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IASET

EFFECT OF CHROMIUM ON SEED GERMINATION AND SEEDLING GROWTH OF GREEN GARM (*PHASEOLS AUREUS* L) AND CHICKPEA (*CICER ARIETINUM* L)

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ABSTRACT

The objective of the study of the Hindon river water quality with respect to Cr contamination, water samples were collected from three different sites (Saharanpur, Baghpat and Gautam Budh Nagar). Cr toxicity in plants depends on its valence state. Cr exists in several oxidation states but the most stable and common forms are Cr (0), Cr (III) and Cr (VI) species. Cr (VI) as being highly mobile is toxic, while Cr (III) as less mobile is less toxic. Cr is taken up by plants through carriers of essential ions such as sulphate. Cr uptake, translocation, and accumulation depend on its speciation, which also conditions its toxicity to plants. Symptoms of Cr toxicity in plants are diverse and include decrease of seed germination, reduction of growth, decrease of yield, inhibition of enzymatic activities, impairment of photosynthesis, nutrient and oxidative imbalance. The germination study was conducted, with two varieties of Green gram (Pusa Ratna and Pusa Visha) and two varieties of Chickpea (Pusa 2085 and Pusa Green 112) under different concentrations of chromium treatment. The effect of different concentrations of hexavalent chromium Cr(VI) (10, 25, 50 and 100 mg/l) on seed germination root and shoot growth on Green gram and Chickpea. The study showed that germination and viability of seeds were negatively affected by elevated chromium concentration. Response of seedlings to chromium was more noticeable than that of seed germination.

KEYWORDS: Chickpea, Chromium, Green Gram, Hndon River, Seed Germination, Seedling Growth

INTRODUCTION

Chromium (Cr) is the 17th most abundant element in the Earth crust (Avudainayagam *et al*., 2003). It occurs naturally as chromite ($FeCr_2O_4$) in ultramafic and serpentine rocks or complexed with other metals in the forms like crocoite ($PbCrO_4$), bentorite Ca6 (Cr, Al)2 (SO4)3 and tarapacaite (K2CrO4), vauquelinite ($CuPb_2CrO_4P\ O_4OH$), among others (Babula *et al*., 2008). Chromium is widely used in industry for plating, alloying, tanning of animal hides, inhibition of corrosion, textile dyes and mordents, pigments, ceramic glazes, refractory bricks, and pressure-treated lumber (Avudainayagam *et al*., 2003). Due to this wide anthropogenic use of Cr, the consequent environmental contamination has been increased and has become an increasing concern since last several years (Zayed and Terry, 2003). Chromium exists in several oxidation states, but the most stable and common forms are Cr (0), the trivalent Cr (III), and the hexavalent Cr (VI) species. Cr (0) is the metallic form, produced in industry and is a solid with high fusion point when used for the manufacturing of steel and other alloys. Cr (VI) in the forms of chromate (CrO_4^{2-}), dichromate ($Cr2O_7^{2-}$) and CrO3 are considered the most toxic forms of chromium, as it presents high oxidizing potential, high solubility, and mobility cross the membranes in living organisms and in the environment. Cr (III) in the forms of oxides, hydroxides, and sulphates is less toxic as it is relatively insoluble in water, presents lower mobility, and is mainly bound to organic matter in soil and aquatic environments. Moreover, Cr(III) forms tends to form hydroxide precipitates with Fe at typical ground water pH values. At high concentrations of oxygen or Mn oxides, Cr (III) can be oxidized to Cr (VI) (Becquer *et al*., 2003; Peralta *et al*., 2009).

Leather industry is the major cause for the high influx of chromium to the biosphere. Hexavalent chromium is used extensively worldwide in various industrial activities is therefore considered a serious environmental pollutant and poses a threat to human health. Its presence in agricultural soils can be attributed to the use of organic wastes as fertilizer and the use of waste water for irrigation (Pillay *et al*., 2003). Chromium like others heavy metals do not degrade biologically, it remains stable for several months in the soil without changing its oxidation state. Cr(VI) is accumulated by plants and its accumulation is biomagnified at different tropic levels through food chain (Kotas and Stasicka, 2000; Rogival *et al*., 2007). High levels of metals in soil can be phytotoxic. Toxicity of Cr to plants depends on its valence state. Hexavalent chromium, Cr(VI) is highly toxic and mobile whereas trivalent chromium, Cr(III) is less toxic (Oliveira, 2012). Toxic effects of Cr on plant growth and development include alterations in the germination process as well as in the growth of roots, stems and leaves, which may affect total dry matter production and yield (Shanker *et al.*, 2005). The phytotoxic levels of chromium in most plants seem to limit its accumulation in the food chain. Because most plants have low Cr concentrations, even when grown on Cr rich soils, the food chain is well protected against Cr toxicity. Chromium interferes with several metabolic processes, causing toxicity to plants as exhibited by reduced seed germination or early seedling development (Sharma *et al*., 1995), growth of roots and biomass, causing chlorosis, photosynthetic impairing and finally, plant death (Scoccianti *et al*., 2006).

Seed germination is the first physiological process affected by Cr(VI). The ability of a seed to germinate in a medium containing Cr(VI) would be indicative of its level of tolerance to this metal (Peralta *et al.*, 2001). The highest risk for human health is when plants develop tolerance mechanisms against metals and when those plants are incorporated into the food chain. Chromium may reach human beings either through polluted drinking water sources or through the food chain or both and its accumulation in higher concentration may lead to cancer and associated health hazards. Although some heavy metals form an essential part of human and plant nutrition, their higher levels of plants uptake cause carcinogenic and mutagenic effects (Goyer., *et al*., 1977). Excessive level of heavy metals in the soil environment adversely affect the germination of seeds, plant growth, alter the level of biomolecules in the cells and interfere with the activities of many key enzymes related to normal metabolic and developmental processes (Zhang *et al*., 2009; Rahoui *et al*., 2010).

The uptake, translocation and accumulation of heavy metals in plants are mediated by integrated network of physiological, biochemical and molecular mechanisms. Generally industrial wastes include heavy metals are one of the major threats for agriculture practices because above critical levels they may turn into toxins and cause inhibition of growth and development for the most of the plant species and at times leads to death also (Weiqiang *et al.*, 2005). Heavy metal stress negatively affects the process associated with biomass accumulation and overall yield in almost all the major field grown crops by damaging several metabolic pathways and if not yield, damage they may get incorporated in our food supply through harvested crops. However plants have some defense mechanism to deal with the excess of heavy metals in the soil by which they can prevent or restrict the uptake of metals or minimize the toxic effects through metal excluders, accumulators and indicators. They may localize selected metals mostly in roots and stems, or they may accumulate and store other metals in non-toxic forms for later distribution and use (Aydinalp and Marinova, 2009).

MATERIALS AND METHODS:

The two varieties of Green gram (*Phaseolus aureus* L.) and two varieties of Chickpea (*Cicer arietinum L.*) were obtained from the Division of Genetics, Indian Agriculture Research Institute, Pusa campus, New Delhi and used as plant material for this study. Chromium stock solution was prepared by dissolving 141.4mg of Potassium dichromate ($K_2Cr_2O_7$) in 100ml of distilled water and was standardized. From this solution aliquots were freshly drawn for each experiment. Distilled water without chromium was used as control.

The seeds of two varieties of Green gram (Pusa Ratna and PusaVisha) and two varieties of Chickpea (Pusa 2085 and Pusa Green 112) of uniform size, color and weight were surface sterilized with 0.1% AR Grade mercuric chloride, $HgCl_2$ solution and washed 5-6 times with distilled water. Fifty seeds of Green gram and Chickpea were placed for each treatment and were placed equi-spacially in sterilized plastic plates, lined with filter paper soaked with different concentrations of chromium solutions. Each treatment was replicated three times. The number of seeds eliminated in each treatment was counted on the 7th day and the total germination percentage was calculated. The emergence of radical was taken as a criterion for germination. The seedlings from each treatment were randomly selected for the measurement of root length and shoot length. The seedlings were separated into root and shoot system. The fresh weights were measured with an electrical weighing balance. Thereafter, the petri dishes containing the fresh seedling were placed in a hot air oven at 80℃ for 24 hours for determination of dry weight of seedling. The dry matters of seedling were measured with electrical balance.

Germination Percentage (GP)

Germinated seeds were counted according to the seedling evaluation procedure as specified in the Handbook of Association of Official Seed Analysts (AOSA, 1983). The number of germinated seeds was recorded. Seven days after germination, the germination percentage (GP) was calculated using the following formula for each replication of the treatment (Tanveer *et al*., 2010), and percentage was calculated as:

$$\textbf{Germination percentage (GP)} = \frac{\text{Germinated seeds}}{\text{Total seeds}} \times 100$$

Statistical Analysis

Each treatment was analyzed with at least three replicates and the Standard Deviation (SD) was calculated. The data were expressed in X $\pm$ SD of three replicates.

RESULTS AND DISCUSSIONS

Effect of Chromium on Seed Germination

The effect on germination of all seeds were trended with chromium accrued from control to 100mg/l with variations depending on Cr(VI) concentration on two varieties of Green gram (Pusa Ratna and Pusa Vishal) and Chickpea (Pusa 2085 and Pusa Green 112) taken for studies.

The phytotoxin effect of chromium research carried out to examine the seed germination percentage of two varieties of Green gram and two varieties of Chickpea were given in Table 1. The results showed that similar phytotoxic effect of chromium on seed germination percentage of Green gram and Chickpea both positive and negative effects were seen on seed germination of Green gram and Chickpea. The maximum percentage of germination of Green gram

88.53±1.36, 88.93±1.07, was found for Pusa Ratna and Pusa Vishal `and Chickpea 86.90±0.60, 84.83±0.99 for Pusa 2085 and Pusa Green 112 were recorded as control respectively.

The germination percentages were recorded to be decreased gradually with progressive increase in Cr concentration. The minimum percentage of germination were recorded at 100 mg/l concentration of Cr on Green gram 54.83±0.95, 53.53±0.71 for Pusa Ratna and Pusa Vishal and Chickpea 47.97±0.49, 42.60±0.62 for Pusa 2085 and Pusa Green 112 respectively. There are several reports on the promontory and inhibitory effect of copper and chromium treatment on various plant species. Symptoms of Cr phytotoxicity include inhibition of seed germination or of early seedling development, reduction of root growth, leaf chlorosis and depressed biomass (Sharma *et al*., 1995). Low concentrations shows growth promotory and higher concentrations shows germination inhibitory effect in four varieties of *Vigna radiata*. Some heavy metals are essential micronutrients for plants but their excess may result in metabolic disorders and growth inhibition in most of the plant species (Claire *et al.*, 2000). Similar experiments were carried out in green gram under the influence of mercury (Jagatheeswari and Ranganathan, 2012), mung bean under lead acetate (Gautam *et al.*, 2008), in green gram under the effect of cobalt (Hussain *et al*., 2007). Increasing concentration of Cr leads to decreasing seed germination was observed in *Hibiscus esculentus* and some important pulses (Amin *et al.*, 2013; Jun *et al.*, 2009). Inhibition of germination percentage at higher concentrations of chromium was observed in soybean (Sidharthan and Lakshmanachary, 1996), mung bean (Rout *et al*., 1997), cowpea (Lalitha *et al.*, 1999), groundnut (Subramani *et al.*, 1999), black gram (Lakshmi and Sundaramoorthy, 2003), green gram (Samantary and Deo, 2004) and paddy, black gram and soybean (Sankar Ganesh *et al.*, 2006b; Sundaramoorthy *et al.*, 2006a,b).

Table 1: Effect of Cr (VI) On Seed Germination Percentage, Root Length, Shoot Length, Of Two Varieties of Green Gram (*Phaseolus Aureus* L) and Two Varieties of Chickpea (*Cicer Arietinum* L.) 7th Day's Seedling under Treatment

Varieties /Treatment Cr(VI) (Mg/L)		Germination Percentage	Root Length (Cm/Seedling)	Shoot Length (Cm/Seedling)
Green gram (Pusa Ratna)	C	88.53 (±1.36)	6.80±0.36	12.87±0.74
	10	88.47 (±0.85)	6.77±0.06	11.93±0.91
	25	73.63 (±0.64)	4.50±0.36	8.60±0.46
	50	63.63 (±0.70)	3.90±0.20	7.03±0.31
	100	54.83 (±0.95)	1.83±0.31	4.87±0.76
Green gram (Pusa Vishal)	C	88.93 (±1.07)	6.80±0.40	12.90±0.78
	10	89.43 (±0.99)	6.97±0.31	11.87±0.80
	25	72.13 (±0.40)	4.53±0.40	9.90±0.40
	50	65.23 (±0.83)	3.50±0.30	7.93±0.87
	100	53.53 (±0.71)	1.47±0.25	5.77±0.55
Chickpea (Pusa 2085)	C	86.90(±0.60)	8.53±0.40	12.07±0.75
	10	86.63(±0.55)	8.47±0.35	11.10±0.92
	25	76.77(±0.42)	6.53±0.35	9.60±0.44
	50	57.80(±0.90)	5.37±0.38	6.50±0.30
	100	47.97(±0.49)	3.73±0.56	5.20±0.56
Chickpea (Pusa Green 112)	C	83.80(±1.05)	8.93±0.35	13.07±0.57
	10	84.83(±0.99)	8.23±0.47	11.60±0.85
	25	68.97(±0.80)	6.57±0.25	8.17±1.52
	50	58.63(±0.86)	5.57±0.31	6.27±0.47
	100	42.60(±0.62)	3.93±0.51	5.37±0.51

Effect of Chromium on Seedling Growth

The root length of Green gram and Chickpea cultivars showed decreasing trends with increase in Cr concentrations are tabulated. Among the cultivars studied the Green gram (Pusa Vishal) exhibited maximum root length at C 6.97±0.31 and minimum length of root was observed at 100 mg/l Cr concentration of 1.47±0.25 of Green gram (Pusa Vishal) cultivars Table 1. In Chickpea the maximum root length of Pusa Green 112 exhibited at C 8.93±0.35 minimum length of root was observed at 100 mg/l Cr concentration of 3.73±0.56 of Pusa 2085 cultivers. Similar results were obtained by (Jamal *et al*., 2006) with two wheat (*Triticum aestivum*) varieties named Anmol and Kiran treated with chromium. This metal has been reported not to inhibit germination but impair the growth of new roots and seedling establishment (Rellén-Álvarez *et al*., 2006). Reductions of 32–57% in sugarcane bud germination was observed with 20 and 80 mg/L Cr, respectively (Jain *et al*., 2000). In fact, roots were observed shorter and brownish and presented less number of roots hairs in chromium-treated plants in contrast to the control, in which thin, elongated roots were formed. The root length and shoot length of *Arachis hypogea* were found to be affected by the increasing concentrations of Cr(VI) (Rajalakshmi *et al*., 2010) have also been reported that root growth was comparatively more inhibited than shoot of rice (*Oryza Sativa* L.) cultivars.

In Green gram the maximum shoot length was found at control 12.90±0.78 for Pusa Vishal and minimum was observed at 100 mg/l for 4.87±0.76 for Pusa Ratna and maximum shoot length in Chickpea (Pusa Green 112) found at control 13.07±0.57 and minimum at 100 mg/l 5.20±0.56 of Pusa 2085 cultivers. There was gradual decrease in shoot and root length with the increase in Cr concentration from 0 to 100 mg/l. The effect of chromium in shoot and root lengths were gradual decrease in melon plant (Akinci and Akinci, 2010). In cobalt treatments gradual decrease in shoot and root lengths with increased cobalt concentrations (Khan *et al.*, 2010) in chick pea. (Abdul Ghani, 2011) has been reported that increasing concentrations of chromium caused significant reduction in root length and shoot length of *brassica juncea* L. (Bishoni, 1993) reported that Cr(VI) did not affect the percentage germination but suppressed the growth of radical and plumule, significantly and its effect was more pronounced on roots than on the shoots. (Arduini *et al*., 2006) have reported that root growth of miscanthus was less affected than shoot growth, but root morphology changed drastically. Also, (Samantary, 2002) have reported that the development of lateral roots and root number was affected by Cr exposure. Moreover, roots of *Zea mays* L. treated with Cr(VI) were shorter and brownish and presented less number of roots hairs (Mallick *et al.*, 2010). Decrease in root growth in presence of Cr(VI) can be explained by inhibition of root cell division and/or elongation, which might have occurred as a result of tissue collapse and consequent incapacity of the roots to absorb water and nutrients from the medium (Barcel´o *et al.*, 1985). The decrease in plant height could be due to the reduced root growth and consequent decreased nutrients and water transport to the higher parts of the plant. Moreover, Cr transport to the aerial part of the plant can directly impact cellular metabolism of shoots contributing to the reduction in plant height (Oliveira, 2012).

Fresh Weight and Dry Weight

The fresh weight and dry weight of root and shoot of seedlings are presented in (Fig 1 and Fig 2). Fresh weight and dry weight also showed decreasing trends with increase in Cr concentration. Green gram (Pusa Ratna) and exhibited maximum fresh weight and dry weight at Control 0.34±0.01, 0.16±0.03 and minimum fresh weight and dry weight were observed at 100 mg/l Cr concentration of 0.14±0.01, 0.05±0.01 of Pusa Vishal cultivars. In Chickpea the maximum fresh weight and dry weight at Control Pusa 2085 0.35±0.01, 0.18±0.03 and minimum fresh weight and dry weight were observed at 100 mg/l Cr concentration of 0.13±0.01, 0.07±0.01 of Pusa Green 112.

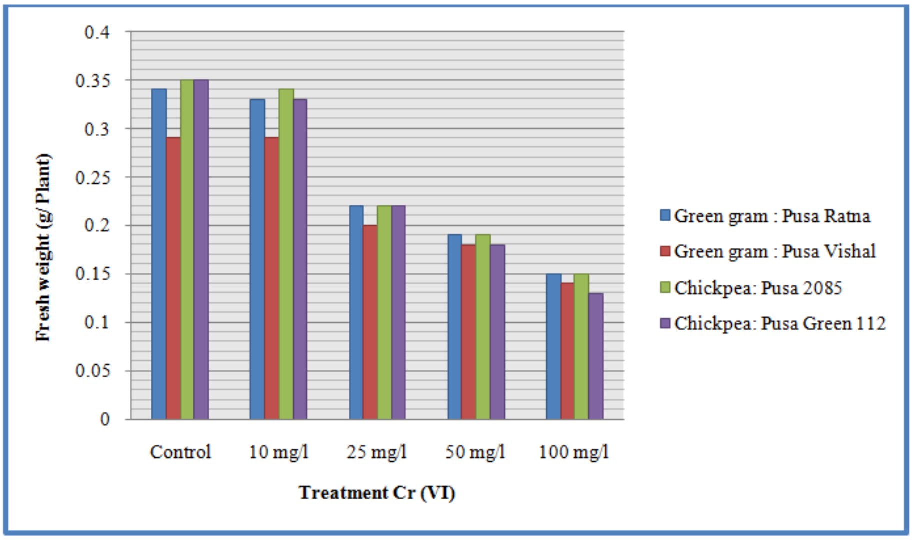

Figure 1: Effect of Cr (VI) on Fresh Weight (G/Plant) of Two Varieties of Green Gram (*Phaseolus Aureus* L) and Two Varieties of Chickpea (*Cicer Arietinum* L.) 7th Day's Seedlings under Treatment

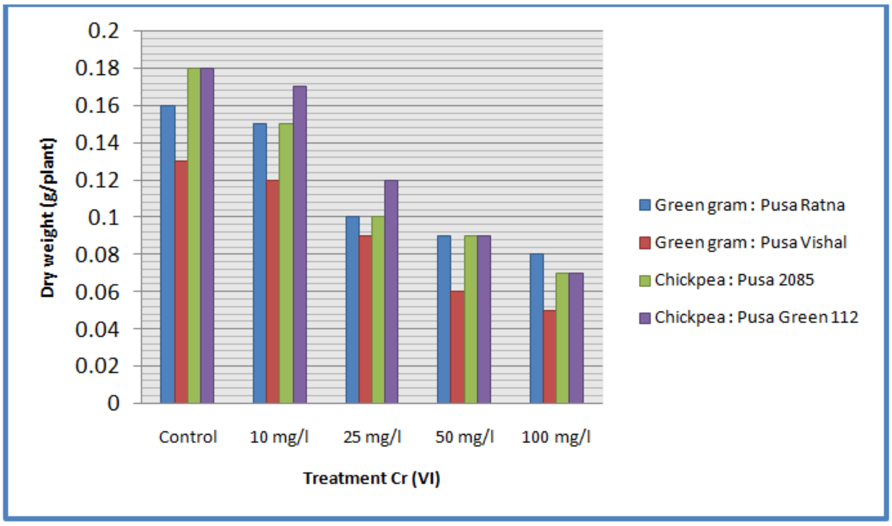

Figure 2: Effect of Cr (VI) On Dry Weight (G/Plant) of Two Varieties of Green Gram (*Phaseolus Aureus* L) and Two Varieties of Chickpea (*Cicer Arietinum* L.) 7th Day's Seedlings under Treatment

CONCLUSIONS

The present investigation revealed that the increase in Cr (VI) concentration causes inhibition of germination and seedling growth as represented by root and shoot length effect. Inhibition effect of Cr (VI) was more pronounced in root length than shoot length of all seeds and was accompanied with morphological changes in root. It can be concluded that the Cr (VI) are toxic to seed. So, this polluted water should be properly treated to remove the Cr (VI) and if it is to be used for irrigation and such simililar uses, it must be treated appropriately.

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