'a' – Chlorophyll a Chl 'b' – Chlorophyll b

