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Chromium Toxicity affects Structural and Non Structural Carbohydrate Value of *Sorghum bicolor* (L.)

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KEYWORDS

Structural and non structural carbohydrate ;Neutral detergent fibre (NDF); Acid detergent fibre (ADF);Chromium; Metal toxicity; Sorghum bicolor.

A B S T R A C T

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 structural and non structural carbohydrate value of sorghum. The present investigation showed that the structural carbohydrate *viz* NDF, ADF,Cellulose, Hemicellulose, lignin and Silica content in leaves and stem are increased and non structural carbohydrate includes Total soluble sugar, Reducing and Non reducing sugar in leaves, stem and root decreased in plant 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.

Introduction

Metals are necessary components of all ecosystems and occur naturally in the earth's crust (Pinto *et al.*, 2003). They appear in a wide range of oxidative states influencing their chemical characteristics and thus their bioavailability and toxicity (Pinto *et al.*, 2003; Verbruggen *et al.*, 2009). 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).Chromium exists in several oxidation states and the impact of its contamination on the physiology of plants depends on the metal speciation, which is responsible for its mobilization, subsequent uptake and resultant toxicity in the plant system (Shanker *et al.*, 2005). The most stable and common forms of Chromium are Cr (III) and Cr (VI) species. Cr toxicity in plants depends on its valence state. Cr (VI) as being highly mobile is toxic, while Cr (III) as less mobile is less toxic (Oliveira, 2012). Chromium is found in all phases of the environment, including air, water and soil. Naturally occurring in soil, chromium ranges from 10 to 50 mg kg^{-1} depending on the parental material. Chromium (VI) is a strong oxidant with a high redox potential in the

range of 1.33-1.38 eV accounting for a rapid and high generation of ROS (Reactive Oxygen Species) and resultant toxicity (Shanker *et al.*, 2004a).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 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.

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.

Materials and Methods

Soil

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.

Plant growth conditions

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. The plant samples from each treatment were collected at different stages *viz.* vegetative (35 DAS), flowering (70-75 DAS), and grain filling (90-100 DAS) stages. The plants were treated with following Cr (VI) concentrations:

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

Material and Methods

Non-structural Carbohydrates

Extraction of sugars

One hundred mg of powdered samples was taken in a 100 ml flat bottomed volumetric flask with 10 ml of 80% ethanol. The flask was kept in water bath ,maintained at 70 °C, for 1 hour and the filtrate was collected in a 25 ml volumetric flask. The extraction procedure was repeated five times. The final volume was made to 25 ml with 80% ethanol.

Total soluble sugars

Total soluble sugars in roots were estimated by the method of Dubois *et al.* (1956).

Reagents

2% phenol (w/v) : prepared by dissolving 2 g phenol in 80 ml of distilled water and volume was made to 100 ml.

Concentrated H_2SO_4 (G.R.)

Procedure

One ml of the diluted sugar extract (1 ml of sugar extract + 9 ml of distilled water) was taken in a test tube. 2 ml of 2% phenol solution was added followed by 5 ml of concentrated H_2SO_4. The test tubes were allowed to cool for 30 minutes. The absorbance of the solution was measured at 490 nm on UV-Vis spectrophotometer (systronic 118). The concentration of total sugars was calculated from the standard curve of glucose prepared simultaneously and expressed as mg g^{-1} dry weight.

Reducing sugars

Reducing sugars were determined by the Nelson Somogyi's method (1944).

Alkaline Copper reagent

Solution A: 25 g anhydrous sodium carbonate, 25 g sodium –potassium tartrate, 20 g sodium bicarbonate and 200 g anhydrous sodium sulfate were dissolved in 800 ml of water and finally the volume was made to 1 litre.

Solution B: 15 % (w/v) $CuSO_4.5H_2O$ containing one or two drops of conc. H_2SO_4 was prepared.25 parts of solution A and 1 part of B were mixed just before use.

Arsenomolybdate colour reagent: 25 g of ammonium molybdate was dissolved in 450 ml of distilled water and 21 ml of conc. H_2SO_4 was added to it. 3.0 g of disodium hydrogen arsenate was dissolved in 25 ml of water and was added to the acidified molybdate solution with constant stirring. The solution, thus prepared, was kept at 37°C for 24-48 h before use.

Procedure

1 ml of alkaline copper reagent was added to the 1 ml of the sugar extract in boiling test tubes. The test tubes were then placed in boiling water bath for 20 minutes. The test tubes were cooled and 1 ml of arsenomolybdate solution was added to it. The volume of each test tube solution was made up to 10 ml using distilled water. Then, absorbance was read at 520 nm using reagent blank containing 1 ml of distilled water in place of sugar extract (rest of the reagents and procedure was same as in case of samples) to set UV-Vis spectrophotometer (systronic-118) at zero O.D. The concentration of reducing sugars was calculated from the standard curve of glucose prepared simultaneously and expressed as mg g^{-1} dry weight.

Non – reducing sugars

The concentration of non-reducing sugars was calculated by subtracting the reducing sugars from the total sugars.

Structural Carbohydrates

Structural carbohydrates viz. acid detergent fibre, cellulose, lignin and silica were

Neutral Detergent Fibre (ADF)

Reagents

Neutral detergent solution: Prepared by dissolving 18.61 g disodium ethylene diamine (EDTA) and 6.81 g sodium borate decahydrate in about 500 ml of distilled

water by heating on a boiling water bath. Then 30 g sodium lauryl sulphate dissolved in about 200 ml of hot distilled water was added.To this 4.56 g anhydrous disodium hydrogen phosphate dissolved in about 100 ml hot distilled water was mixed. After cooling, 10 ml of 2- ethoxy ethanol was added to the mixture and volume made one litre.

Acetone

Sodium sulphite

Procedure

One g dried plant tissue was refluxed with 100 ml of neutral detergent solution containing 0.5 g sodium sulphite in one litre beaker (without spout) for 1h on a refluxing apparatus. The extracted material was filtered through a tared Gooch crucible (G-1) under vaccum. Any residue left in the beaker was transferred to the Gooch crucible with hot distilled water (90-100^{0}C) and filtered again.This washing procedure was repeated twice with hot distilled water and final washing was done with acetone till the filterate was free from colour. The crucible was kept in an oven maintained at 100^{0}C for over night, cooled in a dessicator and weighed again. The gain in the weight of the crucible was expressed as neutral detergent fibre and expressed on percentage basis

Acid Detergent Fibre (ADF)

Reagents

Acid detergent solution: Prepared by dissolving 20 g cetyl trimethyl ammonium bromide (CTAB) in one litre of 1N sulphuric acid with gradual shaking; Acetone

Procedure

One g dried plant sample was refluxed with 100 ml of acid detergent solution in one litre beaker (without spout) for 1 h on a refluxing apparatus. The extracted material was filtered through a weighed Gooch crucible (G-1) under vacuum. Any residue left in the beaker was transferred to the Gooch crucible with hot distilled water (90-100^{0}C) and filtered again. This washing procedure was repeated twice with hot distilled water and final washing was done with acetone till the filtrate was free from color. The crucible with residue was kept in an oven maintained at 100^{0}C for overnight, cooled in a dessicator and again weighed. The residue weight was expressed as acid detergent fibre and expressed on per cent dry weight basis.

Hemicellulose

The hemicellulose content was calculated by subtracting the values of acid detergent fibre from the neutral detergent fibre.

Cellulose, lignin and silica

Reagents

72% sulphuric acid (w/v) Acetone

Procedure

After ADF extraction, the content of crucible was covered with cooled 72% H_2SO_4 (w/v) and stirred with a glass rod to a smooth paste, breaking all lumps.

The crucible were again half filled with 72% H_2SO_4 stirred regularly at an interval of 1 h and kept for 3 h at room temperature. The excess of sulphuric acid was filtered off under vacuum and contents were washed with hot distilled water until free from acid, the glass rods were also rinsed with hot distilled water. The crucibles were kept in an oven at 100^{0}C for overnight, cooled in a dessicator and weighed again. The loss in the weight of crucible was expressed as

cellulose content. Crucibles were then transferred to a muffle furnace and ignited at 550^0C for 3 h. Next day, the crucibles were cooled in a dessicator and weighed again. The loss in the weight was taken as lignin content. The original weight of the crucible was then subtracted from this weight in order to calculate the silica content. The results were expressed on per cent dry weight basis.

Results and discussion

Symptoms

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. Almost similar symptoms were observed by earlier researchers (Panda and Patra, 1997; Samantary *et al.*, 1998; Joshi *et al.*, 1999 and Sandeep Kumar, 2002., sihag *et al.*,2016) in different crops.

Effect of Chromium on Structural carbohydrate

Neutral detergent fibre (NDF)

The perusal of data in Table 1 reveal an increase in NDF content of leaves and stem in Sorghum plant due to increasing concentration of Cr (VI). At 70 DAS, NDF content increased from 68.55 to 69.42 and 72.66 to 74.61 per cent with increasing Cr (VI) levels from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil in leaves and stem, respectively. NDF was more in stem than leaves. With growth NDF content increased in both leaves and stem and become maximum at 90 DAS (Table 1).

Acid detergent fibre (ADF)

Like NDF, it was increased significantly in leaves and stem with varied levels of Cr (VI), upto 4.0 mg Cr (VI) kg^{-1} soil with increasing levels of Cr (VI) from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil it was increased from 36.76 to 39.13 and 39.11 to 41.33 per cent in leaves and stem, respectively at 70 DAS. ADF content was more in stem than leaves. With growth, it was increased in leaves as well as stem and become maximum at 90 DAS. Trend was more or less same in control and Cr (VI) treated plants (Table 1).

Hemicellulose

The effect of various levels of Cr (VI) on hemicellulose content in leaves and stem of sorghum plant was inconsistent (Table 2). Hemicellulose content in leaves of control plants, at 35 and 70 DAS was 34.00 and 31.79 per cent and with 4.0 mg Cr (VI) kg^{-1} soil it becomes 35.45 and 30.29 percent, respectively. In stem, it was 34.80 and 33.55 per cent in control plants and with 4.0 mg Cr (VI) kg^{-1} soil t becomes 34.96 and 33.28 per cent at 35 and 70 DAS, respectively. With growth, it was decreased from 34.00 to 31.25 per cent in leaves of control plants, however in Cr(VI) treated plants no regular trend was observed with growth. In stem of control plants hemicellulose content decreased with growth from 34.80 to 29.20 per cent and in Cr (VI) treated plants, also it was decreased in regular way with advancement of growth stage from 35 to 90 DAS (Table 2).

Cellulose

Cellulose per cent in leaves and stem of sorghum plant increased gradually and significantly .with increasing levels of Cr

(VI) from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil However, in leaves at 35 DAS, it was decreased from 30.52 to 27.65 per cent with increasing concentration of Cr (VI) from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil. Further, at 70 DAS the increase in cellulose content in leaves of control plant and 1.0 mg Cr (VI) kg^{-1} soil Cr (VI) treated plant was non significant. Cellulose content increased from 36.46 to 38.12 per cent in leaves and 36.41 to 38.98 per cent in stem, with increasing levels of Cr (VI) from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil at 70 DAS. In leaves, it was increased continuously and significantly upto growth span of 70 DAS and then decreased slightly at 90 DAS. In stem, it was increased significantly and regularly upto 90 DAS. The trend was same in control as well as Cr (VI) treated plants (Table 2).

Lignin

Lignin content in leaves and stem of sorghum plant increased significantly from 3.06 to 3.55 and 3.57 to 4.26 per cent with increasing Cr (VI) concentration from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil respectively at 70 DAS. At 35 and 90 DAS also, the increasing trend of lignin content was observed with increasing levels of Cr (VI). It was also increased with growth in leaves and stem of control as well as Cr (VI) treated plants and become maximum at 90 DAS.

Silica

Silica content also increased significantly and regularly with increasing concentration of Cr (VI) in leaves and stem. The trend was same at all the stages of growth. Silica content in leaves of control plant was 0.08, 0.28 and 1.23 per cent and it increased to 0.80, 1.02 and 1.66 per cent in leaves of 2.0 mg Cr (VI) kg^{-1} soil treated plants. It was 0.82 and 1.02 per cent at 35 and 70 DAS, respectively in 4.0 mg Cr (VI) kg^{-1} soil treated plants and at 90 DAS plants were unable to survive with 4.0 mg Cr (VI) kg^{-1} soil treatment. In stem also it was increased from 0.17 to 0.37, 0.38 to 0.72 and 1.12 to 1.26 per cent with increasing level of Cr (VI) from 0.0 to 2.0 mg Cr (VI) kg^{-1} soil at 35, 70 and 90 DAS, respectively. Advancement of age also resulted into increase in silica content in leaves and stem of control as well as Cr (VI) treated plants (Table 3).

Non-structural carbohydrates

Non-structural carbohydrates (total soluble sugar, reducing sugar, and non-reducing sugar) were estimated in leaves, stem and root of sorghum plant at 35, 70 and 90 DAS.

Total soluble sugar

Data presented in Table 4, 5 and 6 reveal that increasing concentration of Cr (VI) resulted in a decrease in total soluble sugars in leaves, stem and root of sorghum, at all the stages of growth. Total soluble sugars content decreased from 8.90 to 3.90 per cent dry weight in leaves and from 10.45 to 7.45 per cent dry weight in stem and from 3.75 to 3.10 per cent dry weight in roots, with increasing levels of Cr (VI) from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil at 70 DAS. With growth, total soluble sugar content increased become maximum at 70 DAS and then decreased at 90 DAS. Trend was more or less same in leaves, stem and roots of control as well as Cr (VI) treated plants. It was maximum in stem followed by leaves and root (Table 4, 5 and 6).

Reducing sugar

Reducing sugars also decreased with increasing levels of Cr (VI) from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil. The trend was same in leaves, stem and roots, at all the growth stages. It was decreased from 3.50 to 1.40 per cent in leaves, from 4.65 to 4.02 per cent dry weight in stem and from 2.15 to 1.91 per

cent dry weight in roots, with increasing level of Cr (VI) from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil respectively at 70 DAS. With growth, it was increasing become maximum at 70 DAS and decreased thereafter at 90 DAS (Table 4, 5 and 6).

Non-reducing sugar

Non-reducing sugars in leaves, stem and roots of sorghum plant followed a pattern almost identical to that of total soluble sugars. It was found to decrease with increasing levels of Cr (VI) from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil in leaves, stem and roots of sorghum at all the stages of growth. At 70 DAS, it was decreased from 5.40 to 2.5 0 per cent dry weight in leaves, from 5.80 to 3.13 per cent dry weight in stem and from 1.60 to 1.19 per cent dry weight in roots with increasing concentration of Cr (VI) from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil respectively (Table 4, 5 and 6). Maximum non-reducing sugars was observed at flowering stage (70 DAS) and then decreased at grain filling stage (90 DAS), however, in roots of 4.0 mg Cr (VI) kg^{-1} soil treated plants it was decreased at 70 DAS (Table 4, 5 and 6).

Cell wall metabolism is important component in plant growth, not only because it represent a large proportion of the cell biomass, but also because in determining wall extensibility for cell enlargement (Zhang and Lauchli, 1988). The content of structural carbohydrates *viz.* NDF, ADF, cellulose, lignin and silica of leaves and stem were increased with increase in chromium (VI) concentration, Cr(VI) treatment might have decreased the activity of cellulase enzyme due to distortion of its quaternary structure, resulting in an increase in NDF content which is positively correlated with ADF.

Table.1 Effect of Cr (VI) on neutral detergent fibre and acid detergent fibre (per cent dry weight) in leaves and stem of sorghum plant at different growth stages^

Cr (VI) mg kg^{-1} soil	**Days after sowing**											
	Neutral detergent fibre						**Acid detergent fibre**					
	35	**70**	**90**	**35**	**70**	**90**	**35**	**70**	**90**	**35**	**70**	**90**
	Leaves			**Stem**			**Leaves**			**Stem**		
T_1	62.51	68.55	71.51	69.36	72.66	73.26	28.51	36.76	40.26	34.56	39.11	44.06
1.0	66.58	67.55	72.55	70.31	73.47	74.16	30.14	37.86	40.97	35.11	39.97	45.87
2.0	67.86	68.56	73.06	71.06	74.16	75.27	31.71	38.74	41.74	36.06	40.70	46.60
4.0	68.31	69.42	-	71.76	74.61	-	32.86	39.13	-	36.80	41.33	-
	A	B	A×B	A	B	A×B	A	B	A×B	A	B	A×B
SE (m)	0.01	0.02	0.02	0.02	0.03	0.03	0.04	0.05	0.07	0.02	0.03	0.05
CD at 5%	0.02	0.05	0.04	0.04	0.07	0.08	0.0 8	0.13	0.19	0.05	0.18	0.14

^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 hemicellulose and cellulose (per cent dry weight) in leaves and stem of sorghum plant at different growth stages^

Cr (VI) mg kg^{-1} soil	Days after sowing											
	Hemicellulose						Cellulose					
	35	70	90	35	70	90	35	70	90	35	70	90
	Leaves			Stem			Leaves			Stem		
T_1	34.00	31.79	31.25	34.80	33.55	29.20	30.52	36.46	35.46	32.57	36.41	38.62
1.0	36.44	29.69	31.58	35.2	33.50	28.29	28.97	36.51	36.42	33.16	37.16	39.12
2.0	36.15	29.82	31.32	35.00	33.46	28.67	26.85	37.36	36.82	34.12	38.08	39.16
4.0	35.45	30.29	-	34.96	33.28	-	27.65	38.12	-	34.98	38.98	-
	A	B	A×B	A	B	A×B	A	B	A×B	A	B	A×B
SE (m)	0.09	0.01	0.02	0.02	0.02	0.04	0.06	0.07	013	0.08	0. 10	0. 18
CD at 5%	0.27	0.03	0.06	0.06	0.06	0.12	0.18	0.27	0.39	0.24	0.30	0.54

^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) levels on lignin and silica (per cent dry weight) in leaves and stem of sorghum plant at different growth stages^

Cr (VI) mg kg^{-1} soil	Days after sowing											
	Lignin						Silica					
	35	70	90	35	70	90	35	70	90	35	70	90
	Leaves			Stem			Leaves			Stem		
T_1	1.86	3.06	3.56	2.96	3.57	3.57	0.08	0.28	1.23	0.17	0.38	1.12
1.0	2.11	3.12	3.62	3.57	3.68	3.68	0.39	0.62	1.52	0.26	0.44	1.21
2.0	3.01	3.42	3.96	3.62	4.15	4.15	0.8	1.02	1.66	0.37	0.72	1.26
4.0	3.12	3.55	-	3.72	4.26	-	0.82	1.02	-	0.53	0.75	-
	A	B	A×B	A	B	A×B	A	B	A×B	A	B	A×B
SE (m)	0.09	0.09	0.19	0.08	0.09	0.17	0.08	0.09	0. 17	0.07	0.08	0.14
CD at5%	0.23	0.24	0.49	0.21	0.23	0.45	0.20	0.24	0.46	0.16	0.19	0.37

^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.4 Effect of Cr (VI) on total sugar, reducing sugar and non-reducing sugar (per cent dry weight) in leaves of sorghum plant at different growth stages^

Cr (VI) mg kg^{-1} soil	Days after sowing								
	35	70	90	35	70	90	35	70	90
	Total sugar			Reducing sugar			Non-reducing sugar		
T_1	4.50	8.90	7.78	1.50	3.50	3.20	3.00	5.40	4.58
1.0	4.10	6.75	5.35	1.40	3.10	2.90	2.70	3.65	2.45
2.0	3.50	5.50	4.40	1.10	2.70	2.50	2.40	2.80	1.90
4.0	2.95	3.90	-	0.70	1.40	-	2.25	2.50	-
	A	B	A×B	A	B	A×B	A	B	A×B
SE (m)	0.04	0.07	0.08	0.011	0.20	0.22	0.25	0.28	0.30
CD at 5%	0.09	0.17	0.21	0.29	0.52	0.59	0.64	0.66	0.78

^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.5 Effect of Cr (VI) on total sugar, reducing sugar and non-reducing sugar (per cent dry weight) in stem of sorghum plant at different growth stages^

Cr (VI) mg kg^{-1} soil	Days after sowing								
	35	70	90	35	70	90	35	70	90
	Total sugar			Reducing sugar			Non-reducing sugar		
T_1	8.70	10.45	8.25	3.45	4.65	3.15	5.25	5.80	5.10
1.0	7.45	9.35	7.05	3.24	4.35	3.02	4.21	5.00	4.03
2.0	6.10	7.45	6.55	3.09	4.05	2.98	3.01	3.50	3.31
4.0	5.45	7.15	-	2.85	4.02	-	2.60	3.13	-
	A	B	A×B	A	B	A×B	A	B	A×B
SE (m)	0.01	0.01	0.02	0.01	3.65	0.03	0.01	0.01	0.02
CD at 5%	0.03	0.03	0.06	0.03	5.40	0.09	0.03	0.03	0.06

^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.6 Effect of Cr (VI) levels on total sugar, reducing sugar and non-reducing sugar (per cent dry weight) in root of sorghum plant at different growth stages^

Cr (VI) mg kg^{-1} soil	Days after sowing								
	35	70	90	35	70	90	35	70	90
	Total sugar			Reducing sugar			Non-reducing sugar		
T_1	2.25	3.75	2.95	1.05	2.15	1.85	1.20	1.60	1.10
1.0	2.12	3.67	2.82	0.98	2.09	1.78	1.14	1.58	1.04
2.0	1.95	3.55	2.66	0.90	2.03	1.76	1.05	1.52	1.00
4.0	1.64	3.10	-	0.74	1.91	-	0.90	1.19	-
	A	B	A×B	A	B	A×B	A	B	A×B
SE (m)	0.01	0.01	0.02	1.18	0.20	0.35	0.01	0.01	0.02
CD at 5%	0.03	0.03	0.06	2.56	0.60	1.05	0.03	0.03	0.06

^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

The pattern of structural carbohydrate with growth is in accordance with earlier finding of Sumanlata (1995) and Sandeep Kumar *et al.* (2010). The decrease in total soluble sugars, reducing sugar and non reducing sugar by chromium (VI) treatment, might be due to decrease in rate of photosynthesis which was reflected in reduction in chlorophyll content. Bishnoi *et al.* (1993) reported a simultaneous reduction in chlorophyll content and photosynthesis in chromium treated plants. The observed pattern of sugar content with growth was in accordance with earlier findings of Joshi (1991) in cowpea and sorghum and Sumanlata (1995) in guar. Sihag *et al.*,(2016) reported that chromium adversely affected the antioxidative enzyme activity in *sorghum bicolor*.

Significance of results

On structural carbohydrates

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).

Non-structural carbohydrates (total soluble sugars, non-reducing sugars and reducing sugars) decreased in leaves and

shoots of guar and cowpea with graded levels of chromium (Sumanlata, 1995; Rani *et al.*, 1998).

Conclusion

It is, therefore, concluded that content of structural carbohydrate and non – structural carbohydrate in leaves, root and shoot adversely affected with an increase in Cr (VI) concentration from 0.0 to 4.0 mg Cr (VI) kg^{-1} soil.

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