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Research Article – Environmental Sciences

Effect of various concentrations of chromium polluted soil on biochemical changes of Globe amaranth (*Gomphrena globosa* L.)

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Abstract

An attempt was made to study the effect of different concentrations of chromium on biochemical specifications on response of Globe amaranth (*Gomphrena globosa* L.). The seeds were obtained from Private Agro centre, Villupuram District, Tamil Nadu, India. The healthy seeds of globe amaranth were chosen and used for both laboratory experiments. The various Cr concentrations were used (25, 50, 75 and 100 mg/l). Biochemical parameter such as, protein, amino acid, sugar and proline contents in leaf, stem and root of globe amaranth at 15, 30, 45 and 60 DAS in chromium polluted soil. Among the results protein, sugars and amino acid contents in leaf, stem and root were also significantly decreased in all different concentration of Cr treated as compared to control plants. On the other hand, proline content in leaf, stem and root were increased in all the Cr treated plants as compared to control. To sum up protein, sugars and amino acid were also significantly decreased while, proline content was increased in all the chromium treated plants.

Key words: Globe amaranth, Chromium, protein, sugar and proline

Introduction

Global industrialization and human social and agricultural activities have an effect on environmental pollution and the global ecosystem. This corruption of the ecosystem has a negative effect on human health and on all living organisms. Growing industrialization and environmental pollution from technology have started to affect human health (YagdiI *et al.,* 2000).

Soil pollution by heavy metals, such as cadmium, lead, chromium, and copper, etc. is a problem of concern. Although heavy metals are naturally present in soil, contamination and comes, from local sources: mostly industry (mainly non-ferrous industries, but also power plants and iron, steel and chemical industries), agriculture (irrigation with polluted waters, sewage sludge and fertilizer, especially phosphates, contaminated manure and pesticide containing heavy metals), waste incineration, combustion of fossil fuels and road traffic. Long-range transport of atmospheric pollutants adds to the metals in the natural environment (European Environmental Agency, 1995). In recent years, it has been shown that lead levels in soil and vegetation have increased considerably due to traffic pollution, especially from usage of leaded petrol and exhaust combustion (Cabrera *et al.,* 1999, Vidal *et al.,* 1999, Madejon *et al.,* 2002, Onoanwa and Adoghe, 1997). The problem worsens as daily traffic increases (Oncel *et al.,* 2004).

Heavy metals are known to cause irreversible damage to a number of vital metabolic constituents and important biomolecules. They also injure plant cell membrane. Trace elements are necessary for the normal metabolic functions of a plant, but at relatively higher concentrations these metals are toxic and may severely interfere with physiological and biochemical functions (Chandra and Kulshreshtha, 2004). Chromium (Cr) is a natural element found in the earth's crust. It is one of the heavy metal pollutants produced from tanning, paint and other such industries. Cr shows its

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cytotoxic and phytotoxic effects because it is easily absorbed by the plants from the soil and atmosphere. It accumulates in different parts of the plants (Parmar and Chanda, 2005).

Chromium is widespread heavy metal in the environment. The environment contaminated by the chromium in the way of both natural and anthropogenic sources. Chromium is released into the environment by a large number of industrial operations such as electroplating, chromate manufacturing, leather tanning and wood preservation resistant alloys, mining etc (Papp, 2001). The leather industry is the major cause of high efflux of Cr to the biosphere, accounting for 40% of the total industrial used (Barnhart, 1997). Notably Trivalent chromium (III) and hexavalent chromium (VI) are the two species of chromium which are found naturally in the environment Cr(VI) appears to be more toxic than Cr(III).

Gomphrena species is an edible, commercial ornamental and medicinal plant commonly known as Globe Amaranth or Bachelor Button, belongs to the family Amaranthaceae. It comprises approximately 120 species found in the America, Antartica and Indo-Malaysia. 46 species found in Brazil. Moreover, the Amaranthaceae is a cosmopolian family which occurs at disturbed, arid or saline areas; one of the characteristics that ensure its survival in adverse environments is the operation of C4 pathway of photosynthesis (Báo *et al.,* 2010). It is erect or ascending herbs to 50cm. leaves spiral, deltoid to elliptic ovate. Inflorescence is axillary, slender, thyrsiform spike. Flowering mostly occurs in June to December. Globe amaranth (*Gomphrena globosa* L.) might be one of the plants that can use atmospheric sulfides for its growth (Andrade *et al.,* 2012, Wang *et al.,* 2009).

The main goal of the present study was to investigate in detail the effect of different concentrations of chromium on biochemical specifications on response of Globe amaranth (*Gomphrena globosa* L.). Biochemical parameter such as, protein, amino acid, sugar and proline contents in leaf, stem and root of globe amaranth at 15, 30, 45 and 60 DAS in chromium polluted soil.

Materials and Methods

The present investigation was carried out to the effect of different concentrations of chromium on germination and growth of Globe amaranth (*Gomphrena globosa* L.). The research work comprises of the following aspects, biochemical parameters such as protein, amino acid, sugars, proline of *Gomphrena globosa* L. seeds grown under different concentrations of chromium.

Seed materials

Globe amaranth (*Gomphrena globosa* L.) seeds were obtained from Private Agro centre, Villupuram District, Tamil Nadu, India. The healthy seeds of Globe amaranth were chosen and used for both laboratory and field experiments.

Preparation of chromium solution

Potassium dichromate ($K_2Cr_2O_7$) salt was used as Cr source for the present study. A known weight (2.9583 g) of potassium dichromate salt was dissolved in 1000 ml of distilled water to get a concentration of 1000 ppm as Cr. From this standard solution, the various concentrations (25, 50, 75 and 100 mg/l) of chromium solution were prepared and used both for laboratory and field experiments.

Estimation of protein (Lowry et al., 1951)

Extraction

Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10 ml 20 per cent trichloro acetic acid. The homogenate was centrifuged for 15 min at 600 rpm. The supernatant was discarded. To the pellet, 5 ml of 0.1 N NaOH was added and centrifuged for 5 min. The supernatant was saved and made up to 10 ml with 0.1 N NaOH. This extract was used for the estimation of protein.

Estimation

One ml of the extract was taken in a 10 ml test tube and 5 ml of 'reagent C' (Reagent A: 0.4 g of sodium hydroxide was dissolved in 100 ml of distilled water. To this solution, 2 g of sodium carbonate was added; Reagent B: One per cent of copper sulphate was mixed with equal volume of 2 per cent sodium potassium tartarate; Reagent C: Fifty ml of reagent A and 1 ml of reagent B were taken and mixed and it was prepared freshly at the time of experiment) was added. The solution was mixed and kept in darkness for 10 min. Later, 0.5 ml of folin-phenol reagent (One ml of folin-phenol

reagent was diluted with 2 ml of distilled water) was added and the mixture was kept in dark for 30 min. The sample was read at 660 nm in the UV-Spectrophotometer. The protein contents were expressed in mg g^{-1} fresh weight.

Estimation of sugars (Nelson, 1944)

Extraction

Five hundred mg of plant materials were weighed and macerated in pestle and mortar with 10 ml of 80 per cent ethanol. The homogenate was centrifuged for 10 min at 800 rpm. The supernatant was saved. Then, the ethanol is evaporated in a water bath at 500C. The net content was made up to 20 ml with distilled water and the extract was used for the estimation of reducing sugar.

Estimation

One ml of extract was taken in a 25 ml marked test tube. 1 ml of reagent 'C' was added. Then, the mixture was heated for 20 min at 100°C in a boiling water bath, cooled and 1 ml of arsenomolybdate reagent was added. The solution was thoroughly mixed and diluted to 25 ml with distilled water. The sample was read in a UV-spectrophtometer at 520 nm. The sugar contents were expressed in mg/g fresh weight basis.

Reagent A: Twenty five grams of anhydrous sodium carbonate, 25 g of sodium potassium tartarate, 20 g of sodium bicarbonate and 200 g of anhydrous sodium sulphate were dissolved in 800 ml of distilled water and made up to 1000 ml. Then, it was filtered and stored in a glass stoppered brown bottle.

Reagent B: Fifteen per cent copper sulphate containing 1 or 2 drops of concentrated sulphuric acid.

Reagent C: Fifty ml of reagent A and one ml of reagent B were mixed well and it was prepared freshly at the time of experiment) was added. Then, the mixture was heated for 20 min at 100°C in a boiling water bath, cooled and 1 ml of arsenomolybdate reagent (To 450 ml of distilled water, 25 g of ammonium molybdate, 21 ml of concentrated sulphuric acid were added and 3 g of sodium arsenate was dissolved in 25 ml of distilled water. The mixture was kept in a water bath at 37°C for 24 to 48 hours. The reagent was stored in a glass stoppered brown bottle) was the added. The solution was thoroughly mixed and diluted to 25 ml of distilled water. The sample was read in a UV-Spectrophotometre at 520 nm. The sugar contents were expressed in mg g^{-1} fresh weight.

Non-reducing sugars (Nelson, 1944)

Non-reducing sugars present in ethanol extracts (extraction as in reducing sugars) were hydrolysed with sulphuric acid to reducing sugars. Reducing sugars present in the hydrolysates were estimated following Nelson's method. The differences between the total sugars and the reducing sugars correspond to the reducing sugars.

Hydrolysis

One ml of extract was taken in a test tube and evaporated to dryness in a water bath for 15 min. To the residue, 1 ml of distilled water and 1 ml of 0.1 N sulphuric acid was added. The mixture was hydrolysed by incubating at 49°C for 30 min in a thermostat. The solution was neutralized with 0.1 N NaOH (5 ml) and the methyl red as indicator. To this, 1 ml of reagent C (copper reagent) was added and heated for 20 min, cooled and 1 ml of arsenomolybdate reagent was added. The content was made upto 25 ml and the absorbance was read at 495 nm in a UV-Spectrophotometre. The reducing sugar contents are expressed in mg g^{-1} fresh weight. Blank was prepared with 1 ml of distilled water.

Estimation of amino acids (Moore and Stein, 1948)

Extraction

Five hundred mg of plant materials were weighed and macerated with a pestle and mortar with 10 ml of 80 per cent ethanol. The homogenate was centrifuged for 10 min. The supernatant was saved. The extract was used for the estimation of amino acids.

Estimation

One ml of the extract was pipette out into a test tube. A drop of methyl red indicator was added. The sample was neutralized with 1 ml of 0.1 N sodium hydroxide. To this, 1 ml of ninhydrin reagent was added and mixed thoroughly. The content of the test tube was heated for 20 min. in a boiling water bath. Five ml of the diluents solution was added and heated in water and the contents were mixed thoroughly. Blank was prepared without extract. The absorbance was read at 570 nm in a UV-Spectrophotometre. The amino acid contents are expressed in mg^{-1} fresh weight.

Proline (Bates et al., 1973)

Extraction

300 mg of fresh leaves were homogenized in 10 ml of 3% aqueous sulpho salicylic acid (3 g of sulpho salicylic acid was dissolved in 100 ml of distilled water). The homogenate was centrifuged at 9000 for 15 min. The procedure was repeated with the residue and the filtrates were pooled.

Estimation

2.0 ml of the filtrate was taken and 2.0 ml of acid ninhydrin (1.25 g of ninhydrin was dissolved in a warm mixture of 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid with agitation. The reagent was stable for 24 hrs when stored at 4°C) and 2.0 ml of glacial acetic acid added. The tubes were incubated for 1 hour at 1000C on a water bath. The tubes were transferred to an ice bath to terminate the reaction. 4.0 ml of toluene was added and mixed vigorously for 15 to 20 seconds. The chromophore containing toluene was aspirated from the aqueous phase. It was allowed to reach room temperature and the absorbance measured at 575 nm in UV-Spectrophotometre. A reagent blank was maintained. A standard curve was obtained using a known concentration of authentic proline (5 mg of proline was dissolved in 10 ml of 0.1 N Hydrochloric acids). The proline content was expressed as mg g^{-1} fresh weight.

Results and Discussion

The present investigation deals with detail the effect of different concentrations of chromium on biochemical specifications on response of Globe amaranth (*Gomphrena globosa* L.). Biochemical parameter such as, protein, amino acid, sugar and proline contents in leaf, stem and root of globe amaranth at 15, 30, 45 and 60 DAS in chromium polluted soil.

Biochemical analysis

Proteins

The impact of different concentration of chromium on protein contents in leaf, stem and root of globe amaranth at 15, 30, 45 and 60 DAS is given in Fig. 1. The highest protein content of leaf (3.76, 5.07, 3.9, and 3.28 mg g^{-1} fr. wt), stem (2.06, 3.69, 2.76 and 2.37 mg g^{-1} fr. wt) and root (2.67, 4.08, 3.48 and 2.2 mg g^{-1} fr. wt) were recorded in control plants at 15, 30, 45 and 60 DAS respectively. The lowest protein content of leaf (1.95, 3.32, 2.38 and 1.39 mg g^{-1} fr. wt), stem (0.47, 1.86, 1.02 and 0.66 mg g^{-1} fr.wt) and root (1.09, 2.06, 1.22 and 0.85 mg g^{-1} fr. wt) were recorded in 100 mg/l concentration of chromium treated plants at 15, 30, 45 and 60 DAS respectively. Bakiyaraj *et al* 2014 investigated that *Sesbania* plants in the higher concentration of chromium and cadmium reduce the protein content. The decrease in protein content in *Albizia lebbak* has been interpreted either due to reduced de novo synthesis of proteins or increased decomposition of proteins into amino acids (Tripathi and Tripathi, 1999). Moreover, the decreasing protein content in *Sesbania sesban* supported by Verma *et al*. (2012) the results showed that the soluble protein content decreased in seedlings with increasing concentration of cadmium chloride over the control seedlings. Results of John *et al.* (2008) showed that cadmium treatment (20 mg L^{-1}) resulted in reduction of soluble protein in *L. polyrrhiza.* Mohan and Hosetti (1997) and (Palma *et al*., 2002) found more pronounced decrease in the protein content with cadmium when compared to lead treatment in *L. minor.* The decrease in protein content in *L. polyrrhiza* was caused by enhanced protein degradation process as a result of increased protease activity.

Reducing sugar

The impact of different concentration of chromium on reducing sugar contents in leaf, stem and root of globe amaranth at 15, 30, 45 and 60 DAS is given in Fig. 2. The highest reducing sugar content of leaf (3.98, 6.73, 5.19 and 4.23 mg g^{-1} fr.wt), stem (3.20, 5.18, 4.28 and 3.08 mg g^{-1} fr.wt) and root (3.16, 4.79, 3.98 and 2.89 mg g^{-1} fr.wt) were recorded in control plants at 15, 30, 45 and 60 DAS respectively. The lowest reducing sugar content of leaf (1.45, 2.75, 2.09 and 1.67 2.mg.g^{-1} fr.wt), stem (1.08, 1.66, 1.8 and 0.96 mg.g^{-1} fr.wt) and root (0.54, 1.75, 0.86 and 0.38 mg.g^{-1} fr. wt) were recorded in 100 mg/l concentration of chromium treated plants at 15, 30, 45 and 60 DAS respectively.

Non-Reducing sugar

The effect of different concentration of chromium on non-reducing sugar contents in leaf, stem and root of globe amaranth at 15, 30, 45 and 60 DAS is given in Fig. 3. The highest non-reducing sugar content of leaf (2.93, 4.15, 2.86 and 1.98 mg g^{-1} fr. wt), stem (2.17, 3.42, 2.76 and 1.56 mg g^{-1} fr. wt) and

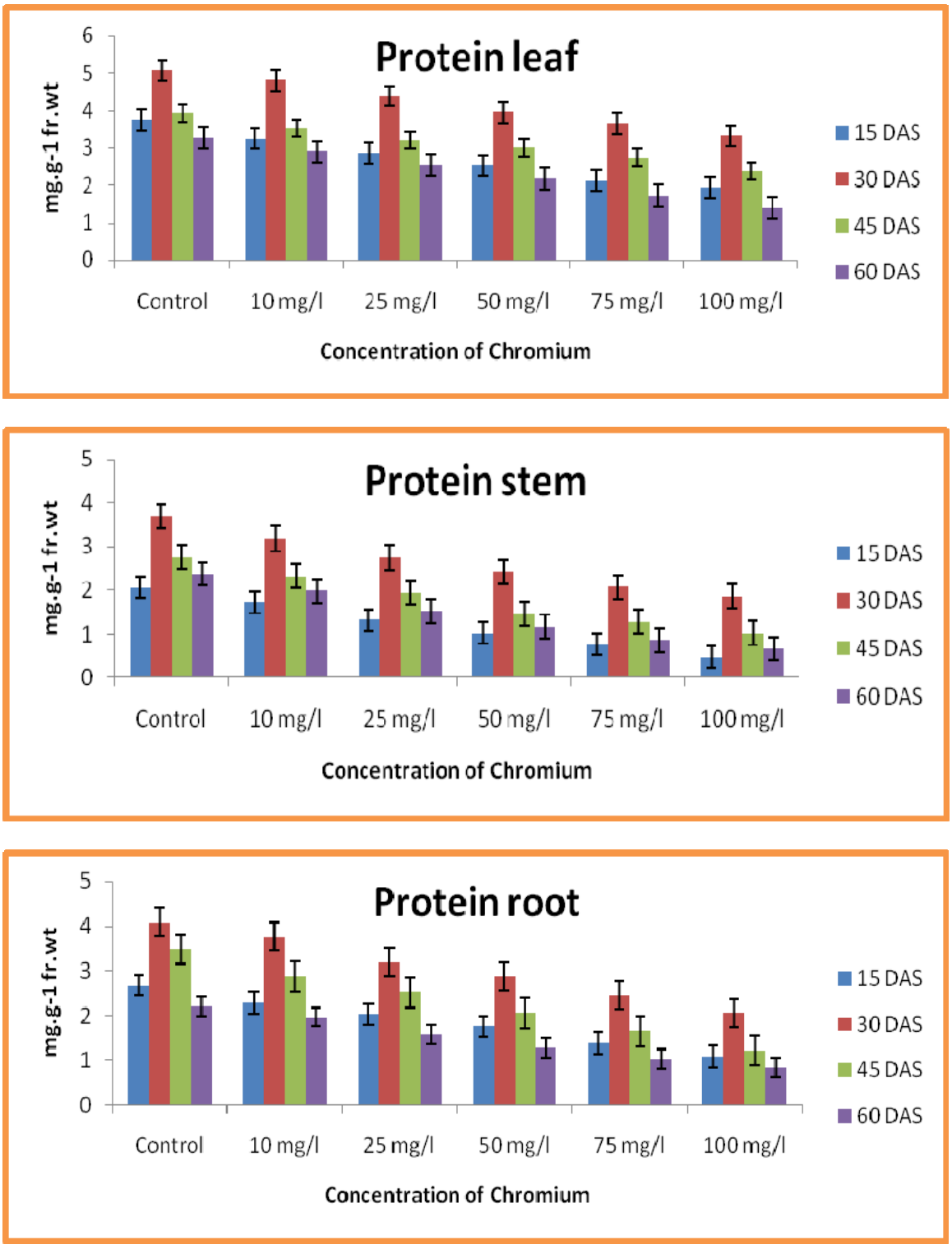
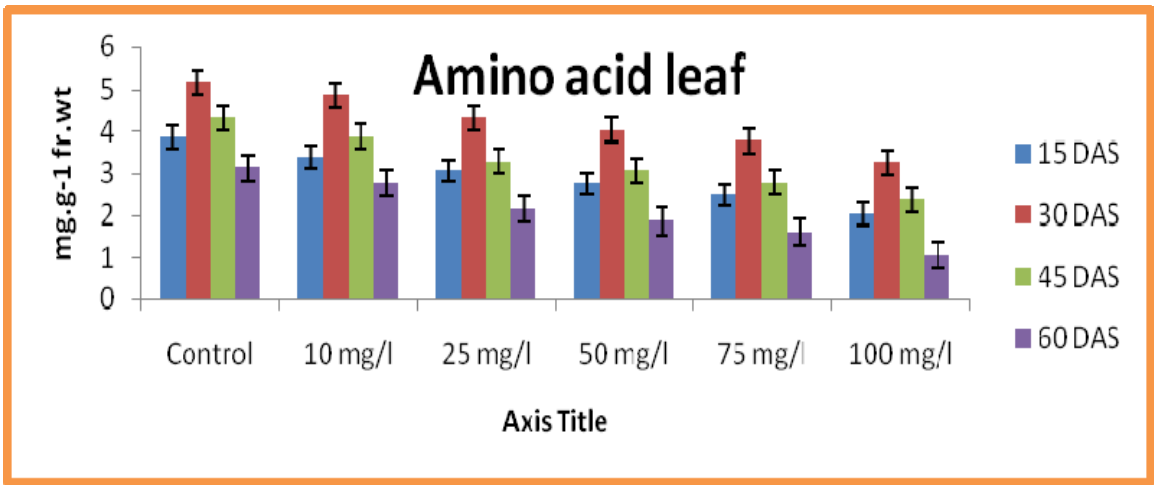
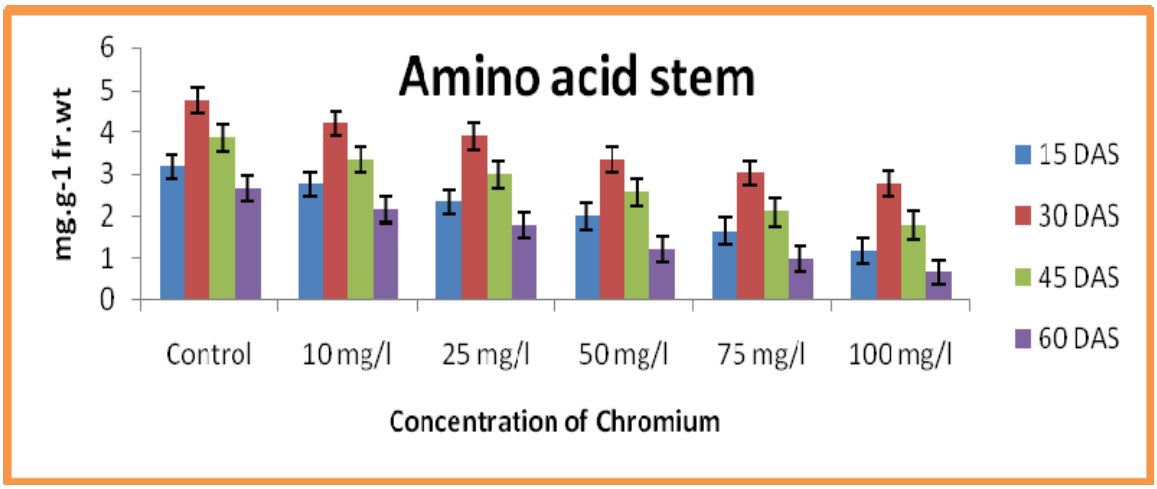


Figure 1. Effect of different concentrations of chromium on protein content of *Gomphrena globosa* L. seedlings





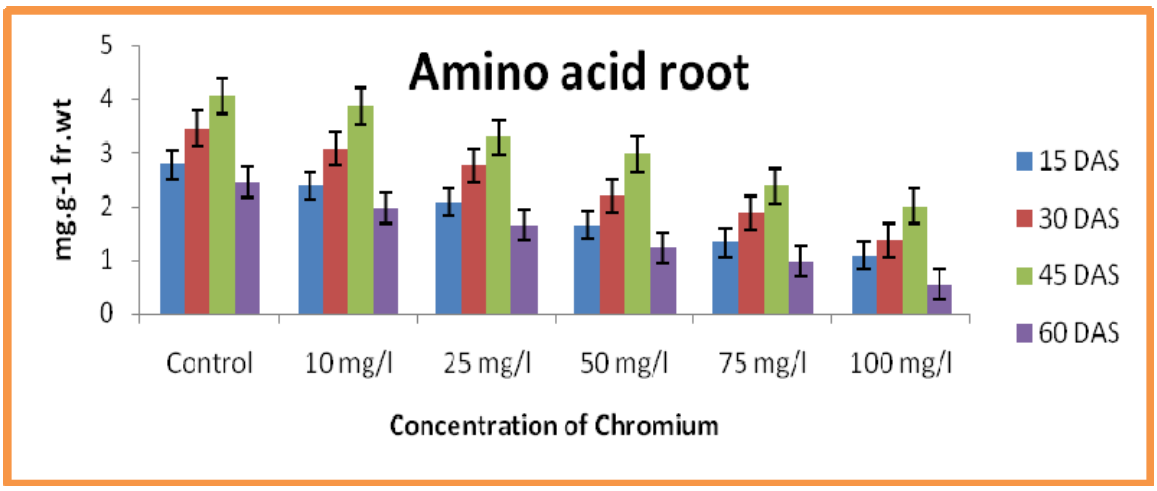


Figure 2. Effect of different concentrations of chromium on amino acid content of *Gomphrena globosa* L. seedlings

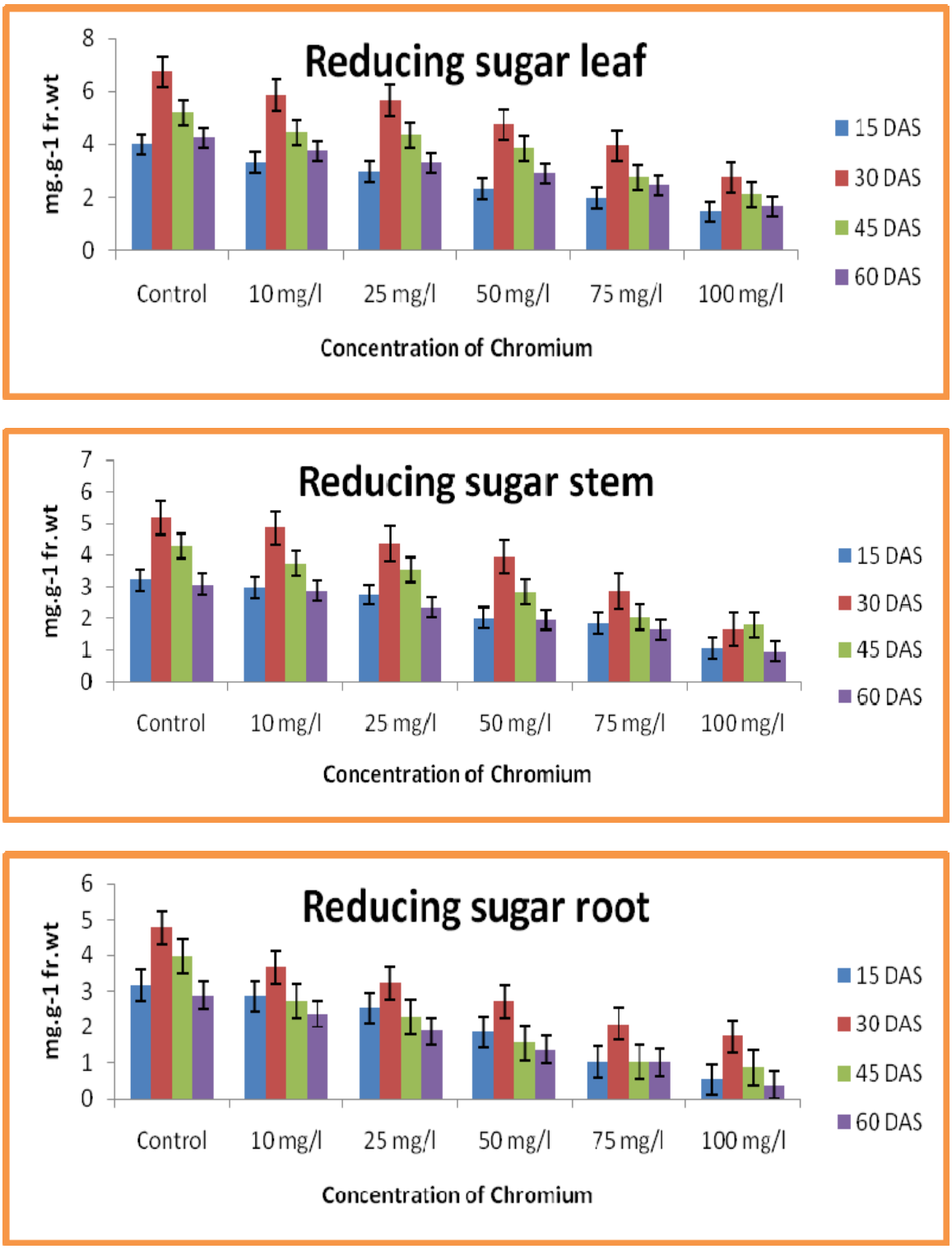


Figure 3. Effect of different concentrations of chromium on protein content of *Gomphrena globosa* L. seedlings

root (1.92, 3.09, 2.37 and 1.28 mg g^{-1} fr. wt) were recorded in control plants at 15, 30, 45 and 60 DAS respectively. The lowest non-reducing sugar content of leaf (0.86, 1.94, 1.55 and 0.26 mg g^{-1} fr.wt), stem (0.62, 1.54, 1.02 and 0.19 mg g^{-1} fr.wt) and root (0.54, 1.22, 0.94 and 0.1 mg g^{-1} fr.wt) were recorded in 100 mg/l concentration of chromium treated plants at 15, 30, 45 and 60 DAS respectively. Additionally, the chromium and cadmium treated *Sesbania sesban* biochemical like reducing, non-reducing sugars, total sugar and starch contents were decreased in higher concentration when compared to control (Bakiyaraj *et al*., 2014). The same results were observed by following authors. Similarly, total sugars, starch, protein and were also decreased with the increasing the mercury concentrations. Studies have proved that amino acids can directly or indirectly influence the physiological activities of the plant (Sharma, 1985; Shafiq and Iqbal, 2005; Street *et al*., 2007). Moreover, soluble sugar, an important constituent is manufactured during photosynthesis and broken down during respiration by plants. All metals have decreased the content with increasing concentration as reported in agricultural crops (Hemalatha *et al*.,1997). Such inhibition of photosynthesis in higher plants by heavy metals have been reported (Becher and Hofner, 1994). The low sugar levels may be due to lowered synthesis or diversion of the metabolites to other synthetic processes.

Amino acids

The influence of various concentrations of chromium on amino acid contents in leaf, stem and root of globe amaranth at 15, 30, 45 and 60 DAS is given Fig. 4. The highest amino acids content of leaf (3.86, 5.18, 4.32 and 3.15 mg g^{-1} fr.wt), stem (3.19, 4.76, 3.86 and 2.65 mg g^{-1} fr.wt) and root (2.77, 3.45, 4.06 and 2.44 mg g^{-1} fr.wt) were recorded in control plants at 15, 30, 45 and 60 DAS respectively. The lowest amino acids content of leaf (2.04, 3.26, 2.36 and 1.05 mg g^{-1} fr. wt), stem (1.18, 2.77, 1.78 and 0.65 mg g^{-1} fr.wt) and root (1.08, 2.0, 1.36 and 0.54 mg g^{-1} fr.wt) were recorded in 100 mg/l concentration of chromium treated plants at 15, 30, 45 and 60 DAS respectively. Furthermore, an increase in amino acid content in maize plants was observed in response to cadmium (Narwal and Singh, 1993) in *Cajanus cajan* (pigeon pea) (Patnaik and Mohanty, 2014) to cadmium and mercury it may be due to excess cellular concentration of cadmium either inhibits the utilization of amino acid or promotes protein hydrolysis, affecting the normal balance of cellular proteins (Tendon and Srivastava, 2004). The increased amino acid content with chromium and cadmium treatment may be due to the increased proteolysis of the cellular proteins or de novo synthesis of amino acid. Accumulation of amino acid might be due to several possibilities: it might be the result of malfunctioning of respiratory activity due to membrane damage (Vazquez *et al*., 1987), resulting in the accumulation of several TCA cycle compounds, such as 2-oxyglutrate that promote the synthesis of specific amino acids; increase in the levels of sulfur amino acids, activity of the enzymes involved in their synthesis (Rauser, 1999); a reduced protein synthesis that contribute to the accumulation of amino acid, particularly at high concentration (Costa *et al*., 1997).

Proline

The influence of various concentrations of chromium on proline contents in leaf, stem and root of globe amaranth at 15, 30, 45 and 60 DAS is given Fig. 5. The highest proline content of leaf (3.74, 4.19, 5.08 and 5.43 mg g^{-1} fr. wt), stem (3.21, 3.82, 4.42 and 4.67 mg g^{-1} fr. wt) and root (2.34, 3.19, 3.52 and 4.17 mg g^{-1} fr. wt) were recorded in 100 mg/l concentration of chromium treated plants at 15, 30, 45 and 60 DAS respectively. The lowest proline content of leaf (1.35, 2.09, 2.57 and 3.27 mg g^{-1} fr. wt), stem (0.94, 1.42, 1.97 and 2.41 mg g^{-1} fr. wt) and root (0.54, 1.17, 1.58 and 1.92 mg g^{-1} fr. wt) were recorded in control plants at 15, 30, 45 and 60 DAS respectively. Notably, Vijayarengan (2012b) stated that marked increase in proline content was observed in rice plant treated with cadmium (10-50 mg kg^{-1}), due to different stress, particularly drought and salinity, as common observation. But it is the fact that metal stress also increased the proline content, which has been meagerly reported in the literature. In addition to that, Proline, an amino acid is well known to get accumulated in wide variety of organisms ranging from bacteria to higher plants on exposure to abiotic stress (Saradhi and Alia Vani 1993). Proline accumulation may contribute to osmotic adjustment at the cellular level and enzyme protection stabilizing the structure of macromolecules and organelles. Increase in proline content may be either due to de novo synthesis or decreased

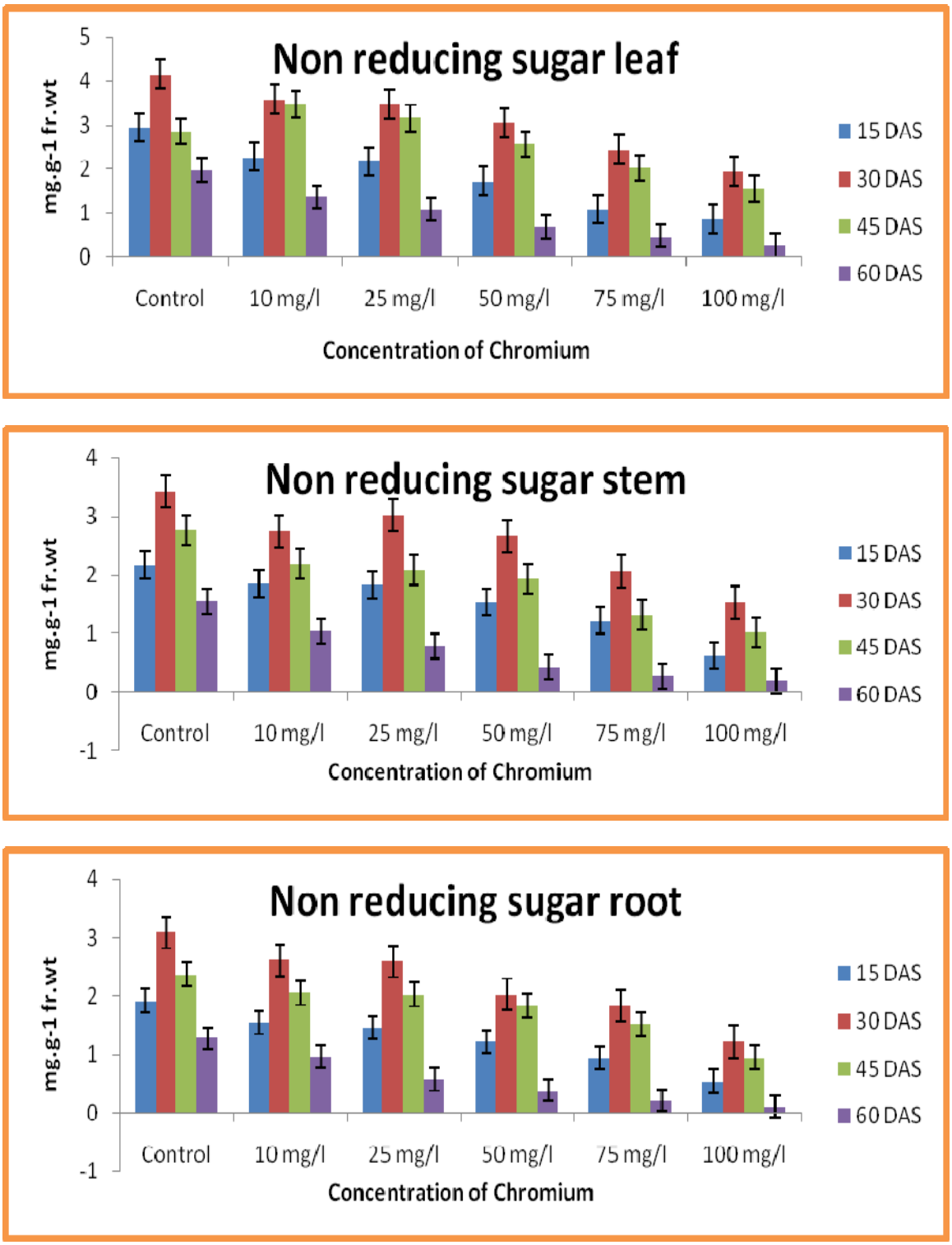
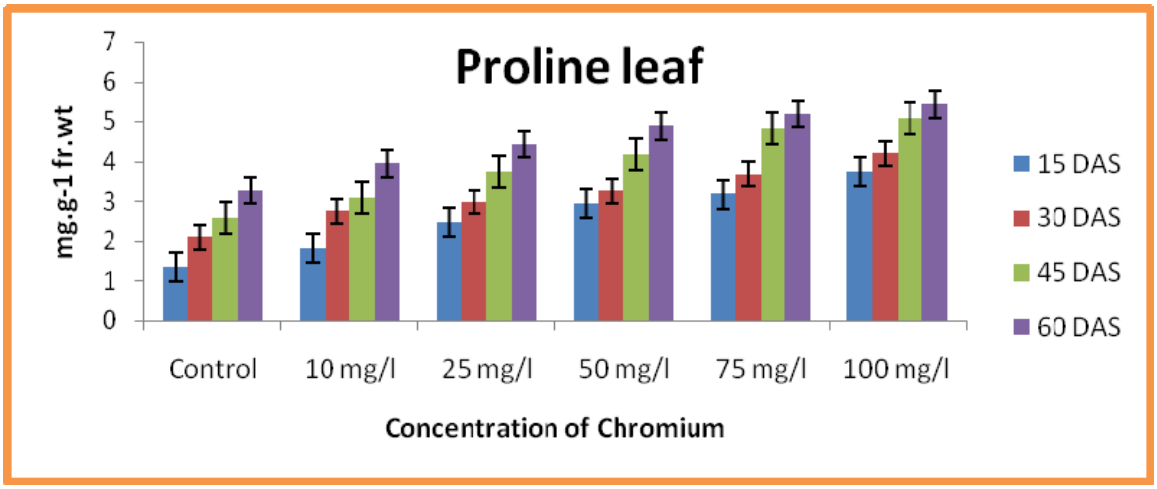
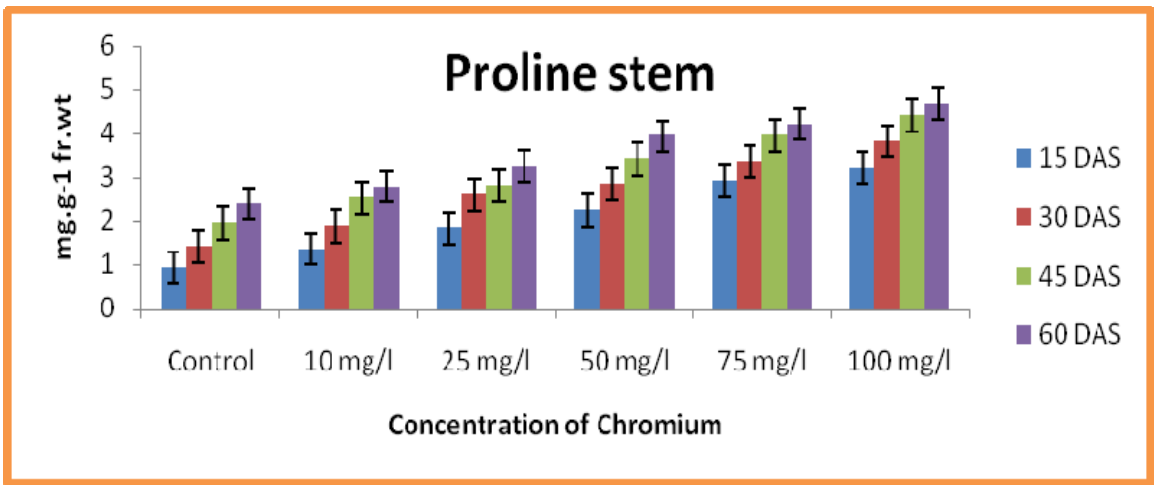


Figure 4. Effect of different concentrations of chromium on non-reducing sugar content of *Gomphrena globosa* L. seedlings





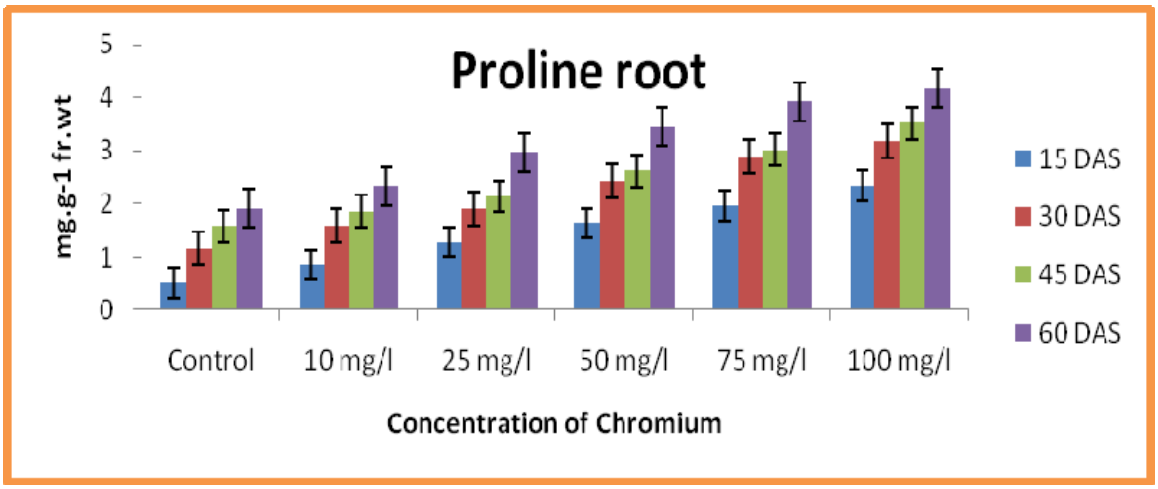


Figure 5. Effect of different concentrations of chromium on protein content of *Gomphrena globosa* L. seedling

degradation (Kasai *et al*., 1998). Also, Rastgoo and Alemzadeh (2011) observed in *Aeluropus littoralis*, increase in proline level as a proteinogenic amino acid, in all treatments reinforces the hypothesis that this amino acid by detoxification reactive oxygen species causes tolerance to heavy metals. Therefore, the accumulation of proline can be considered as an indicator of tolerance to heavy metal stress.

Conclusion

From this experiment, it can be concluded that the heavy metal contaminated soil is toxic to crop. The increasing chromium concentrations (10, 25, 50, 75 and 100 mg/l) with the biochemical parameter such as, protein, sugars and amino acid contents were also significantly decreased in all different concentration of Cr treated as compared to control. The proline content was increasing with increasing concentration of Cr treated plants as compared to control at 15, 30, 45 and 60 DAS in chromium polluted soil. The phytoremediation technology can be applied to treat the contaminated water and soil. The government organization, public and private sectors should join their hand to protect our environment for the better living of our future generation.

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