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**Title: Evaluation of toxic effects of hexavalent chromium on the yield and quality of Sorghum**

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**Novelty Statement:** Nowadays there are great losses of agriculture and food security due to heavy metal contaminated conditions. An increase in heavy metal contamination due to human activities are becoming an attentive problem for food and agriculture security in the world. Human activities are increasing heavy metal contamination in agricultural soil, water, and air which are reaching plants directly or indirectly which inturn causing major loss of agricultural crops and deficiency of food. In the current scenario of the environment, chromium contamination is changing very fast and creating problems for the scientist in developing better yielding variety under chromium toxicity. In this experiment, the better yielding variety is screened for their potential under the lowest to the highest concentration of chromium causing toxicity. The toxic effects appeared at 1ppm concentration of Cr and 4ppm chromium concentration appeared lethal dose to sorghum. Various important parameters were studied which should be studied while investigating chromium toxicity in agricultural crops.

**Abstract:** Sorghum is a multipurpose crop, but Cr(VI) toxicity influenced the production of crop and have established highlighted courtesy, due to robust toxicity and a comparatively less known mode of action. Many reports approve

the negative impact of Cr(VI) on plants. Yet, it is not clear that, at what concentrations, Cr(VI) inhibits growth and nutrient quality of Sorghum. In the present research, toxic effects appeared after 1ppm of Cr treatment. The plant growth decreased 15 – 20% and nutritional quality decreased 30 – 40% significantly with increasing concentration of Cr(VI). Toxic components increased 14 – 16% with increasing concentration of Cr(VI) in both the varieties (HJ 541 and SSG 59-3). Chromium was accumulated more in roots followed by shoots with increasing Cr(VI) treatments (0-4 ppm). Chromium at 4ppm level was becoming lethal to Sorghum. Sorghum cultivar SSG 59-3 was more tolerable to Cr toxicity than HJ 541. Chromium accumulated in Sorghum and increased HCN content and at higher doses adversely affects the nutritive values and growth making it toxic for animal consumption. These findings may be useful for scheming a mitigation strategy for chromium toxicity.

**Keywords:** Chromium; Oxidative stress; Sorghum; Biomass; Toxicity; Adversely, Nutritive, Heavy metal, Tolerant.

## 1. Introduction:

Sorghum (*Sorghum bicolor* L.) is a multipurpose crop grown for food, animal feed, and industrial resolves. Sorghum belongs to the Poaceae family and is a C<sub>4</sub> plant that usually grows under hot and dry conditions. It is considered more tolerant of different stresses, such as drought, salinity, heat, and swamping, compared to other cereal crops (Bibi *et al.* 2012; Hefny *et al.* 2013; Menezes *et al.* 2014). However, the production of Sorghum is influenced by heavy metal (HM) stress. Among HM, Cr toxicity has highlighted a big loss in agriculture, due to vigorous toxicity and a relatively less known mode of action (Singh *et al.* 2013). The wide industrial use of chromium (Cr VI) makes it a very attentive environmental pollutant. Contamination of soil and water by Cr is increasing day by day in the current world due to excessive use of Cr by various anthropogenic activities (Gill *et al.* 2015; Mathur *et al.* 2016). Mainly Cr(VI) reaches Sorghum by the use of fertilizers and irrigation water containing industrial waste with Cr (Xu *et al.* 2009). Chromium exists in two forms that are stable in the living system. They are the trivalent Cr(III) and the hexavalent Cr(VI) forms, though there are different other forms of Cr they are unstable and small lived-in living systems. Hexavalent Cr is considered the most toxic form of Cr, which usually exist associated with oxygen as chromate ( $\text{CrO}_4^{2-}$ ) or dichromate ( $\text{Cr}_2\text{O}_7^{2-}$ ) oxyanions. Both chromates and dichromates are broadly involved in leather processing industries, refractory steel industries, boring industries, coating and washing agents industries, catalytic industries, and in the chemicals like chromic acid manufacturing industries. Chromium composites are highly toxic to plants and are harmful to their growth and development. The presence of Cr(VI) in the cellular environment

influences plant growth and development. The oxidizing nature and generation of free radicals during the reduction process of hexavalent Cr to trivalent Cr occurring inside the cell produces serious damages to cellular compounds. The oxidative stress due to Cr toxicity produces reactive oxygen species (ROS) in the cell which causes oxidative injury which in turn demolish the discriminating mechanism among inorganic nutrient uptake this permits larger quantities of Cr(VI) to enter roots passively and further movement of Cr(VI) to shoots causing oxidative injury to the photosynthetic and mitochondrial apparatus, ultimately refunded in poor growth and yield. Moreover, the formation of ROS can damage the creation of biomolecules, such as sugar, proteins, and structural carbohydrates, thereby, interfering with plant progress, nutrition value, and carbohydrate metabolism (Gill *et al.* 2010). Thus, the general opposing effect of Cr(VI) toxicity on plant development could be impairment in the absorption of minerals and water resulting in insufficiency in shoots (Sangwan *et al.* 2014). It has been observed that Cr(VI) toxicity causes wilting of tops, destruction of roots, chlorosis of young leaves, reduced photosynthesis, and finally plant death (Sharma *et al.* 2003; Scoccianti *et al.* 2006). Though the excess chromium produces toxic indications in plants, such as distressed photosynthesis (loss of biomass in a plant), chlorosis and destruction to the stem and roots (Chandra *et al.* 2009) there are some reports about chromium supply in very minute quantities to the soil may improve plant yields (Van *et al.* 1968). A number of other reports (Srivastava *et al.* 1999; Cervantes *et al.* 2001; Shanker *et al.* 2005; Banks *et al.* 2006; Wyszowski *et al.* 2010) approve the negative impact of chromium on plants. Yet, it is not clear that, at what concentrations, Cr causes the ultrastructural variations in chloroplasts and inhibits the photosynthesis (Ali *et al.* 2013a; Ali *et al.* 2013b). Therefore, by keeping in view the above information, present research work was planned to evaluate the effect of varying Cr(VI) levels on the growth and nutritive quality of Sorghum and its accumulation in various plant parts at different stages of growth viz. 35 days after sowing (DAS) and 95 DAS. The levels of Cr(VI) viz. 2ppm and 4ppm were decided after screening the crop for survival under different Cr concentrations and the toxic effects in Sorghum appeared after 0.05ppm Cr concentration. Sorghum is generally utilized as a fodder purpose mainly at two stages which were selected for the analysis in the present research work. Sorghum cultivars were selected on the bases of difference that HJ 541 is a single-cut while SSG 59-3 is a multi-cut cultivar and HJ 541 is less sweet than SSG 59-3. This research article can be useful among other ways which are involved in analyzing heavy metal toxicity problem in other agricultural crops and animals.

## 2. Results:

The present research work was carried out to analyze the effect of Cr(VI) toxicity on growth and quality of

Sorghum (fodder crop) for animal feed. Chromium could harm the animal's which are consuming the plants grown in Cr(VI) polluted soils directly or indirectly and it may also enter humans and carnivores animals which are consuming these animals. During the present experiment, Sorghum varieties viz. HJ 541 and SSG 59-3 were tested for their suitability as an animal feed, when grown in Cr(VI) polluted soil, by giving an artificial Cr(VI) toxic stress. Plant growth was measured in terms of chlorophyll, biomass, root-shoot length, number of leaves and tillers. The nutritive value was measured in terms of various quality parameters such as structural and non-structural carbohydrates, IVDMD, proline and crude protein. The toxicants level was determined in terms of Cr(VI) and HCN content. The observations and results obtained during the present experiment are as follows -

### **2.1 Effect of Cr(VI) toxicity on growth and development of Sorghum plants:**

Plant growth and development were measured in terms of parameters viz. plant biomass, chlorophyll content, root length, plant height, number of leaves and tillers. Chromium (VI) toxicity significantly reduced plant growth and development with its increasing concentrations (from 0 – 4 ppm) in both the varieties at both the stages (35 and 95 DAS). The results presented in figure 1 demonstrate that leaf fresh weight decreased significantly from 0.70% to 1.18% and 0.23% to 0.31% in HJ 541 and SSG 59-3 variety respectively at 2ppm and 4ppm Cr(VI) toxicity as compared to control at 35 DAS. The decrease in leaf fresh weight was more at 4ppm Cr(VI) toxicity in both the varieties at both the stages. Variety HJ 541 showed more decrease (14.61%) than SSG 59-3 variety (5.65%) at 95 DAS. Similarly, leaf dry weight decreased significantly from 0.15% to 0.24% and 0.05% to 0.17% in HJ 541 and SSG 59-3 variety respectively at 2ppm and 4ppm Cr(VI) toxicity as compared to control at 35 DAS. The decrease of leaf dry weight was 1.64% to 3.34% and 3.15% to 6.22% in HJ 541 and SSG 59-3 variety respectively at 2ppm and 4ppm Cr(VI) toxicity as compared to control at 95 DAS. Similar trends were observed for chlorophyll content in figure 2. The results showed that the decrease in chlorophyll content was more in HJ 541 (21.93%) than in SSG 59-3 variety (10.44%) at 95 DAS. Variety HJ 541 showed a higher decrease in chlorophyll content than SSG 59-3 variety at both the stages under 4ppm of Cr(VI) toxicity. Results indicated a similar pattern for root length, shoot length, plant height, number of leaves and tillers as for plant biomass and chlorophyll content under Cr(VI) toxicity at both the stages in both the varieties (figure 3,4,5,6 and 7). The higher decrease was observed in HJ 541 than SSG 59-3 variety under 2ppm and 4ppm Cr(VI) toxicity at both the stages. The decrease was more at 4ppm Cr(VI) toxicity in both the varieties at both the stages as compared to control.

### **2.2 Effect of Cr(VI) stress on nutrient values of Sorghum plant for animal feed:**

The nutrient values of Sorghum were studied in terms of parameters viz. acid detergent fiber (ADF), neutral detergent fiber (NDF), proline content, *in-vitro* dry matter digestibility (IVDMD), total sugar, grain yield and crude protein. Chromium (VI) toxicity significantly reduced the nutrient quality of Sorghum with its increasing concentrations (from 0 – 4 ppm) in both the varieties (HJ 541 and SSG 59-3) at both the stages (35 and 95 DAS). The ADF content was increased significantly with increasing levels of Cr(VI) toxicity in both the varieties at both the stages (figure 8). In the present study, it was observed that the content of ADF increased significantly by 2.44 and 2.11 times as compared to control at 35 and 95 DAS respectively in HJ 541 variety at 4 ppm Cr(VI) toxicity. The increase in ADF content of variety SSG 59-3 was 2.34 and 2.36 times as compared to control at 35 and 95 DAS respectively at 4ppm Cr(VI) toxicity. The NDF content was also increased significantly with increasing levels of Cr(VI) in both the varieties at both the stages (figure 8). It was observed that the NDF content in Sorghum was increased by 1.16 and 2.91 times at 35 and 95 DAS respectively in HJ 541 variety under 4ppm Cr(VI) toxicity as compared to control. While the increase in NDF content of SSG 59-3 variety was 1.11 and 1.25 times at 35 and 95 DAS respectively under 4ppm Cr(VI) toxicity. Similarly, the proline content of Sorghum was also increased significantly with increasing levels of Cr(VI) at both the stages in both the varieties as compared to control (figure 9). The proline content in variety SSG 59-3 was more than HJ 541 variety at both the stages in control as well as Cr(VI) treated plants. In contrast to ADF and NDF content the IVDMD of Sorghum was decreased significantly with increasing concentration of Cr(VI) at both the stages in both the varieties as compared to control (figure 8). The decrease was 2.11 and 1.70 times in HJ 541 variety and 1.40 and 1.30 times in SSG 59-3 variety at 35 and 95 DAS respectively; under 4ppm Cr(VI) toxicity as compared to control. The highest decrease in IVDMD was observed in HJ 541 than SSG 59-3 variety at both the stages under 4ppm Cr(VI) toxicity as compared to control. Sorghum was observed more digestible at 35 DAS than 95 DAS in both the varieties in control as well as under Cr(VI) toxicity. Similar to IVDMD, the total sugar, grain yield (figure 10) and crude protein content (figure 11) of Sorghum were decreased significantly with increasing concentrations of Cr(VI) in both the varieties at both the stages as compared to control. The decrease in grain yield was more in HJ 541 than SSG 59-3 variety under Cr(VI) toxicity. The decrease in crude protein content was more at 4ppm Cr(VI) concentration (2.60 and 1.70 times) in HJ 541 variety and (2.40 and 1.44 times) in SSG 59-3 variety at both the stages (35 and 95 DAS) respectively; as compared to control.

### **2.3 Effect of Cr(VI) stress on the content of toxic components in Sorghum:**

The present research investigates the content of two toxic components viz. hydrocyanic acid (HCN) content

and Cr(VI) content present in the Sorghum. It was observed that the Cr(VI) content in Sorghum (roots and shoots) increased significantly (14 to 16%) with the increase in the supply of Cr(VI) in both the varieties at both the stages as compared to control (figure 12). The Cr(VI) content of roots and shoots increased with an increase in the growth stage in both the varieties. Roots had accumulated more Cr(VI) than shoots in both the varieties at both the stages. Similar to Cr(VI) content, the HCN content also increased significantly (3.47 times and 2.67 times) at 4ppm of Cr(VI) toxicity in both the varieties (HJ 541 and SSG 59-3) respectively; at 35 DAS as compared to control (figure 13). Both Cr(VI) content and HCN content were observed higher in HJ 541 variety than SSG 59-3 variety in control as well as under Cr(VI) toxicity. These results showed that SSG 59-3 variety was safer than HJ 541 variety for animal consumption. In the present experiment, the SSG 59-3 variety showed a more tolerant nature to Cr(VI) toxicity than HJ 541 variety.

### 3. Discussion:

Heavy metals are communal and damaging environmental dangers, come across by plants. Plant uptake of excessive concentrations of heavy metals generates stress resulting in serious physiologic, structural and biochemical disturbances. Now a day's environmental pollution is suppressing the yield of crops. Contamination of soil by toxic metals and metalloids is a major concern all over the world. Among heavy metals, Cr(VI) toxicity has become a serious problem all over the world. Chromium (VI) pollution has produced many negative effects on plants and animal health. Chromium (VI) toxicity also affects the quality parameters in plants (Kumar *et al.* 2010). Chromium (VI) toxicity causes morphophysiological changes such as discoloration of leaves, curling of leaves, chlorosis, necrosis and lowering of biomass in plants (Kahle *et al.* 1993; Han *et al.* 2004; Sihag *et al.* 2018; Amin *et al.* 2018). Chromium (VI) toxicity may produce long term impacts in the biotic environment (Tauchnitz *et al.* 1983; Obata *et al.* 1997). It might be due to the easy solubility of Cr(VI) in soil and effortless engrossment of Cr(VI) in plant roots (Yadav *et al.* 2010). In the present experiment also the toxic effect of Cr(VI) reduced the plant growth and development as evident from decreased plant biomass, chlorophyll content, root length, plant height, number of leaves and tillers with the addition of Cr(VI) at 2 and 4ppm levels at both the stages in both the varieties. It might be due to the demolition of the discriminating mechanism among inorganic nutrients uptake by plant caused by increased Cr(VI) accumulation in the plant tissues which resulted in the oxidative injury to absorption system of the plant. It causes impairment in the absorption of essential nutrients by the plant which in turn decreased plant growth. These findings of present experiment are in agreement with various other researchers reported in other species such as Rice, Barley and Sorghum

(Mei *et al.* 2002; Ganesh *et al.* 2008; Ali *et al.* 2011; Qiu *et al.* 2013; Kasmiyati *et al.* 2016). Similar, results were observed in Indian mustard by Diwan *et al.* 2012 under Cr(VI) toxicity. Deterioration in Mustard (*Brassica juncea*) plant growth under Cd and Pb toxicity has also observed by Ali *et al.* 2011 and Sheetal *et al.* 2016; respectively. They observed Pb to be more toxic than Cd in *B. juncea*.

In the present experiment forage quality of Sorghum was shown in terms of ADF, NDF, IVDMD, total sugars and crude protein present in the plant. The amount of structural carbohydrates (ADF and NDF) present in Sorghum governs its digestibility property by the animals. Sangwan *et al.* 2014 has been reported ADF is less digestible than NDF. During the present experiment both ADF and NDF increased under Cr(VI) toxicity but the increase in ADF was more than the NDF content which resulted in decreased digestibility (IVDMD). The IVDMD of Sorghum was decreased with the plant age as was evident from increased content of ADF and NDF with the plant age. Sumanlata *et al.* 1999 and Sangwan *et al.* 2014 obtained similar results of decreased IVDMD in guar and cluster bean (*Cyamopsis tetragonoloba* L.) respectively; under Cr(VI) toxicity. During present experiment, the increase in ADF and NDF content in Sorghum under Cr(VI) stress might be due to increased lignification and silicification as a defense mechanism against stressful conditions in plant cells which in turn imparts firmness and rigidity to the cell-wall and protects the cell from osmotic burst. It resulted in the increased fiber components of the cell-wall which makes the plants less digestible for animals (Luthra *et al.* 1988; Luthra *et al.* 1989; Navarro *et al.* 1997; Macfarlane *et al.* 2002). Similar results were also reported in Persian clover grown in medium containing chromium treated water by Stockdale *et al.* 1993.

In the present experiment, the number of total sugars, crude protein and grain yield decreased under Cr(VI) toxicity. Similar observations were reported by Arora *et al.* 1975 during their research work on dietary evaluation in Sorghum. It might be due to less absorption of nitrogen by the plant under Cr(VI) toxicity as nitrogen is utilized directly in protein synthesis by the plant. Hence as the nitrogen absorption decreased and Cr(VI) accumulation increased in the plant which in turn resulted in decreased crude protein content and given rise to decreased overall plant growth in terms of sugars, proteins and yield. These results were also supported by the findings of Sumanlata *et al.* 1999 in guar and Zayed *et al.* 1998 in vegetable crops.

In this experiment, it was observed that proline content increased with increasing concentration of Cr(VI) toxicity. These results were also supported by the results of Singh *et al.* 2012 in black gram (*Vigna mungo* L.) seedlings grown under Pb and Ni toxicity. Many scientists have been reviewed that plants have a common strategy of

accumulation of osmolytes such as polyamines, polyols and amino acids (proline) to fight against stressful conditions for example Cr(VI) toxicity (Bassi *et al.* 1993; Costa *et al.* 1994; Ali *et al.* 2011). Proline also acts as a quencher of ROS and chelator of heavy metals which in turn helps in dismissing stress (Farago *et al.* 1979; Saradhi *et al.* 1991; Sears *et al.* 2013; Kumar *et al.* 2017). Aggarwal *et al.* 2011 reported that pre-treatment of proline in *Solanum nigrum* under Cd stress encouraged the creation of phytochelatins and quenched the ROS generated due to Cd stress. Similarly, increased content of endogenous proline in bean seedlings on exogenous proline treatment provoked the toxic effects of selenium (Hall *et al.* 1997; Wyszowski *et al.* 2013). Hence, in the present experiment also the increase in proline content under Cr(VI) toxicity in Sorghum might be a part of the defence mechanism of the plant to combat oxidative stress created by Cr toxicity.

The results of the present experiment shown that the toxic components viz. Cr(VI) and hydrocyanic acid (HCN) content increased in Sorghum with increasing concentrations of Cr(VI) toxicity. The Cr content in roots was greater than the Cr content found in shoots of Sorghum. Moreover, the increasing rate of Cr content was more in roots as compared to shoots. It might be due to slow transport of Cr(VI) from roots to shoots as was reported by Kumar *et al.* 2010 and Kumar *et al.* 2019 in Sorghum. Crowe *et al.* 2001 and Shanker *et al.* 2004 reported that the impounding of Cr(VI) into the vacuoles of the roots might be the reason for poor transport of Cr(VI) from roots to shoots. Additionally, in a plant cell Cr(VI) is taken up inertly and retained by cation exchange sites of the cell-wall roots and shoots (El *et al.* 2012). Consequently, Cr(VI) could be halted more in the roots as compared to shoots. A similar, pattern of Cr(VI) uptake such as extreme uptake by roots followed by stem and leaves was also reported by Sharma and Sharma. 1993 and Gheju *et al.* 2009. They observed that Cr(VI) was least mobilized in roots which in turn resulted in lesser content in stem and leaves.

The HCN is produced extremely at 35 DAS growth stage in the Sorghum plant that's why it was measured at this stage only (Taneja *et al.* 1983). Sorghum crop is very poisonous to the animals who consume it at this stage due to excessive HCN production and the fatal dose of HCN is 200ppm (Mcbee *et al.* 1980). The nitrogen manuring frostiness and water stress improved the HCN content in Sorghum, but the supply of phosphorus, irrigation, plant oldness and ensiling generally reduced the HCN levels in Sorghum plants (Arora *et al.* 1977; Talanova *et al.* 2000). Similar to Cr(VI) content the HCN content was also increased with increasing Cr(VI) toxicity in both the varieties. But variety HJ 541 showed higher HCN and Cr(VI) content than variety SSG 59-3 under Cr(VI) toxicity. The rise in HCN content under Cr(VI) toxicity might be due to the reduction in the activity of the  $\beta$ -glucosidase enzyme which



in turn increased the glycosides availability used for HCN synthesis (Gebrehiwot *et al.* 2001). The other reason might be the creation of dehydrated conditions, decreased plant growth and biomass under Cr(VI) toxicity (Gebrehiwot *et al.* 2001). In this way the nutrient quality of Sorghum was decreased under Cr toxic stress and even the Sorghum may become lethal to organisms who consumed it.

#### **4. Conclusion:**

Sorghum is used as grain as well as a fodder crop in the world. It has great economic importance. It may be utilized as a better substitute for food materials in drought-prone areas. From the present research work it may be concluded that Cr(VI) may produce harmful effects in the living system on earth as evident from the impacts of Cr(VI) toxicity on plant growth and nutritional quality of Sorghum which in turn can affect the health of livestock directly or indirectly. During the present experiment, the exogenous application of Cr(VI) in Sorghum resulted in decreased plant growth, nutritional quality and increased toxic components (Cr and HCN) as compared to control. Toxic effects in sorghum plants appeared after 1ppm of hexavalent Cr treatment in soil. It was observed that 4ppm level of Cr(VI) in soil was becoming lethal to Sorghum. Among both the cultivars, HJ 541 was found more sensitive to Cr(VI) toxicity than SSG 59-3. Thus Cr(VI) toxicity must be resolved by keeping in mind the nutritional quality and yield of the crop. Suitable strategies must be utilized to overcome Cr(VI) toxicity in crop plants so that nutritional quality and plant growth can be restored. The investigational plan and explanations of this study may be used for the scheming of a mitigation strategy for chromium toxicity.

#### **5. Methods:**

The present research was conducted on two Sorghum (*Sorghum bicolor* L.) varieties viz. HJ 541 and SSG 59-3. The corresponding author himself undertook the formal identification of the plant material used in this study. The author maintained the treatments, noted down the stages of growth, proper nutrient, and daily caring of the crop, and prepared the samples properly. The specimens were demolished by the university authority after the completion of the research work. All types of experimental conditions were maintained properly by the corresponding author.

##### **5.1 Experimental design and raising of the crop:**

A pot experiment was conducted by using complete randomized design, in the screen house of Biochemistry department, Chaudhary Charan Singh Haryana Agricultural University (CCSHAU) Hisar, India. Seeds of Sorghum were collected from the Forage Section, Department of Genetics and Plant Breeding, CCSHAU. The plants were

raised in earthenware pots filled with acid washed, chromium free 5 kg sandy loam soil in naturally lit net house. The physiologic and biochemical properties of soil used to fill the pots are mentioned in Table 1. Seeds of uniform size were selected and surface sterilized with 0.01 % mercuric chloride ( $\text{HgCl}_2$ ) solution for two minutes, followed by complete washing of the seeds with distilled water. After proper washing, ten seeds per pot were sown at a depth of 5 cm in each pot. All pots were irrigated with equal quantities of water and nutrient solution as per recommended POP. Three replications were maintained for each treatment. After seedling emergence, thinning was done to six seedlings per pot. Further thinning was done after 35 days of sowing (DAS) up to three plants per pot.

### 5.2 Exposure with Cr(VI) toxicity:

On the basis of earlier experiment conducted by Ali *et al.* 2011; two different levels (2 and 4 ppm) of Cr(VI) were prepared with distilled water by using  $\text{K}_2\text{Cr}_2\text{O}_7 \cdot 7\text{H}_2\text{O}$  formulation obtained from Sigma Ltd. This preparation was supplied directly in soil in each pot before sowing respectively.

### 5.3 Sampling and Analysis:

The plant samples from each treatment, were collected at 35 DAS and 95 DAS, for both the cultivars. The samples for hydrocyanic acid (HCN) analysis were collected at 35 DAS only. All the samples were maintained at 4 to 10 °C temperature for complete analysis or as per the requirement of the parameter. A complete plant from each replication was collected and carefully washed with distilled water. Plant growth and its attributes were measured. The plant samples were divided in to root, shoot and leaves for further analysis.

### 5.4 Plant growth and biomass determination:

Root and shoot length was measured in centimeters (cm) by using a non-commercial scale of five meters in size. Same scale was used for the measurement of plant height (cm), from the base to the top most part of plant. Results for number of leaves and tillers per plant were expressed as the mean of simply counted leaves and tillers number from each replication, respectively. One whole plant was used for fresh weight determination and same plant sample were kept in a hot air oven (70°C) for determination of dry weight per plant in gram (g) from each replication. Chlorophyll content was estimated in third leaf of the plant using the Konica Minolta Chlorophyll Meter SPAD-502Plus.

### 5.5 Determination of Nutritional Parameters:

Structural carbohydrates *viz.* neutral detergent fiber (NDF) and acid detergent fiber (ADF) were estimated by using the method of Van Soest *et al.* 1968. Nonstructural carbohydrates *viz.* total sugar content was determined by

using method of Dubois *et al.* 1956. Grain yield was determined on 100 grains weight basis. Hundred grains from each replication were selected randomly and weighed separately for each treatment. The grain yield in % was expressed as the mean weight of different grain weights taken separately for both varieties and treatments. *In vitro* dry matter digestibility (IVDMD) was determined by the method of Barnes *et al.* 1971. Crude protein was estimated by conventional Micro-Kjeldahl method (984.13) of AOAC (1995). Proline content in straw sample was analyzed by the method off Bates *et al.* 1973.

### 5.6 Determination of Toxic constituent:

In order to measure the HCN content, fresh green inner most portion of the collar from upper most portion of the growing tip of the plant was collected and chopped with blades and dipped in chloroform and provided with a whatman number – 4 filter paper saturated with alaline picrate solution in air tight conditions, overnight. The change in colour was read at 515 nm wavelength spectrophotometrically and the HCN content was calculated by using a standard curve described by Hogg and Ahlgren. 1942. The amount of HCN was expressed on dry weight basis. Chromium concentration in root and straw sample was analyzed separately by following the method off Sahuquillo *et al.* 1995. The samples were digested in digesting mixture consist of HNO<sub>3</sub> and HClO<sub>4</sub> in 3:1 ratio (v/v). The digested solution was mixed with DTPA solution and the Cr content was determined by using atomic absorption spectrophotometry and expressed in ppm.

### 5.7 Statistical analysis:

All the results were statistically analyzed by using IBM SPSS Statistics 23 software for windows along with post hoc tukey test. On the basis of CD values, differences between the treatment doses were evaluated. The interactions were found to be significant with P at  $\leq 0.05$ .

### Abbreviations:

2ppm =  $2 \times 10^{-3}$  g Cr(VI)/kg soil

4ppm =  $4 \times 10^{-3}$  g Cr(VI)/kg soil

Chromium = (Cr VI)

DAS = Days after sowing

dS = Deci-siemens

EC = Electrical conductivity

HCN = Hydrocyanic acid

IVDMD = *In-vitro* dry matter digestibility

OC = Organic carbon

P<sup>H</sup> = Potential of hydrogen

POP = Package of practices

ppm = parts per million

## **6. Declarations:**

### **Ethics approval and consent to participate**

The author approves that there are no ethical issues regarding the materials and publication of this research work in the journal Heliyon by any authority.

### **Consent to publish**

This section is not applicable to this manuscript as it shares all original work and no copied work in any form from anywhere.

### **Availability of data and materials**

All data generated or analysed during this research work are included in this published article.

### **Competing interest**

The author declares that he has no conflicts of interest and he has no known competing for financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### **Author contributions**

P.K. conceptualize and conducted the experiments, performed the statistical analysis, and drafted the complete manuscript by preparing all the graphs, figures, and tables. Thereafter reviewed the manuscript and finally, approved the manuscript.

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## Figure Legends

**Fig. 1.** Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on biomass (fresh weight and dry weight) of the Sorghum plants (HJ 541 and SSG 59-3) at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

**Fig. 2.** Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on chlorophyll content of the Sorghum plants (HJ 541 and SSG 59-3) at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

**Fig. 3.** Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on root length per plant of Sorghum (HJ 541 and SSG 59-3) at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

**Fig. 4.** Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on shoot length per plant of Sorghum (HJ 541 and SSG 59-3) at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

**Fig. 5.** Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on height of Sorghum (HJ 541 and SSG 59-3) plants at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

**Fig. 6.** Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on number of leaves per plant of Sorghum (HJ 541 and SSG 59-3) at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

**Fig. 7.** Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on number of tillers per plant of Sorghum (HJ 541 and SSG 59-3) at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

**Fig. 8.** Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on ADF, NDF and IVDMD of Sorghum (HJ 541 and SSG 59-3) plants at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

**Fig. 9.** Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on proline content of Sorghum (HJ 541 and SSG 59-3) plants at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

**Fig. 10.** Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on sugar content and grain yield of Sorghum (HJ 541 and SSG 59-3) plants at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

**Fig. 11.** Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on crude protein content of Sorghum (HJ 541 and SSG 59-3) plants at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

**Fig. 12.** Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on chromium (Cr) content in root samples (RS) and shoot samples (SS) of Sorghum (HJ 541 and SSG 59-3) plants at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

**Fig. 13.** Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on hydrocyanic acid (HCN) content of Sorghum (HJ 541 and SSG 59-3) plants at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

### Table Legends

**Table 1:** Properties of soil used for the experiment.

Tables

Table 1: Properties of soil used for the experiment

Property	Value & unit	Evaluation
Texture	-	Sandy loam
Sand	71.70	-
Silt	18.96	-
Clay	9.34	-
p <sup>H</sup>	8.2	Basic
OC	0.32	Low
EC	0.17 DS/meter	Normal
Water retention capacity of soil	4.67 Inches	-
Nitrogen (N)	3 mg/kg soil	Low
Phosphorus (P)	8 mg/kg soil	Low
Potassium (K)	84 mg/kg soil	Normal
Zink (Zn)	0.61 mg/kg soil	Normal
Iron (Fe)	0.7 mg/kg soil	Low
Copper (Cu)	0.18 mg/kg soil	Normal
Manganese (Mn)	2.73 mg/kg soil	Normal
Chromium (Cr)	0.016 mg/kg soil	Low

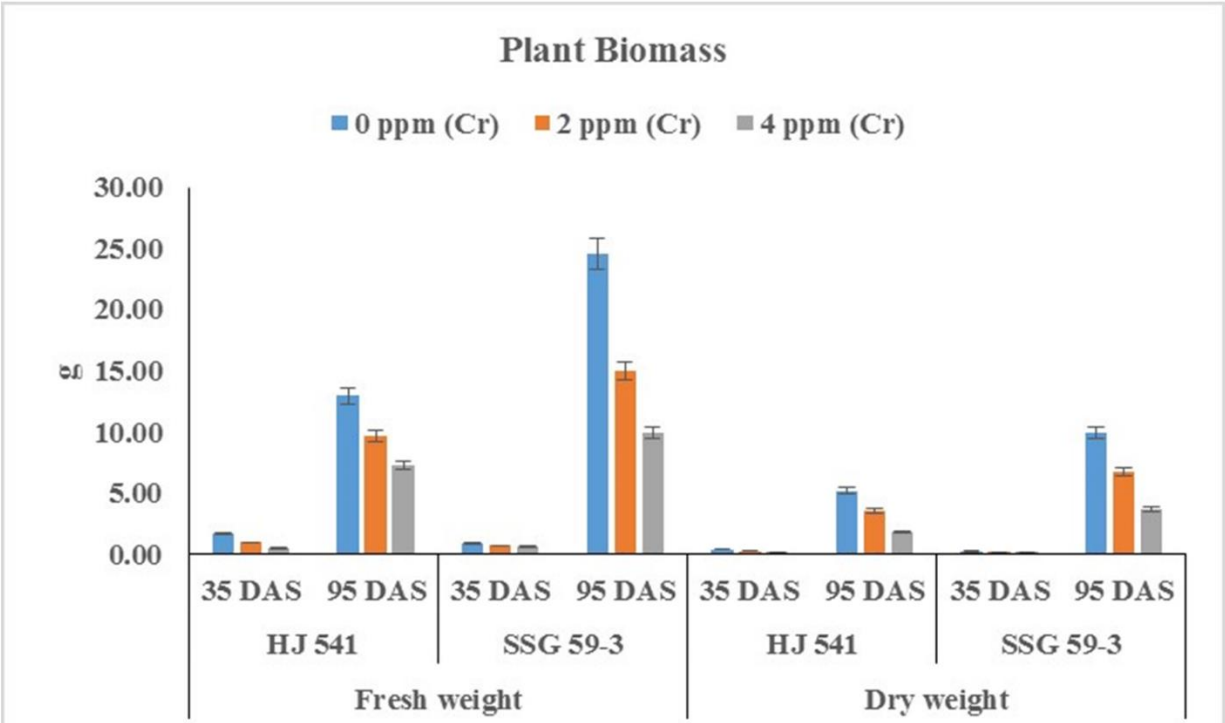


Fig. 1. Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on biomass (fresh weight and dry weight) of the sorghum plants (HJ 541 and SSG 59-3) at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

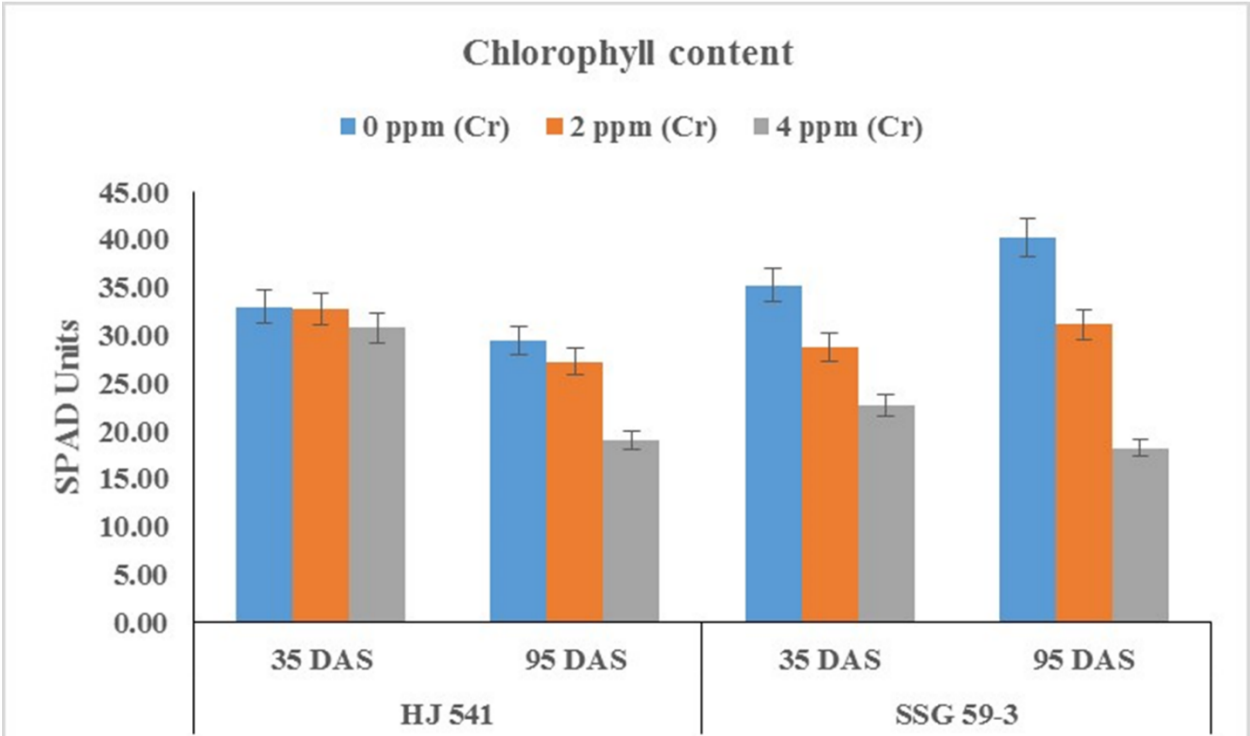


Fig. 2. Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on chlorophyll content of the sorghum plants (HJ 541 and SSG 59-3) at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

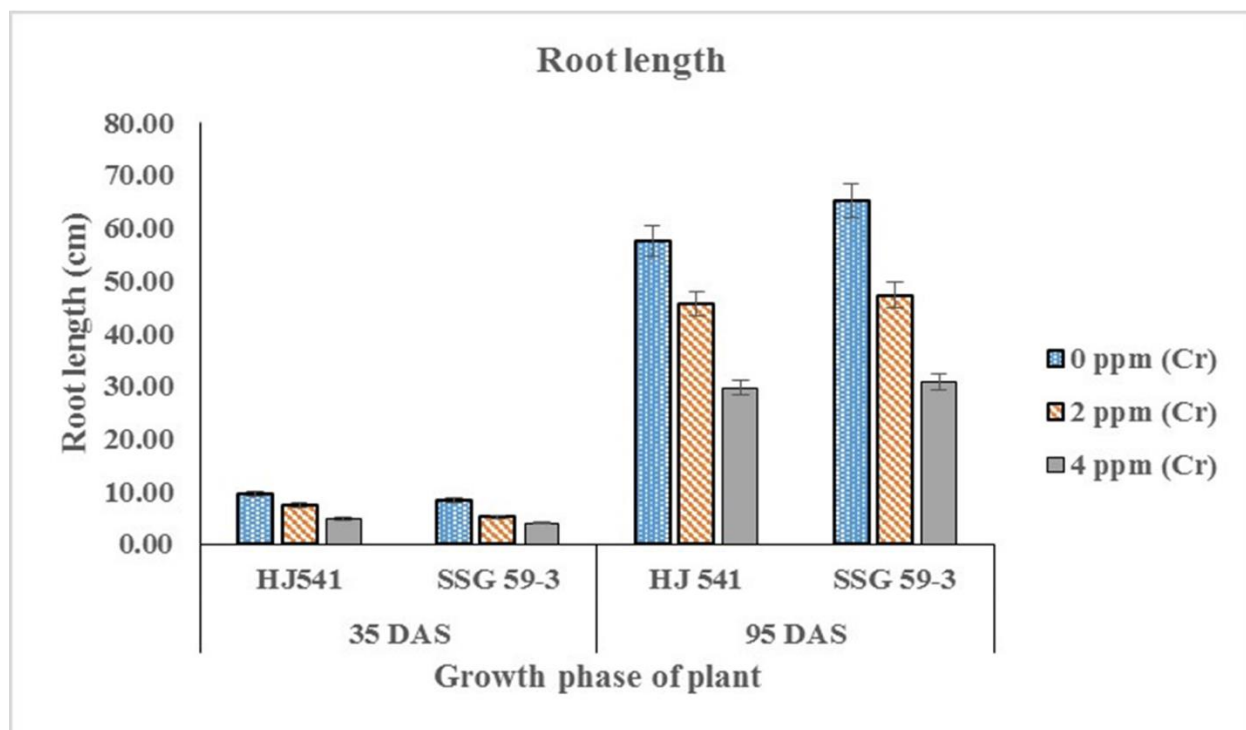


Fig. 3. Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on root length per plant of sorghum (HJ 541 and SSG 59-3) at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

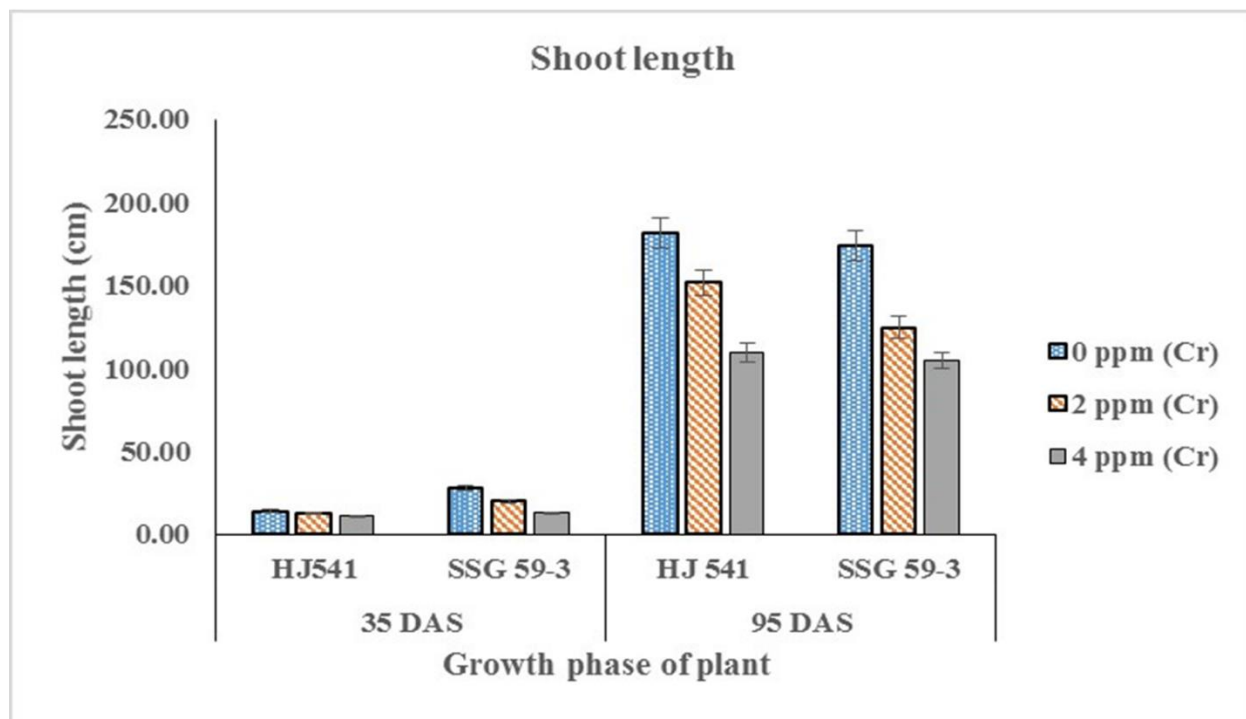


Fig. 4. Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on shoot length per plant of sorghum (HJ 541 and SSG 59-3) at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

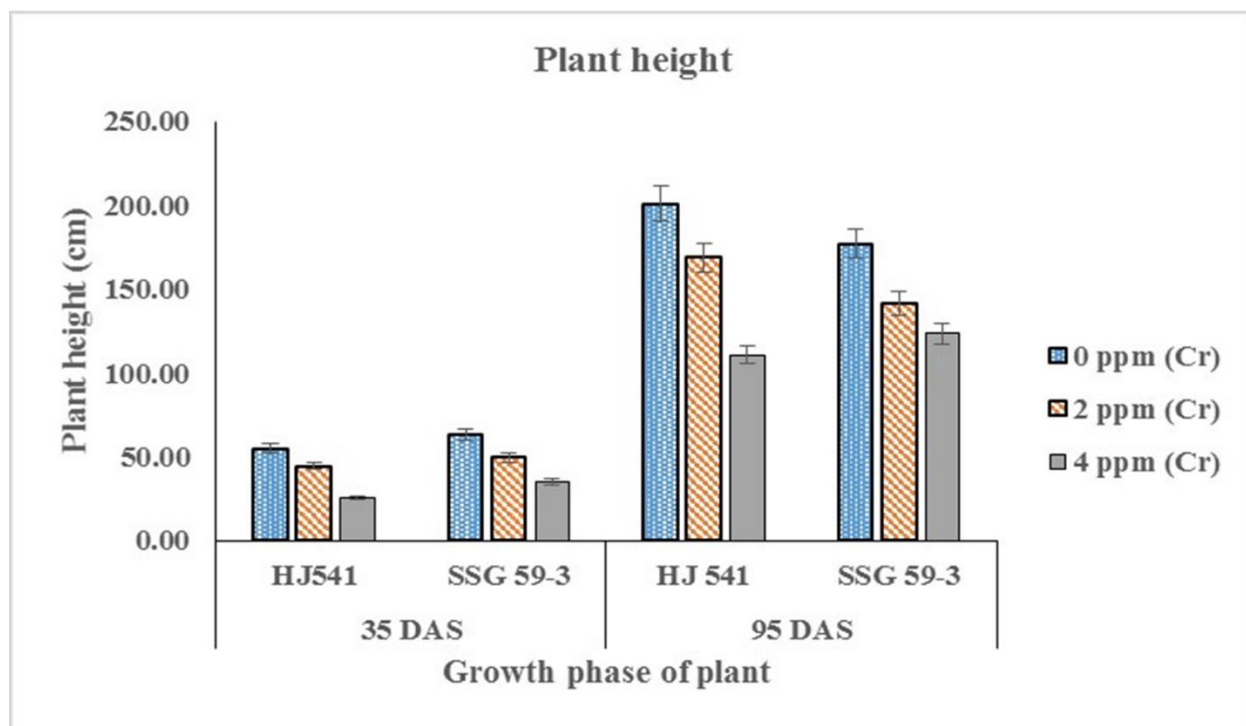


Fig. 5. Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on height of sorghum (HJ 541 and SSG 59-3) plants at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. From three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

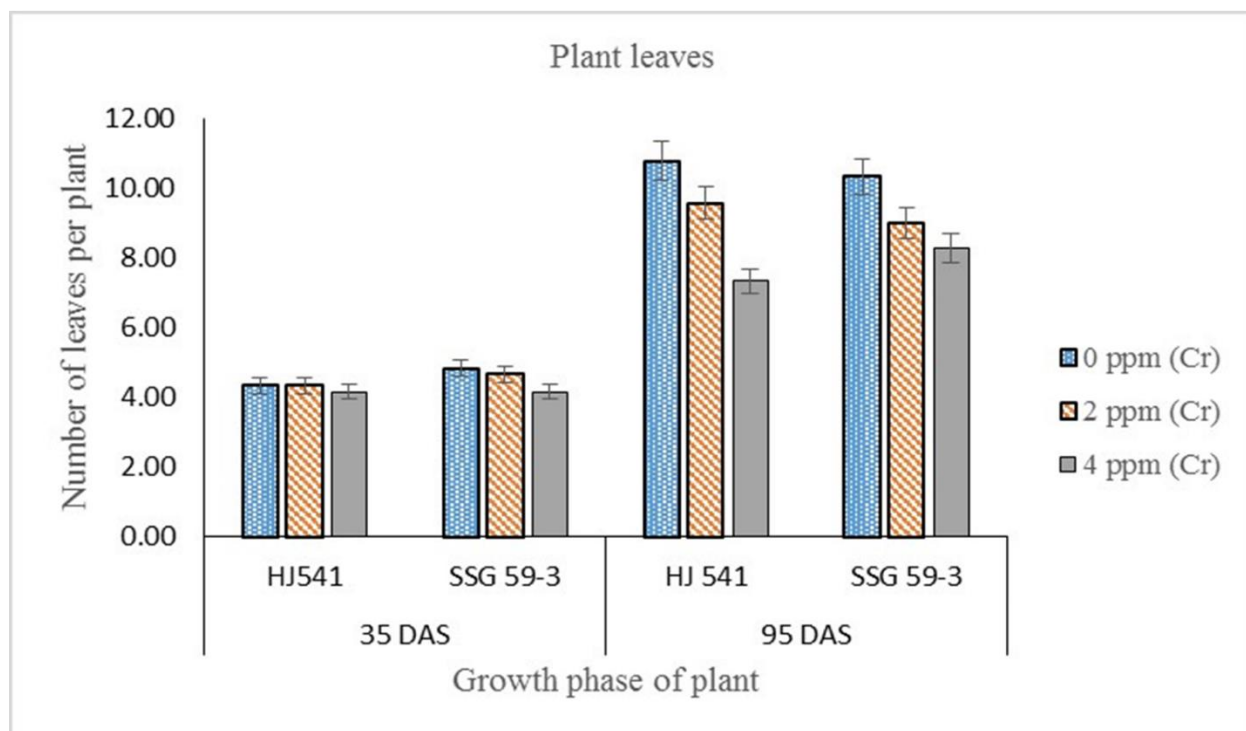


Fig. 6. Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on number of leaves per plant of sorghum (HJ 541 and SSG 59-3) at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).



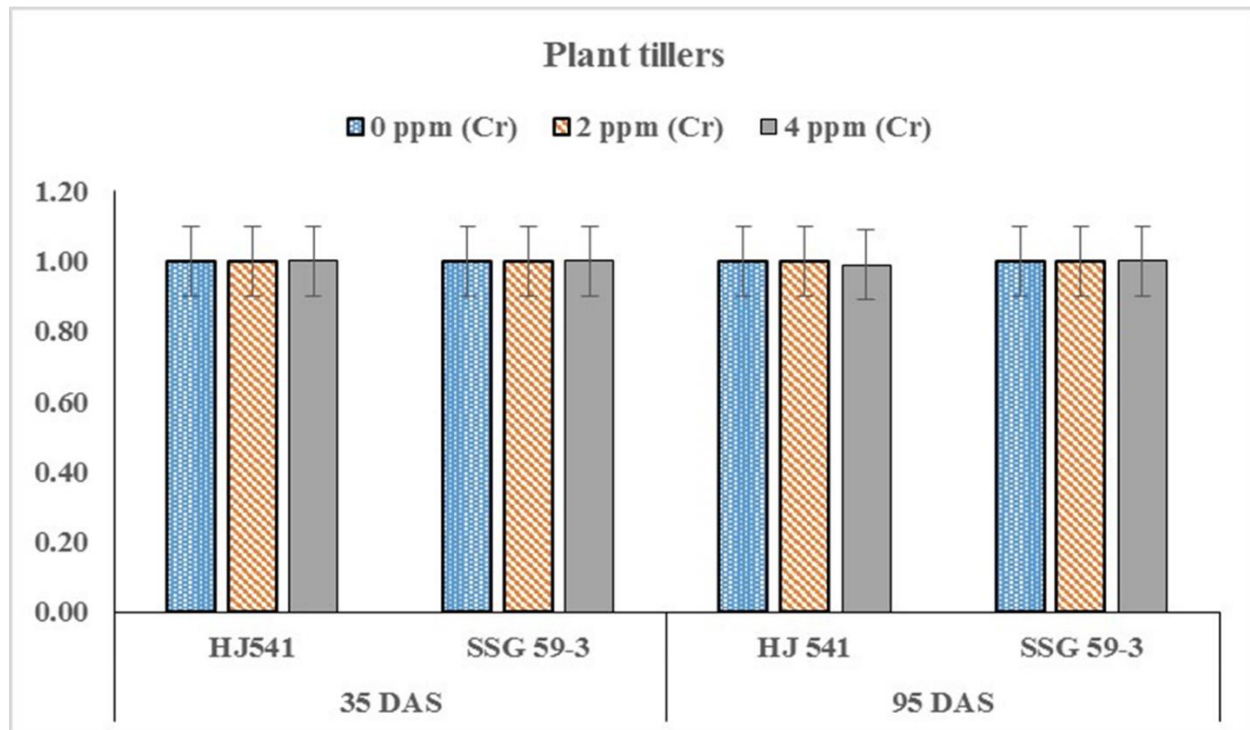


Fig. 7. Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on number of tillers per plant of sorghum (HJ 541 and SSG 59-3) at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

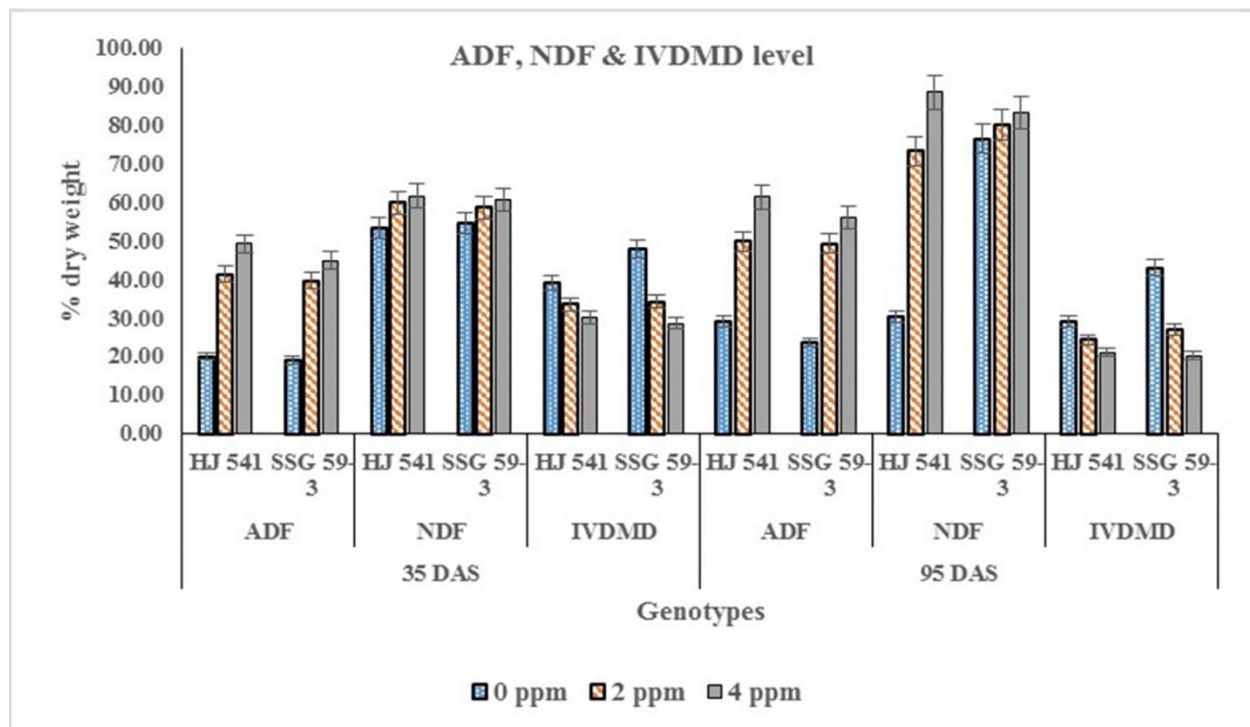


Fig. 8. Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on ADF, NDF and IVDMD of sorghum (HJ 541 and SSG 59-3) plants at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

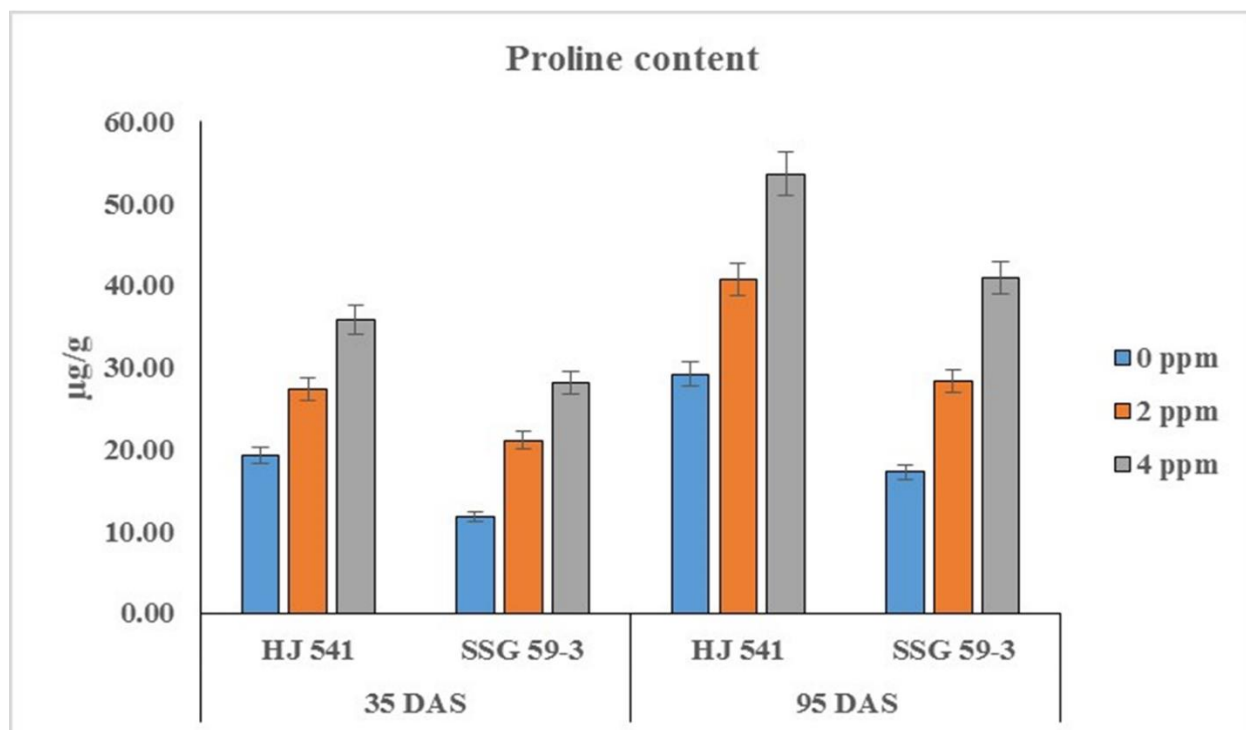


Fig. 9. Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on proline content of sorghum (HJ 541 and SSG 59-3) plants at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

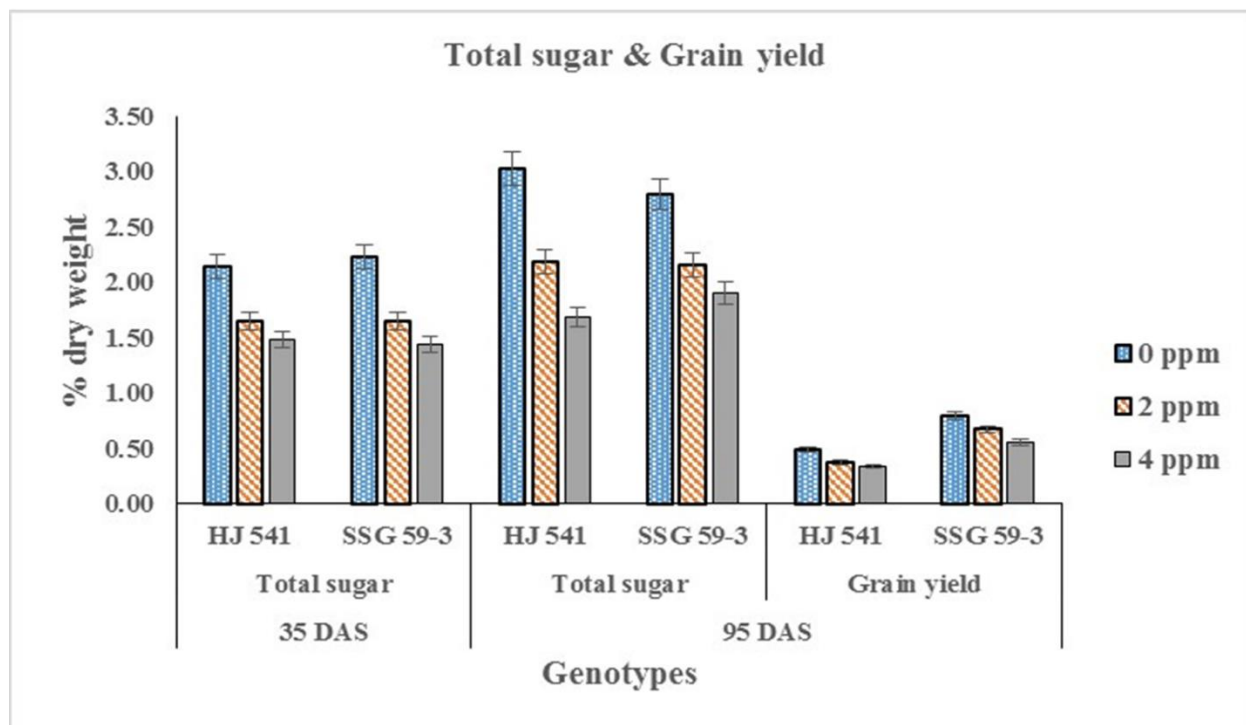


Fig. 10. Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on sugar content and grain yield of sorghum (HJ 541 and SSG 59-3) plants at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

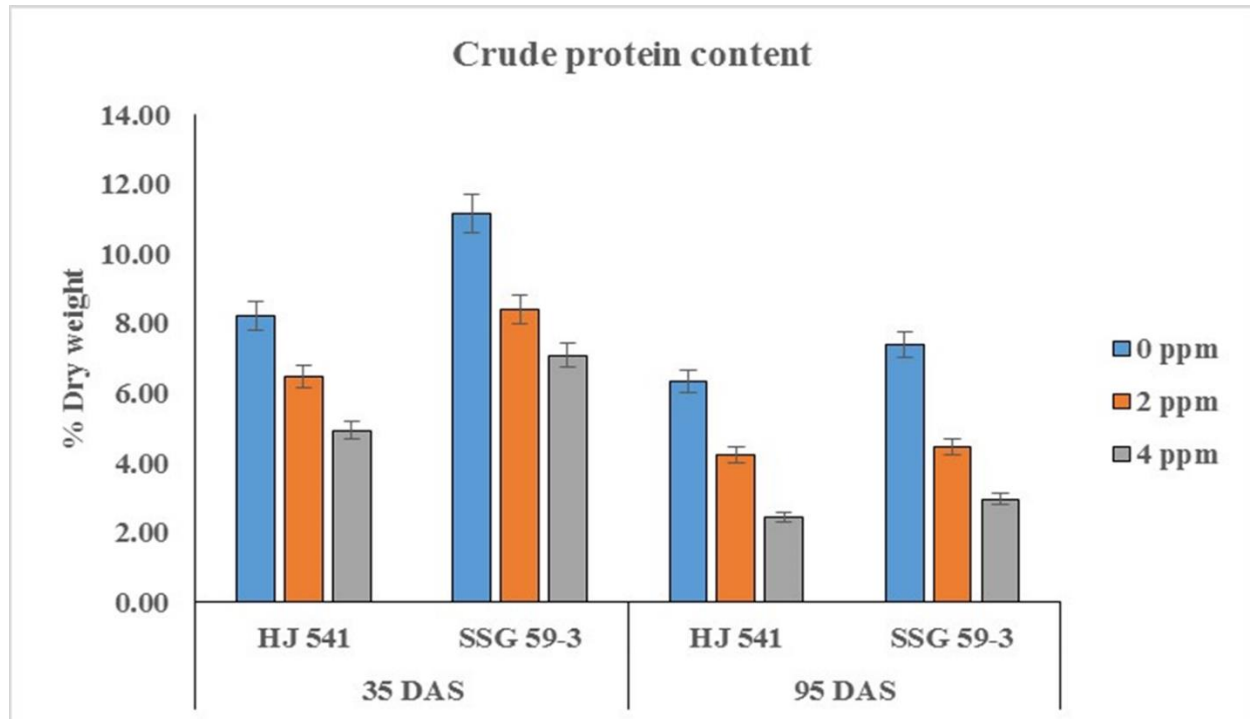


Fig. 11. Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on crude protein content of sorghum (HJ 541 and SSG 59-3) plants at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

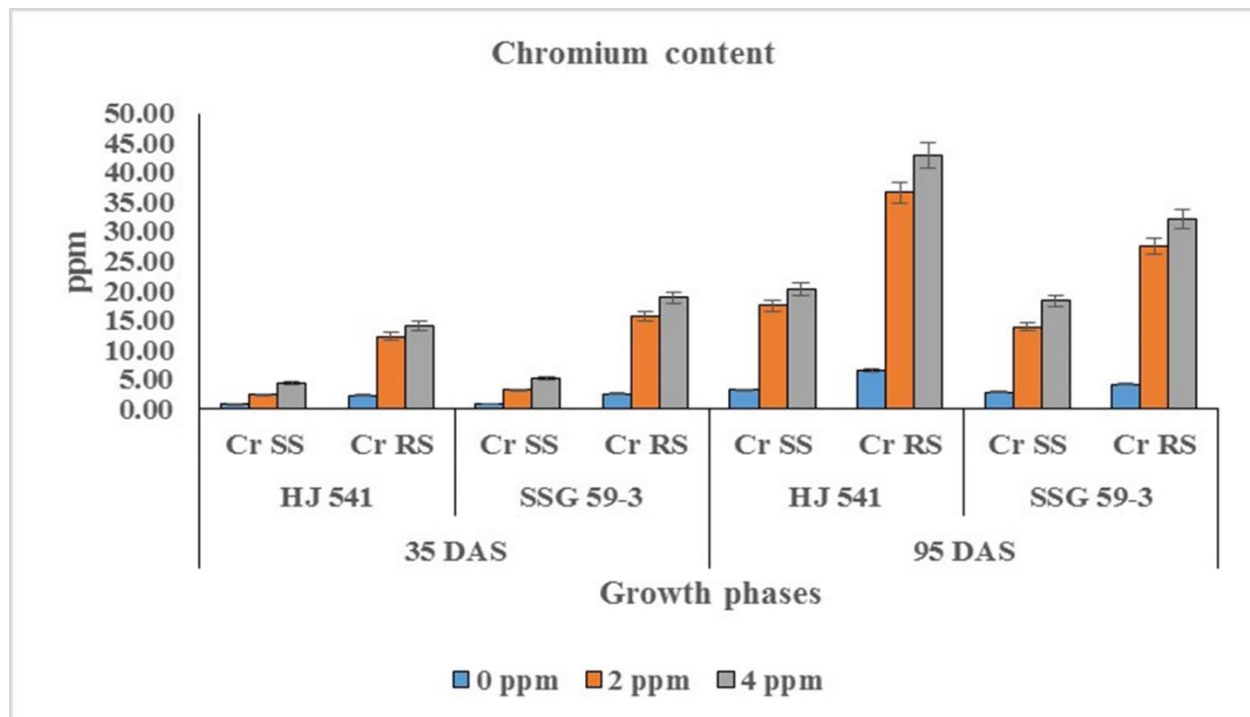


Fig. 12. Effect of Cr(VI) toxicity (0, 2 and 4 ppm) on chromium (Cr) content in root samples (RS) and shoot samples (SS) of sorghum (HJ 541 and SSG 59-3) plants at 35 and 95 DAS. Values represent the mean  $\pm$  S.E. from three independent experiments. Significance difference was at  $P \leq 0.05$  (ANOVA).

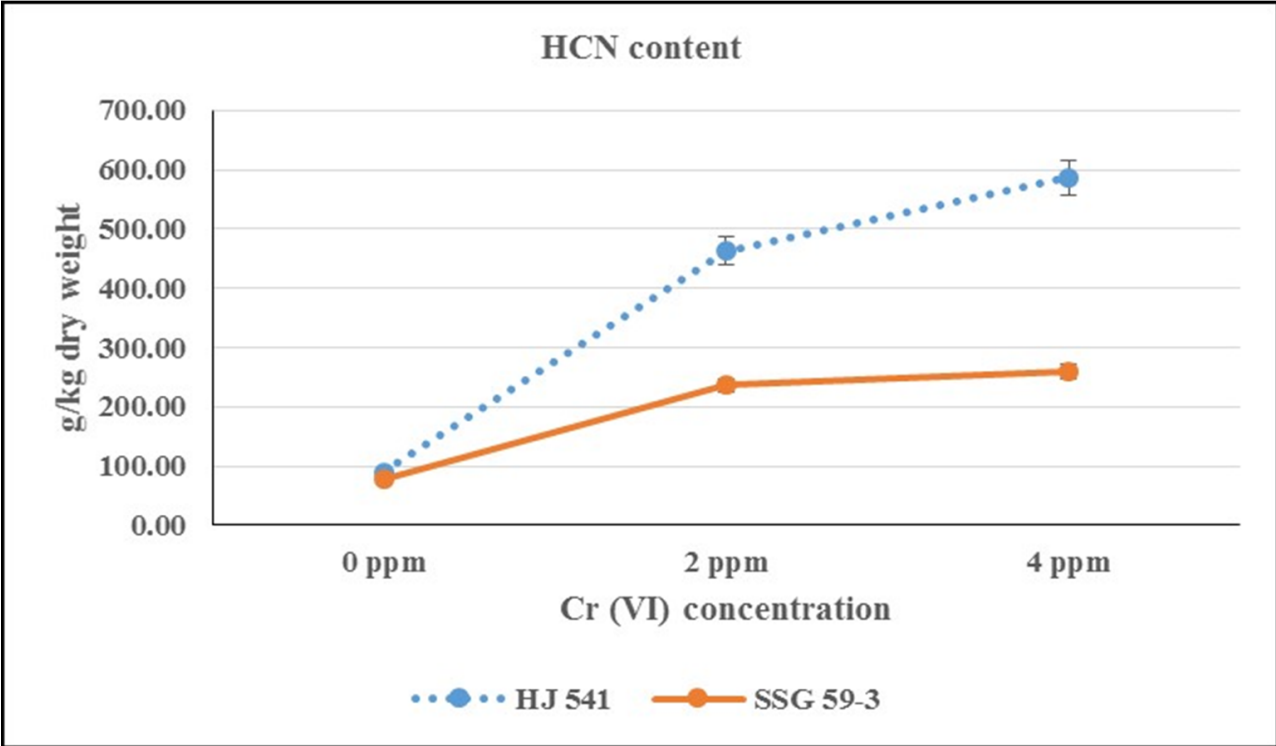


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