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Biochemistry

Chromium (VI) Affected Nutritive Value of Forage Clusterbean (*Cyamopsis Tetragonoloba* L.)

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Abstract

Hexavalent chromium is highly reactive and shown to be toxic for plants at higher concentrations. In present study, a pot experiment was conducted with important forage crop 'clusterbean' to determine the toxic effect of hexavalent chromium on its nutritive value on advancement of plant growth. The plants were grown in soil containing varying chromium concentration ranging from 0.0-4.0 mg Cr (VI) kg^{-1} soil. Nutritive parameters viz structural carbohydrates, protein content and *in vitro* dry matter digestibility were studied in different plant parts and growth stages. Toxic effects of hexavalent chromium were reflected by no survival of plants at 4.0 mg Cr (VI) kg^{-1} soil, upto 28% reduction in protein content and high variation in structural carbohydrates contents. Irrespective of these changes, the *in vitro* dry matter digestibility largely remains unaffected with very less change (0-6%). The study concludes that hexavalent chromium adversely affected nutritive value of clusterbean at higher concentrations.

Highlights

- Toxic effect of hexavalent chromium were maximum at concentration of 4.0 mg Cr (VI) kg^{-1} soil
- Content of ADF, NDF, lignin and silica increased with increased chromium concentration
- Least variation was reported for *in vitro* dry matter digestibility

Keywords: Chromium toxicity, Nutritive value, Clusterbean, Forage

Introduction

Increase of world population has resulted in the environmental pollution due to increased use of chemical fertilizers, toxic industrial effluents and various other human activities. Heavy metals pollution in different environmental regimes is also considered to be a threat to human health, animal nutrition and plant health. Hexavalent Chromium (Cr) is a highly reactive heavy metal with risk to plants and animals. Contamination of the environment by Cr (VI) is a major area of concern in recent years as it interferes with several metabolic processes causing toxicity to plants (Panda and Choudhury 2005; Shanker *et al.*, 2005).

Cr is used on a large scale in many different industries like metallurgical, electroplating, paints, pigments, tanning and paper industries (Oliveira, 2012). Large quantities of Cr compounds are discharged in liquid, solid, and gaseous wastes into environment due to these industrial activities following significant adverse biological and ecological effects (Maiti *et al*., 2012). Its presence in agricultural soils can be attributed to the use of organic wastes as fertilizer and the use of waste water for irrigation (Davies *et al*., 2002). Cr enters the food chain through consumption of plant material. Increased Cr concentration in plants adversely affects several biological parameters leading to loss of vegetation and sometimes land becomes barren (Dube *et al*., 2003). According to Shanker *et al*. (2005), the effects of Cr toxicity includes reduced productivity and growth of plants due to reduced photosynthesis and limiting enzyme activities. At higher concentrations, Cr (VI) adversely affected the photosynthetic pigments (Kumar *et al*., 2004), nitrogen metabolism (Kumar and Joshi, 2008), nutritive value and protein content in plants under Cr (VI) stress (Kumar *et al.,* 2010).

Much of human diet depend directly from plants products like fruits and vegetables or indirectly as fodder given to livestock. Accumulation of heavy metals in the edible parts of plants represents a direct pathway for their incorporation into the human food chain (Puschenreiter *et al*., 2005). Clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.), also known as Guar (Belongs to the family *Leguminaceae*) is a drought hardy crop, widely grown in rainfed condition in arid and semiarid regions of tropical India during *kharif* season. It is a good source of carbohydrates, protein, fibre and minerals like calcium, phosphorus and iron and contains appreciable amount of vitamin C. It also provides nutritive concentrate and fodder for cattle and adds to the fertility of soil, by fixing considerable amount of atmospheric nitrogen. Young pods are eaten as a vegetable, and seeds as cattle feed in India and Pakistan where the crop is also used as fodder and green manure. It is also used in paper industry, postage, stamps, textile, food products, e.g. bakery etc (Kumar and Singh, 2002).

In India, Cr (VI) contamination is a major problem around various industries using Cr compounds, which causes considerable negative impact on crop production (Chandra and Kulshreshtha 2004). In Haryana (A major guar producing state), Sonepat, Panipat, Dharuhera, Gurgaon, Yamunanagar, Faridabad and Shahabad are the main industrial areas, where poor plant growth of field crops has been observed (Kumar and Joshi, 2008). Hence, present investigation was designed and conducted to investigate the impact of varying Cr (VI) concentrations on forage quality including protein, dietary fibres and *in vitro* dry matter digestibility of clusterbean in various plant parts at different growth stages.

Material and methods

Chemicals, reagents and Soil

The chemicals and reagents used during the present investigation were of analytical grade. 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% ; 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+}, 3.6 mg kg^{-1} soil; EC, 1.5; $CaCO_3$ 3.5%; Cr^{2+}, 0.01 mg kg^{-1} soil; texture – sandy loam.

Plant growth conditions

Seeds of clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.) cv HG 2-20 were procured from Forage Section, Department of Genetics and Plant Breeding, C.C.S. Haryana Agricultural University, Hisar and raised in pots filled with 5 kg of sandy loam soil in a naturally lit net house. Its characteristics were : pH (1:2) 8.50; organic carbon, 0.22% ; 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+}, 3.6 mg kg^{-1} soil; EC, 1.5; $CaCO_3$ 3.5%; Cr^{2+}, 0.01 mg kg^{-1} soil; texture – sandy loam. The pots were lined with polythene bags and the soil in each pot was treated with different levels of Cr in the form of potassium dichromate i.e. 0.0, 0.5, 1.0, 2.0 and 4.0 mg kg^{-1} soil. The seeds were surface sterilized with mercuric chloride and after proper washing with distilled water, inoculated with *Rhizobium* culture. Equal amount of nutrient solution was supplied at weekly interval to each pot. The plants were irrigated with equal quantities of tap water as and when required.

Environmental condition

The temperature and relative humidity during the experiment ranged from 11.0 to 35.6°C and 34.5 to 95.2 %, respectively. The light intensity ranged from 36100 to 84000 lux.

Plant sampling

Plants were harvested at three stages of growth, i.e. vegetative (30 Days after sowing i.e. 30 DAS), flowering (50 DAS), and grain filling (65 DAS) stages. Immediately after harvest, the plant samples were dissected into leaves and stem 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.

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Chemical analysis

Protein content (% dry weight basis) in various plant parts was estimated by conventional Micro-Kjeldahl method (AOAC, 1995). Structural carbohydrates viz. neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, and hemicellulose were estimated in leaves and shoots according to Goering and Van Soest (1970) without addition of sodium sulphite. NDF was assayed without a heat stable amylase and expressed inclusive of residual ash. ADF was also expressed inclusive of residual ash. Sulphuric acid lignin (sa) and silica were determined by the method of Goering and Van Soest (1970). The hemicellulose content was estimated in same sample in sequential manner. *In vitro* dry matter digestibility was determined in three replicates (three separate run) by the method followed by Vogler *et al*. (2009). Above chemical analysis were done in plants grown in Cr concentration in the range of 0.0-2.0 mg Cr (VI) kg^{-1} soil only, as 4.0 mg Cr (VI) kg^{-1} soil was found to be lethal and plants did not survived after 20 DAS.

Statistical analysis

A two-factorial ANOVA in complete randomized block design was used to confirm the validity of the data using OPSTAT software available on CCSHAU website home Page (http://hau.ernet.in/opstat.html). In table, standard error (SE) of mean and critical difference (CD) at 5% values for treatment (A), stages (B) and interaction between treatments and stages (A×B) are given. The values used in graphs are mean of three replicates and shown as ±standard error.

Results

Effect of Cr (VI) on crude protein content

The crude protein content (% dry weight) in leaves and stem of Cr treated plants was found to be decreased with increasing Cr levels from 0.0 to 2.0 mg Cr (VI) kg^{-1} soil. A maximum reduction of protein content was observed at 2.0 mg Cr (VI) kg^{-1} soil. Compared to control, the crude protein content at 50 DAS decreased by 9.6, 14.5 and 21.3 % in leaves, and 8.0, 15.3 and 24.5 % in stem of clusterbean plants treated with 0.5, 1.0 and 2.0 mg Cr (VI) kg^{-1} soil, respectively. Its amount was found more in leaves than stem of control as well as Cr treated plants. Progress of growth, resulted into an increase in crude protein content with maximum value at 50 DAS and a thereafter decrease at 65 DAS (Fig. 1).

Effect of Cr (VI) on structural carbohydrate

Structural carbohydrates viz. NDF, ADF, hemicelluloses, cellulose, lignin and silica were estimated in leaves and stem as these are consumed for live-stock feeding.

Neutral detergent fiber content

The NDF content (% dry weight), in Cr treated plants was found to increase with increasing levels of Cr (VI) at each stage of growth (Fig. 2). The NDF content increased by

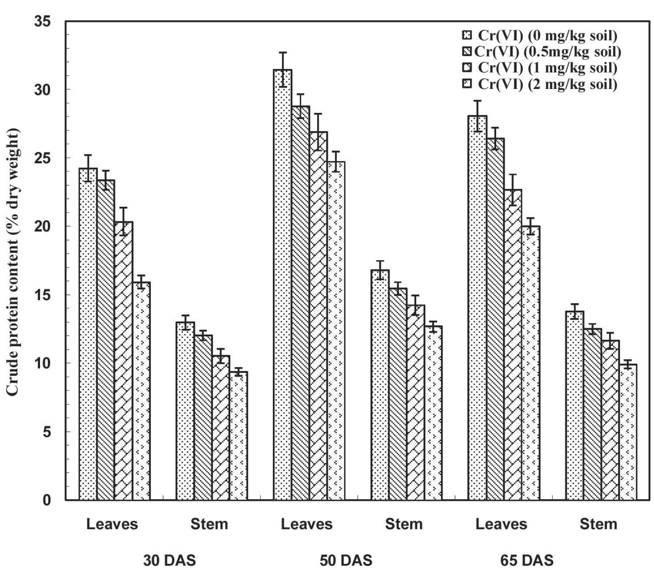

Fig. 1: Effect of Cr (VI) on crude protein content (% dry weight) in clusterbean plant parts at different stages of growth

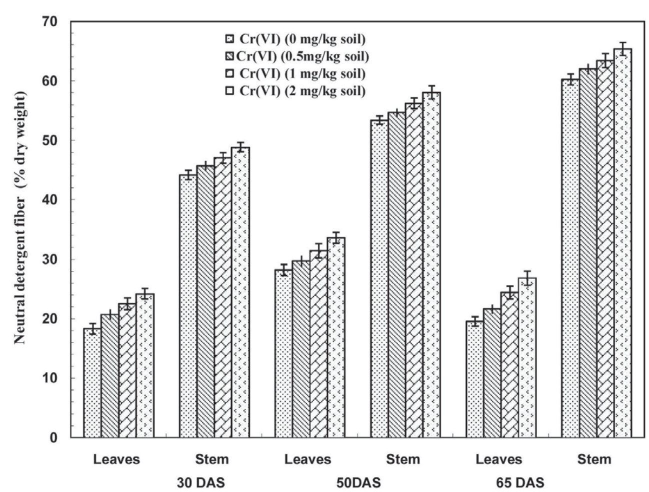

Fig. 2: Effect of Cr (VI) on neutral detergent fiber (% dry weight) in clusterbean plant parts at different stages of growth

32.08, 19.15 and 37.30 % in leaves of 2.0 mg Cr (VI) kg^{-1} soil treated plants at 30, 50 and 65 DAS, respectively. Maximum increase was noticed in leaves of plants treated with 2.0 mg Cr (VI) kg^{-1} soil. In leaves the NDF fraction in each treatment increased with the growth of plants up to 50 DAS and then declined at 65 DAS. In case of stem, the NDF content (% dry weight) increased by 10.58, 8.57 and 8.54 % at 30, 50 and 65 DAS, respectively (Figure 2). Same trend was noticed for NDF in stems as it was observed in leaves i.e. it also increased with increasing concentration of Cr. However, the NDF content in stem, increased with advancement of growth of plants from 30 to 65 DAS. Stem had more NDF content than leaves at all the stages of growth.

Acid detergent fiber content

Like NDF, ADF content (% dry weight) also increased significantly in leaves and stem with increasing levels of Cr (VI) at all growth stages (Figure 3). As compared to control, its content increased by 28.8, 21.6 and 35.8 % in leaves and 14.2, 13.5 and 10.1 % in stem, at 30, 50 and 65 DAS, respectively. Maximum ADF content in leaves and stem was observed at 50 and 65 DAS, respectively, in all the treatments.

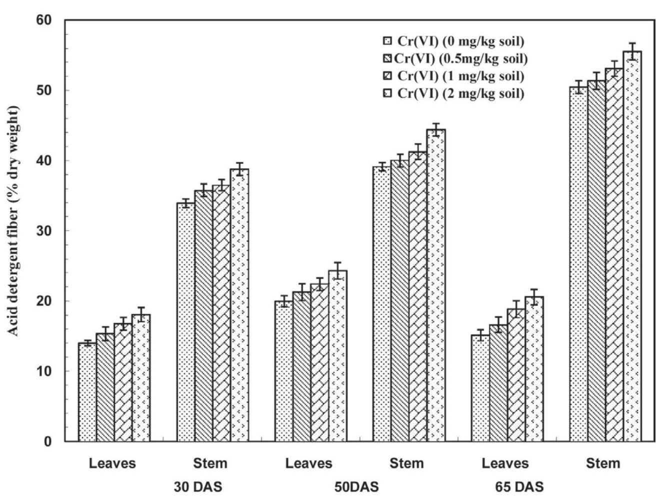

Fig. 3; Effect of Cr (VI) on acid detergent fiber (% dry weight) in clusterbean plant parts at different stages of growth

Hemicellulose content

The effect of various levels of Cr (VI) on hemicellulose content in leaves and stem of clusterbean plant was found to be inconsistent (Figure 4). With increasing level of Cr (VI) in soil, it was increased by 42.1, 13.0 and 42.3 % in leaves over the control at 30, 50 and 65 DAS, respectively. Highest level of hemicelluloses content was observed at 50 DAS, in leaves. However, no regular trend in the hemicellulose content in stem of clusterbean plant was observed with increasing levels of Cr treatment (Figure 4). Maximum hemicellulose content was noticed at 50 DAS and then decreased at 65 DAS.

Cellulose content

Data presented in figure 5 revealed that cellulose content (% dry weight) in leaves and stem increased significantly and gradually with increasing concentration of Cr (VI) i.e. from 0.0 to 2.0 mg Cr (VI) kg^{-1} soil with highest value at

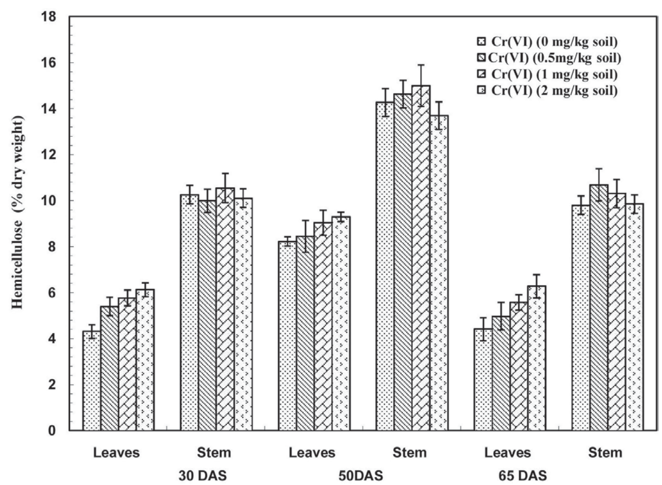

Fig. 4: Effect of Cr (VI) on hemicellulose (% dry weight) content in clusterbean plant parts at different stages of growth

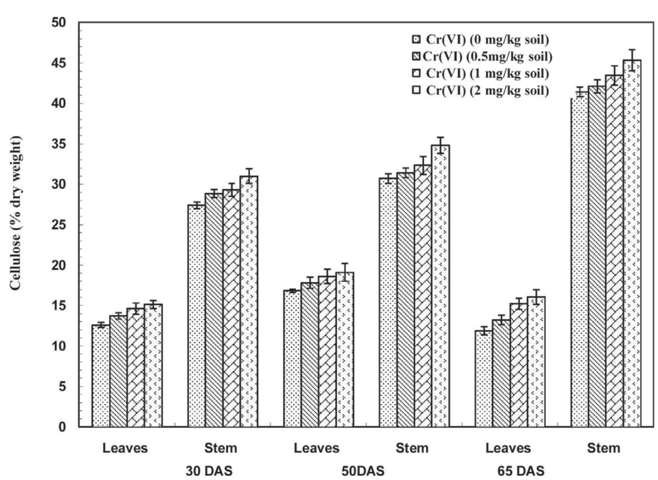

Fig. 5: Effect of Cr (VI) on cellulose (% dry weight) content in clusterbean plant parts at different stages of growth

2.0 mg Cr (VI) kg^{-1} soil at all stages of growth (Figure 5). It increased by 3.1, 7.7 and 13.6 % in leaves and 7.1, 9.8 and 11.7 % in stem at 50 DAS in 0.5, 1.0 and 2.0 mg Cr (VI) kg^{-1} soil treated plants over the control. In leaves, cellulose content increased with the advancement of growth of plant from 30 to 50 DAS and then decreased at 65 DAS. However, in stem it increased continuously up to 65 DAS. The percentage of cellulose was more in stems as compared to leaves.

Lignin content

An increase of lignin content in leaves and stem was observed with progress of growth from 30 to 65 DAS. The lignin content in plants grown in 2.0 mg Cr (VI) kg^{-1} soil increased from 85, 83 and 32 % in leaves and 10, 12.5 and 12 % in stem at 30, 50 and 65 DAS, respectively, as compared to control (0 mg Cr (VI) kg^{-1} soil) (Figure 6). The lignin content was found to be more in stem than in leaves.

Silica content

The data presented in Figure 7 indicates that silica content in leaves and stem increased with increasing levels of Cr (VI) at different stages of growth. As compared to control, silica content in plants grown in 2.0 mg Cr (VI) kg^{-1} soil increased from 430, 26 and 82 % in leaves, and 100, 20 and 42 % in stem at 30, 50 and 65 DAS, respectively. This shows a high increase in silica content at vegetative stage and grain filling stage with increase in Cr concentration in soil. The silica content in leaves and stem increased significantly with plant age and attained a maximum value at 65 DAS.

In vitro *dry matter digestibility*

Data on *in vitro* dry matter digestibility (IVDMD) of leaves and stem revealed a continuous decrease in its content with increase in concentration of Cr (VI) in soil at all the stages of growth (Table 1). The *in vitro* dry matter digestibility of

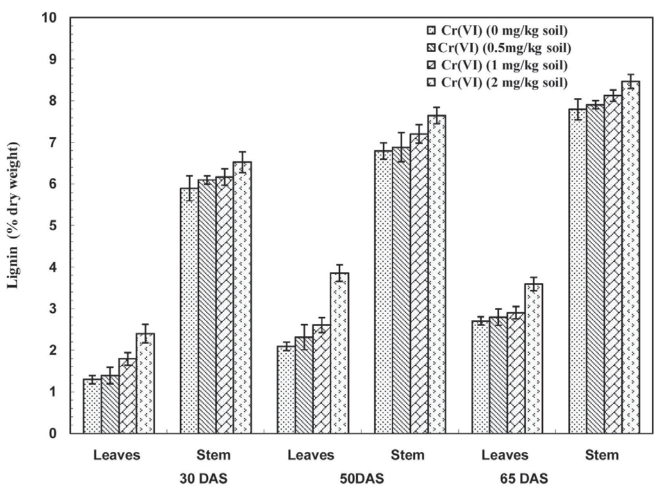

Fig. 6: Effect of Cr (VI) levels on lignin (% dry weight) content in clusterbean plant parts at different stages of growth

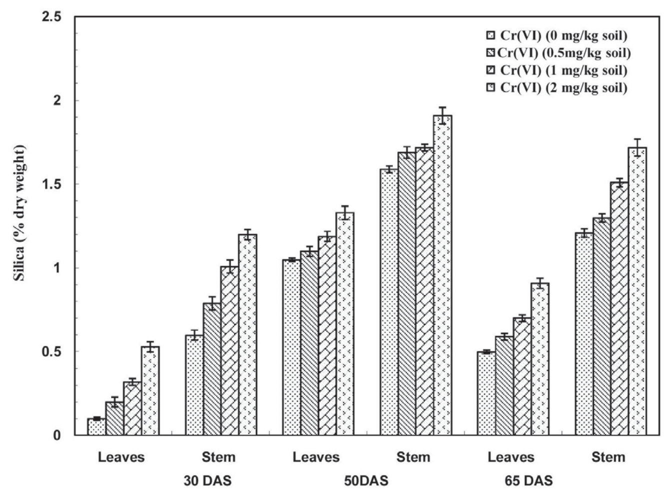

Fig. 7: Effect of Cr (VI) levels on silica (% dry weight) content in clusterbean plant parts at different stages of growth

Table 1: Effect of Cr (Vi) on *in Vitro* Dry Matter Digestibility (% Dry Weight Basis) in Clusterbean Plant Plants at Different Stages of Growth

Cr (VI) treatment (mg kg^{-1} soil)	Days after sowing (DAS)					
	30	50 Leaves	65	30	50 Stem	65
0.0	75.20	72.35	68.15	68.75	60.62	54.75
0.5	74.76	73.76	67.77	68.15	60.0	54.06
1.0	73.15	70.35	66.44	67.37	59.26	53.37
2.0	71.24	68.45	64.35	65.34	57.35	51.47
	A	B	A×B	A	B	A×B
SE (m)	0.01	0.01	0.03	0.005	0.005	0.009
CD at 5%	0.04	0.05	0.09	0.013	0.015	0.027

Cr treated plants was lowered by 5.26, 5.39 and 5.58 % in leaves and 4.96, 5.34 and 5.99 % in stem at 30, 50 and 65 DAS, over their control. Maximum reduction in IVDMD of leaves and stem was observed at 65 DAS. Both leaves and stem during early stages of growth (30-50 DAS) were found to be more digestible than during later stage of growth (65 DAS).

Discussion

Cr significantly reduced the protein content of clusterbean plants. These results are in agreement with earlier reports of Cr (VI) toxicity to protein content in sorghum. It has been suggested that decrease in crude protein content with Cr (VI) application is mainly due to the stunted plant growth, which might have resulted in less utilization of nitrogen (Kumar *et al.,* 2010).

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 trend of increased NDF, ADF, cellulose, hemicellulose, lignin and silica content in Cr-stressed clusterbean plant leaves, stems and roots with increasing Cr (VI) concentrations as well as with plant growth and development is in accordance to reports of Cr affected nutritive value of sorghum plants (Kumar *et al.*, 2010). 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. Cr in soil leads to decrease of stem length and subsequently increase of NDF %age. This could be the reason for increase in NDF content. Lignification decreases the cell-wall plasticity and, therefore, reduces the cell growth (Schutzendubel and Polle 2002). It is undoubtedly true that Cr stress retards the plant growth through its influence on the accumulation of lignin, which is considered as one of the constituents of the secondary cell wall. The accumulation of insoluble phenols, such as lignin, in the secondary cell wall was reported in plants exposed to heavy metals and could be associated with an increase in the activity of lignifying peroxidases (Schutzendubel *et al.,* 2001).

A marked decline in IVDMD in Cr-stressed clusterbean plants as compared to control was in accordance to earlier report of Cr (VI) toxicity on sorghum plants (Kumar *et al.,* 2010). The decrease in IVDMD is mainly due to the increase in fiber components (NDF and ADF) and lower cell content. This reduction in the digestibility coefficient could also be due to the enzymatic losses associated with breakdown of non-structural and reduced mean retention times of digesting material in the rumen (Stockdale *et al.*, 1993). Moreover, IVDMD primarily depends upon the concentration of cellulose and hemicelluloses, which in turn influenced by the degree of lignification, silicification and fiber components (Tessema *et al.*, 2004). Furthermore, it has been reported that tannins can also reduce digestibility (Alonso-Diaza *et al.,* 2009).

Conclusion

The study clearly revealed and concluded that clusterbean plants were not survived in soil with 4.0 mg Cr (VI) kg^{-1} soil and even 2.0 mg Cr (VI) kg^{-1} soil was highly toxic and adverse for nutritive value of forage clusterbean as decreased protein content and increased structural carbohydrates. Increased structural carbohydrates (NDF and ADF) leads to further decreased IVDMD and make it undesirable for use in animal feed. Therefore, Cr (VI) toxicity must be alleviated considering nutritional importance of crop for nutritional security to animals and further studies are recommended on use of suitable amelioration strategies for Cr (VI) toxicity to overcome these toxic effects on nutritive value and plant health. The experimental plan and observations of this study may be used for designing of an alleviation strategy for chromium toxicity.

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