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Toxic affects of chromium on some quality parameters of *Sorghum bicolor* (L.)

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Abstract: A pot experiment was conducted to determine the effects of varying Cr (VI) levels [0.0–4.0 mg Cr (VI) kg^{-1} soil in the form of potassium dichromate] on the some quality parameters of sorghum *Sorghum bicolor* (L.). Quality parameters was measured in terms of protein content, chlorophyll content, and IVDMD (*In vitro dry matter digestibility*) content at different growth stages, *i.e.* 35 DAS, 70 DAS and 90 DAS (Days after sowing) that were adversely affected with an increase in Cr (VI) levels from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil. The decline in protein content 4.67 g/kg dry weight at T_4 was observed as compared to control (T_1) (8.96 g/kg dry weight basis) at 35 DAS in leaves , Total chlorophyll content declined from 3.25 mg g^{-1} fresh weight (T_1) to 2.40 mg g^{-1} fresh weight (T_4) at 35 DAS and IVDMD content declined from 43.60 to 33.60 per cent dry weight basis with increment in chromium concentration. It is concluded that Cr (VI) at higher doses (4.0 mg Cr (VI) kg^{-1} soil) adversly affects the quality parameters of Forage sorghum *Sorghum bicolor* (L.) however, quality parameters are responsible for nutritive value of sorghum *Sorghum bicolor* (L.).

Keywords: Chlorophyll content, Chromium toxicity, Quality parameters, *Sorghum bicolor*

INTRODUCTION

Human and animals are directly dependent upon plants for their survival because plants are the prime source of food, fibre and drugs. Plants in turn affected by a number of natural phenomenon. The environmental factor effects plant productivity and among them heavy metal stress reduces it. Agricultural soils occasionally contain phytotoxic levels of heavy metals, but more frequently they accumulate it as a result of various developmental and economic activities of mankind (Wagner, 1993; Kumar, 2010).Certain metals such as iron (Fe), copper (Cu) and zinc (Zn) are considered as essential nutrients to plants and are needed for photosynthesis and as cofactors for many enzymes (Kovacik *et al.*, 2010; Shanmugam *et al.*, 2011). It is well documented that Cr is a toxic agent for the growth and development of plants (Zou *et al*.,2006; Mohanty and patra.,2013). In addition, it is known as one of the causes of environmental pollution (Singh *et al.,* 2013). In plants, Cr is found in the forms of trivalent Cr^{3+} and hexavalent Cr^{6+} species, where the former is of lower toxicity than the latter. Cr is transported and accumulated via carrier ions such as sulfate or iron and is not directly absorbed by plants (Singh *et al.,*2013 and Gajalakshmi *et al.,* 2012) . Moreover, under reducing condition, Cr^{6+} is converted to its more toxic form Cr^{3+}, which can indirectly influence and change soil pH to both alkalinity or acidity extremes, depending on prevailing condition in soil subsurface (Hawley *et al.,*2004). This phenomenon might perturb the nutrients bioavailability and their sorption by plants. The highest concentration of Cr occurs in the root rather than other parts of plants (Kumar and Maiti, 2013). Cr (VI) as being highly mobile is toxic, while Cr (III) as less mobile is less toxic (Oliveira, 2012). Chromium toxicity in plants is observed at multiple levels, from reduced yield, through effects on leaf and root growth, to inhibition on enzymatic activities and mutagenesis. There are many studies on Cr toxicity in crop plants. Chromium significantly affects the metabolism and antioxidative mechanism of plants such as barley (*Hordeum vulgare*) (Ali *et al*., 2004), cauliflower (Chatterjee and Chatterjee, 2000), wheat (*Triticum aestivum* cv. HD2204) (Sharma *et al*., 1995), maize (*Zea mays*) (Sharma and Pant, 1994) and sorghum (Sihag *et al.,*2016).

Sorghum (*Sorghum bicolor* L.) moench is the world's fifth most important cereal crop, after rice, corn, wheat and barley; and the third leading crop in the USA. It belongs to family *Gramineae,* and also known as 'durra', 'chari' or 'jowar'. Sorghum is cultivated for food, feed, fodder and the production of alcoholic beverage, but extensively grown for fodder in north India during *kharif* season due to its greater adaptability, high fodder yield, better palatability, quality and digestibility. So, the present investigation was aimed to

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find out phytotoxic effects of chromium (VI) on quality parameters and mineral content of sorghum which is an important fodder crop.

MATERIALS AND METHODS

Experimental site and treatment: A nutrient deficient loamy sand soil from Regional Research Station, Gangwa block of Hisar district was used in the present study. Its characteristics were : pH (1:2) 8.50; organic carbon, 0.22%; EC 1.5; N 4.0 mg kg^{-1} soil; P 13.0 mg kg^{-1} soil; K 163 mg kg^{-1} soil; Zn^{2+} 0.61 mg kg^{-1} soil; Fe^{2+} 0.9 mg kg^{-1} soil; Cu^{2+} 0.18 mg kg^{-1} soil; Mn^{2+} 3.65 mg kg^{-1} soil; Cr^{2+} 0.01 mg kg^{-1} soil.Seeds of *Sorghum bicolor* (cv. HJS-541) were procured from Forage section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar and raised in earthen pots filled with 5 kg of chromium free sandy loam soil in a naturally lit net house. The soil in each pot was treated with different levels of chromium (VI) (0.0, 1.0, 2.0 and 4.0 mg Cr (VI) kg^{-1} soil) in the form of potassium dichromate. At weekly intervals, the plants were supplied with equal quantities (250ml) of nutrient solution.

T_1 = control (No treatment)
T_2 = 1.0 mg Cr (VI) kg^{-1} soil
T_3 = 2.0 mg Cr (VI) kg^{-1} soil
T_4 = 4.0 mg Cr (VI) kg^{-1} soil

Plant sampling: Plants were harvested at three stages of growth, *i.e.* vegetative (35 DAS), 50% flowering (70 DAS) and grain filling (90 DAS) stages. Immediately after harvest, the plant samples were dissected into leaves, shoots (stem + petiole), and roots sun dried and kept in a hot air oven (70 °C) followed by grinding in a Micro-Wiley mill (2 mm sieve) purchased from Scientific Equipment Works (SEW), New Delhi, India. The plants treated with Cr (VI) concentration 4.0 mg Cr (VI) kg^{-1} soil died after 70 DAS, therefore, all the observations were recorded up to 2.0 mg Cr (VI) kg^{-1} soil .

Chemical analysis

Photosynthetic pigments: The photosynthetic pigments i.e. total chlorophyll, chlorophyll 'a' and chlorophyll 'b' afirst fully expanded leaf were extracted and estimated as per the method of Hiscox and Israelstum (1979).

***In vitro* dry matter digestibility (IVDMD):** IVDMD was determined in three replicates (three separate run) by the method of Barnes et al. (1971).

Protein content: Protein content (g/kg dry weight basis) in various plant parts was estimated by conventional Micro-Kjeldahl method.

Statistical analysis: The experimental data was analyzed by the application of CRD design using OPSTAT software available on CCSHAU Home Page (Sheoran, O.P.)

RESULTS

Symptoms: Visual observations revealed no toxicity symptoms on aerial parts were observed at low concentration (1.0 mg Cr (VI) Kg^{-1}) of chromium (VI). However, yellowing of leaves, tip burning and inter veinal chlorosis, changing to brown patches which spread throughout the leaves started to appear after 20 DAS at 2.0 mg Cr (VI) kg^{-1} soil. Chlorosis followed by yellowing of leaves and finally a brittle condition followed by abscission of leaves was observed at 4.0 mg Cr (VI) kg^{-1} soil.

Effect of chromium on total chlorophyll content: Perusal of data in Table 1 indicates that total chlorophyll and chlorophyll-a and b content decreased with increasing concentration of Cr (VI) in leaves of sorghum plant , decline effect was observed at all the stages of growth i.e. 35, 70 and 90 DAS. At 70 DAS, total chlorophyll content decreased from 4.61 to 4.04 mg g^{-1} fresh weight and chlorophyll a decreased from 2.88 to 1.98 mg g^{-1} fresh weight, chlorophyll b content decreased from 1.73 to 2.06 mg g^{-1} fresh weight with increasing concentration of Cr (VI) from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil However, with 1.0 mg Cr (VI) kg^{-1} soil total chlorophyll content increased at 70 and 90 DAS and then decreased with further increase in Cr (VI) concentration. As compared to control plants, chlorophyll b content also decreased from 1.48 to 1.03 mg g^{-1} fresh weight of leaves, with increasing concen-

Table 1. Effect of Cr (VI) on total chlorophyll and chlorophyll-a and b (mg g^{-1} fresh weight) in leaves of sorghum (*Sorghum bicolor* (L.) plant at different growth stages.

Cr (VI) mg kg^{-1} soil	Days after sowing								
	35	70	90	35	70	90	35	70	90
	Total chlorophyll			Chlorophyll-a			Chlorophyll-b		
T_1	3.25	4.61	2.90	1.77	2.88	1.45	1.48	1.73	1.45
1.0	3.12	5.10	2.91	1.69	2.44	1.41	1.43	2.66	1.50
2.0	2.73	4.45	2.32	1.57	2.00	1.26	1.16	2.45	1.06
4.0	2.40	4.04	-	1.37	1.98	-	1.03	2.06	-
	A	B	A×B	A	B	A×B	A	B	A×B
SE (m)	0.09	0. 10	0. 17	0. 03	0.03	0.05	0.09	0. 10	0. 17
CD at 5%	0. 26	0. 30	0. 51	0. 08	0. 09	0. 16	0. 26	0. 30	0. 51

Each value is the mean of three determinations. T_1= control, *Plants unable to survive with 4.0 mg Cr (VI) kg^{-1} soil at 90 DAS, A = Treatment, B = Stages, A×B = Interaction

Table 2. Effect of Cr (VI) on crude protein content (per cent dry weight) in leaves, stem and root of sorghum *(Sorghum bicolor* (L.) plant at different growth stages.

Cr (VI) mg kg^{-1} soil	Days after sowing								
	35	70	90	35	70	90	35	70	90
	Leaves			Stem			Root		
T_1	8.96	11.54	10.11	5.69	7.89	4.13	2.35	4.57	3.32
1.0	6.16	7.91	6.44	4.46	5.67	4.88	2.07	3.57	3.35
2.0	6.03	7.46	6.25	3.88	4.68	3.88	1.87	2.77	2.46
4.0	4.67	5.73	-	3.35	3.68	-	1.45	1.88	-
	A	B	A×B	A	B	A×B	A	B	A×B
SE (m)	0.03	0.03	0.06	0.03	0.04	0.06	0.03	0.03	0.05
CD at 5%	0.07	0.08	0. 13	0.09	0.07	0.10	0.08	0.09	0.13

Each value is the mean of three determinations, T_1= control, *Plants unable to survive with 4 .0 mg Cr (VI) kg^{-1} soil at 90 DAS, A = Treatment, B = Stages, A×B = Interaction

Table 3. Effect of Cr (VI) on *in vitro* dry matter digestibility (per cent dry weight basis) in leaves and stem of sorghum (*Sorghum bicolor* (L.)plants at different growth stages.

Cr (VI) mg kg^{-1} soil	Days after sowing					
	35	70	90	35	70	90
	Leaves			Stem		
T_1	55.60	51.80	48.40	43.60	42.40	38.60
1.0	54.4	48.50	46.83	40.20	37.40	35.50
2.0	50.40	48.50	46.83	38.40	34.40	32.40
4.0	48.80	46.40	-	33.60	31.40	-
	A	B	A×B	A	B	A×B
SE (m)	0.07	0.09	0.15	0.08	0.09	0.16
CD at 5%	0.16	0.24	0.42	0.21	0.23	0.36

Each value is the mean of three determinations, T_1= control, *Plants unable to survive with 4.0 mg Cr (VI) kg^{-1} soil at 90 DAS, A = Treatment, B = Stages, A×B = Interaction

tration of Cr (VI) from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil at 35 DAS then with further increase in Cr (VI) concentration. Progress of growth, resulted into increase in total chlorophyll, chlorophyll-a and b content, had a maximum value at 70 DAS and showed a declining trend at 90 DAS (Table 1).

Effect of Cr (VI) on crude protein content: Crude protein (Per cent dry weight) content in various organs of sorghum plant decreased with increasing levels of Cr (VI) from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil at all the stages of growth. It was decreased from 11.54 to 5.73, 7.89 to 3.68 and 4.57 to 1.88 percent, with increasing level of Cr (VI) from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil in leaves, stem and roots, respectively at 70 DAS. Crude protein percent was highest in leaves followed by stem and roots. With growth, it was increased and become maximum at 70 DAS and then decreased at 90 DAS (Table 2).

Effect of Cr (VI) on *in vitro* dry matter digestibility: *In vitro* dry matter digestibility (IVDMD) of leaves and stem decreased continuously and markedly with increasing concentration of Cr (VI) from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil. In leaves of control plant, it was 55.6, 51.8 and 48.4 per cent and decreased to 50.4, 48.5 and 46.8 per cent in 2.0 mg Cr (VI) kg^{-1} soil treated plants at 35, 70 and 90 DAS, respectively. In leaves of 4.0 mg Cr (VI) kg^{-1} soil treated plants, it was further decreased to 48.8 and 46.40 percent at 35 and 70 DAS and plants were died at 90 DAS with 4.0 mg Cr (VI) kg^{-1} soil. Similarly, IVDMD of stem also decreased from 43.60 to 33.60 and 42.40 to 31.40 per cent, with increasing concentration of Cr (VI) from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil at 35 and 70 DAS, respectively. IVDMD of leaves was more than stem and it was decreased with advancement of maturity from 35 to 90 DAS, in both leaves and stem (Table 3).

DISCUSSION

Effect of chromium (VI) toxicity on photosynthetic pigments: Total chlorophyll, chlorophyll a and chlorophyll b content in fresh leaves were decreased significantly by application of Cr (VI) on sorghum plant, at all the stages of growth. The decrease in chlorophyll content was also reported in sunflower (Zengin and Munzuroglu,2006) and in almond (Elloumi et al., 2007) The observed decrease in photosynthetic pigments in Pea (*Pisum sativum* L.) seedlings with increasing Cr concentration is in accordance with the results of Bishnoi *et al.* (1993) and Joshi *et al.* (1999) in legumes. This decrease could be due to imparted biosynthesis of chlorophyll or increased activity of chlorophyllase. This contention gets the support from the work of Vajpayee *et al.* (2000), who observed that Cr application had impaired the biosynthesis of chlorophyll by inhibiting the activities of delta-amino levulenic acid dehydratase (ALAD). Diwan *et al.* (2012) also observed decrease in chlorophyll content by Cr (VI) application in Indian mustard. John *et al*., (2009) studied that the plant exhibited a decline in growth, chlorophyll content and carotenoids with Cd and Pb but Cd was found to be more detrimental than Pb treatment in *B. juncea*. Sheetal *et al.* (2016) in their study revealed that as the contamination level increased significant reductions were observed in biomass, photosynthetic rate and chlorophyll a and b contents as compared to control plants in *mustard Mustard (Brassica juncea)* var. *Pusa Jaikisan*. Singh *et al*. (2012) studied the effect of Pb and Ni on chlorophyll a, b, carotenoids and proline content of Black gram (*Vigna mungo* L.) seedlings were evaluated under 10, 50 and 100 µM concentration. These concentrations significantly affected chlorophyll, carotenoid and proline content of Black gram as compared to control.

Nagajyoti *et al.* (2008) reported the reduction in Chlorophyll a, Chl. b and total chlorophyll in groundnut by industrial effluents containing heavy metals. Chlorophyll content decreased in leaves exposed to Cd stress were also reported in *V. mungo* (Singh *et al.,* 2008). Reduced chlorophyll content due to nickel toxicity in different plant species such as in *green gram* has been well documented (Pandey and Pathak, 2006). Shanker (2003) reported the, decrease in the chlorophyll *a/b* ratio brought about by Cr and indicated that Cr toxicity possibly reduces size of the peripheral part of the antenna complex. The decrease in chlorophyll *b* due to Cr could be due to the destabilization and degradation of the proteins of the peripheral part. The inactivation of enzymes involved in the chlorophyll biosynthetic pathway could also contribute to the general reduction in chlorophyll content in most plants under Cr stress. Further, stimulation of the activity of peroxidase by Cr (VI) application might result into peroxidative damage of thylakoids membrane and thus results into decrease in chlorophyll content also observed by Sinha *et al* ., (2005) in spinach. Bera *et al*. (1999) studied the effect of Cr present in tannery effluent on chloroplast pigment content in mung bean and reported that irrespective of concentration, chlorophyll a, chlorophyll b and total chlorophyll decreased in 6-day-old mung bean seedlings as compared to control. Chlorophyll content was high in tolerant calluses in terms of survival under high Cr concentration in a study of Cr and Ni tolerance in *E. colona* (Samantaray *et al*., 2001). Chlorophyll content decreased as a marked effect of various concentrations of different Cr compounds [Cr (III) and Cr(VI)] in *Triticum aestivum* (Sharma and Sharma, 1996).

Cauliflower (cv. Maghi) grown in refined sand with complete nutrition (control) and at 0.5 mM each of Co, Cr and Cu showed drastic decrease in chlorophylls a and b in leaves in the order Co>Cu>Cr (Chatterjee and Chatterjee, 2000). The influence of 1 and 2 mg L^{-1} Cr (VI) on *Salvinia minima* decreased chlorophylls a and b and carotenoid concentrations significantly (Nichols *et al*., 2000). The decrease in the chlorophyll a/b ratio (Shanker, 2003) brought about by Cr indicates that Cr toxicity possibly reduces size of the peripheral part of the antenna complex. The decrease in chlorophyll b due to Cr could be due to the destabilization and degradation of the proteins of the peripheral part. The inactivation of enzymes involved in the chlorophyll biosynthetic pathway could also contribute to the general reduction in chlorophyll content in most plants under Cr stress.

Effect of chromium (VI) toxicity on crude protein content: The observed decrease in crude protein content in leaves, stem and root by Cr (VI) application might be either due to leaching of nitrogen from plant or due to damage of enzymes responsible for nitrogen uptakes. Further, the results are in accordance with the earlier findings in guar (Sumanlata, 1995), sunflower (Mehta, 1996) brassica (Bardwaj, 1998) and sorghum (Sandeep Kumar *et al*., 2010) for the heavy metal chromium. Our studies of soluble protein content coincides with the findings of Singh and Sinha (2005) who found decrease in soluble protein content in B. juncea when grown on various amendments of tannery waste containing heavy metals. Decrease in the protein content has also been found in aquatic plants when treated with metalliferous wastewater. However, increased concentration of salt stress increases protein content in Pisum sativum (Ahmad and Jhon, 2005). Ahmad et al., (2006) reported the increase in protein content with lower concentrations of salt (Na_2CO_3) and decrease with higher concentration of salt (Na_2CO_3) in mulberry. John *et al*., (2009) reported that the protein content was decreased by Cd (900 µM) to 95% and 44% by Pb (1500 µM) at the flowering stage in *Brassica juncea L.* The decrease in protein content in *L. polyrrhiza* may be caused by enhanced protein degradation process as a result of increased protease activity (Palma et al. 2002) that is found to increase under stress conditions. John *et al.,* (2008) studied that increase in concentration of Cd and Pb in Duckweed plants (*Lemna polyrrhiza* L.) decreased the protein content.

***In vitro* dry matter digestibility (IVDMD):** Forage quality varies with the varieties, species and plant parts and is also influenced by growth stage, soil fertility and environment in which forages are grown (Luthra and Joshi, 2002). Forage quality is a function of nutrients concentration of the forage, its intake, digestibility and partitioning of metabolized products with in the animal. Stockdale (1993) observed a reduction in the digestibility coefficient of Persian clover grown in chromium containing water, structural carbohydrates viz. NDF (neutral detergent fibre), ADF (acid detergent fibre), hemicellulose, cellulose and lignin contents increased, shoots of guar and cowpea plants were also found to be more fibrous than leaves. Further, the observed decrease *in vitro* dry mater digestibility (IVDMD) by 14.5 and 12.8 per cent in leaves and shoots of cowpea at 4 ppm Cr (VI), respectively was mainly due to the corresponding increase in fibre components i.e. NDF and ADF (Rani *et al.*, 1998). A similar decrease in IVDMD of guar leaves and shoots with chromium application was reported by Sumanlata *et al.* (1999).*In vitro* dry matter digestively which takes into accounts all the Known and unknown factors being negatively effected by Cr (VI) was chiefly dependent upon the concentration of cellulose and hemicellulose which in turn is influenced by the degree of lignifications and silification. Silica and lignin content have also been found to reduce the IVDMD (Kumar *et al*., 2010). In the present study also the decrease in IVDMD of leaves and stem is owing to presence of higher content of lignified fiber in Cr (VI) treated plants. Due to toxic effects of chromium progressive increase in structural carbohydrates and decrease in IVDMD with age of plants (in leaves and stem) was in accordance with earlier findings in guar Sumanlata (1995) and in Sorghum Sandeep Kumar *et al*., (2010). Further, at high concentration of chromium the higher digestibility at pre flowering stage in leaves and stem was mainly due to less lignification of cell wall and higher cell content in guar Sumanlata (1995) and in Sorghum Sandeep Kumar *et al*., (2010) also observed similar results.

Conclusion

The Cr absorption abilities for different parts of the plants varied. Roots accumulated more Cr (VI) followed by leaves and stems. Protein content and Total cholrophyll content (chlorophyll a and chlorophyll b) decreased in all plant parts whereas, IVDMD of leaves and shoots decreased mainly due to increase in fibre components (NDF and ADF). So, we concluded that Cr (VI) application adversely affected the quality parameters of forage sorghum by decreasing IVDMD, protein and cholorophyll content.

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