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Evaluation of Chromium Toxicity on Growth, Physiological Activity and Yield Potential of Sunflower (*Helianthus annuus* L.) Plants; An Emphasis on Phytoremediation

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Abstract

Metal pollution is a key environmental issue across the globe. Our environment is seriously under the threat of different heavy metals including As, Cr, Cd, Cu, Hg, Ni, Pb, and Zn. Heavy metals upon exceeding safe limits become part of food chain and affect growing plants and consumers; and ultimately affect consumer's health at large. Amongst, since long chromium is widely used metal in different industries like tannery, textile, fertilizers, electroplating, steel etc. and has become a serious environmental and agricultural pollutant. Present investigation has been set to evaluate chromium toxicity in sunflower test varieties along with phytoremediation potential of these test varieties. Chromium application in the soil (50-500 mg/kg) found to be toxic for germination time, plant growth and biomass. Different photosynthetic parameters were negatively affected under Cr stress. Likewise, the content of chlorophyll and protein were also decreased significantly following decrease in plant minerals (NPK and Mg). However, sunflower varieties were able to accumulate proline content showing their osmolyte accumulation strategy against Cr metal. Different yield parameters were assessed under Cr treatment. A declining trend in seed yield and harvest index was observed. Cr effect was increasing with its increased level in the soil medium. Both varieties revealed the toxic effect of Cr metal, however, both showed a higher Cr uptake potential from the contaminated soil. This shows sunflower varieties to be a good phytoremediation tool.

Keywords: Global Issue; Metal Toxiciy; Plant Growth; Mineral Status; Yield; Phytoremediation

1. Introduction:

Global environmental pollution is the most emerging issue of present modern era. Our ecology is seriously threatened by the toxicity of heavy metals and their compounds. These toxic heavy metals put negative impacts on flora and fauna species (Asif *et al.*, 2020). Heavy metal pollutants are naturally occurring

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substances that, when exposed to the intact environment have hazardous consequences and interfere with normal activity of life. Industrial growth combined with rising community demands have resulted in accumulation of such harmful metal elements into the environment (Hill, 2020). The presence of heavy metal in soil undermines the entire ecosystem by contaminating the food chain (Karim *et al.,* 2015; Musilova *et al.,* 2016). Economy of Pakistan (75%) depends on agricultural soil. In Pakistan, maximum permissible quantities of hazardous heavy metals recommended by WHO in both ground and surface water are frequently exceeded (Asif *et al.,* 2020). Our agricultural soil is contaminated by excessive use of fertilizers, pesticides, and wastewater effluents. Additionally, it may endanger the safety of food, feed and health of consumers as well (Cachada *et al.,* 2018; Chabbi *et al.,* 2021).

The environment is being upset by the uncontrolled release of industrial effluents containing hazardous heavy metals including chromium (Cr) abundantly. Chromium frequently exists in dichromate and hexa-chromate ions, is extremely hazardous (DesMarias and Costa, 2019). It is the most commonly used heavy metal with numerous industrial applications like electroplating, cement, textile, wood preservatives, paints and pigments, and in the dyeing, leather-tanning, canning and steel fabrication industries (Ishchenko, 2018; Vaiopoulou & Gikas, 2020). Cr enriched industrial discharge into freshwater bodies and sewage wastewater may result in accumulation of Cr in agro lands upon irrigation (Drangert *et al.,* 2018; Singh *et al.,* 2021). Cr^{6+} has a density 7.19 g/cm^3 and is placed in group-A by the Environmental Protection Agency (EPA) and is ranked as a serious environmental and carcinogenic pollutant. Both Cr^{6+} and Cr^{3+} forms are causes of allergic contact dermatitis, skin allergy, increasing risk of lung cancer irritants, respiratory, reproduction, digestive and excretory system (Tahir *et al.,* 2010; Alvarez *et al.,* 2021).

Chromium upon its transfer to growing plants becomes accumulated in plant tissues, depressingly affects plant metabolism and stunts crop growth and yield (Shankar *et al.,* 2009; Pardhan, 2017; Ugwu, 2020). Chromium imposes inhibitory effects on seed germination, seedling growth, and plant length (Hafiz & Ma, 2021; Kyari *et al.,* 2022). Higher levels of Cr^{6+} results in chlorosis, chlorophyll degradation, photosynthetic mutilation, decreased plant biomass production, affected protein synthesis, and ultimately causes plant death (Borna, 2016; Shahid, 2017; Farid, 2018) through generation of free radicals / ROS (Ertani *et al.,* 2017; Ranieri, 2020). Moreover, this metal is responsible to induce hormonal imbalance, cytotoxicity, and genotoxicity and altered signal transduction in the intact plants and inhibits plant growth and development (O'Brien & Benkova, 2013; Chebeir, 2016; Jobby, 2018). Studies have also reveled that Cr affected plant growth is due to alteration in the ultra-structure of the chloroplast and cell membrane, abnormal cell division and cell cycle, affected root anatomy, imbalanced water and minerals accumulation, abnormal enzyme activity and nitrogen assimilation, production of free oxygen radicals (ROS) and malfunctioning of antioxidant defense system (Ali, 2015; Farooq *et al.,* 2016; Reale *et al.,* 2016; Anjum *et al.,* 2017; Masciarelli, 2017; Ugwu, 2020).

In present modern era, different modern techniques are suggested in using some physical, chemical, and biological approaches to clean agro soils from heavy metals (Ashraf *et al.,* 2017). However, phytoremediation is the most efficient, eco-friendly, and cheapest that involves only the use of certain plants to extract and reduce the heavy metals from the intact environment. But, on the other side, phytoremediation potential quite depends on the plant's inbuilt capacity of metal extraction (Jacob *et al.,* 2018; Yan *et al.,* 2020; Gavrilescu, 2022). Plant species that have the potential of accumulating a major portion of metals from the soil are ranked as hyper-accumulators. According to earlier documentation (Clemens, 2006; Ozay & Mammadov, 2013; da Conceicao *et al.,* 2016), hyper-accumulator plant species are the most efficient in the removal of metals. Therefore, in present investigation sunflower plants from two different varieties have been used to assess their phytoremediation potential. Heavy metal pollutants are naturally occurring substances that, when exposed to the intact environment have hazardous consequences and interfere with normal activity of life. Industrial growth combined with rising community demands have resulted in accumulation of such harmful metal elements into the environment (Hill, 2020). The presence of heavy metal in soil undermines the entire ecosystem by contaminating the food chain (Karim *et al.,* 2015; Musilova *et al.,* 2016). Economy of Pakistan (75%) depends on agricultural soil. In Pakistan, maximum permissible quantities of hazardous heavy metals recommended by WHO in both ground and surface water are frequently exceeded (Asif *et al.,* 2020). Our agricultural soil is contaminated by excessive use of fertilizers, pesticides, and wastewater effluents. Additionally, it may endanger the safety of food, feed and health of consumers as well (Cachada *et al.,* 2018; Chabbi *et al.,* 2021).

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1.1. Objectives:

- To evaluate Cr toxicity on growth, physiology and yield of sunflower plants.
- To evaluate metal accumulation rate in sunflower plants.
- To evaluate phytoremediation potential of sunflower plants.

2. Materials and Methods:

2.1. Seed Germination, Germination Time and Radicle Length:

Seed germination test was employed under laboratory conditions. Sunflower seeds were placed in 12 cm petri-plates and were arrayed viz. Cr concentrations 50, 100, 150, 250, 350, and 500 ppm. For sufficient absorption of moisture content, each petri-plate was fitted with two filter papers (Whatman # 42) and 15 ml of respective treatment. The metal free control treatment was initiated with distilled water whereas; the subsequent metal treatment was executed according to treatment plan (50-500 ppm) from stock-solution prepared with distilled water. Seeds with 2 mm emergence of radicle were recorded to be germinated and observation for percent germination continued till final 10th day of germination. Length of radicle was measured with a scale alignment (Akinci & Akinci, 2010).

2.2. Growth and Biomass Attributes:

2.2.1. Pot-Experimental Trail:

Different plant growth attributes were evaluated under Cr treatment by conducting a pot-experimental trial. Sunflower hybrids {Hysun-33 (SF1) and SF-5009 (SF2)} were potted in Bio-Park (Bahauddin Zakariya University, Multan-Pakistan), and final data was observed at yielding stage. Different Cr concentrations (50, 100, 150, 250, 350, 400 & 500 mg kg-1 of pot soil) were weighed and mixed with the pot soil. Each treatment (including control) comprised of seven replicates for 112 earthen pots of 16-inch diameter were arranged in a completely randomized block design (CRBD). Pots were filled with sandy loam and humified soil characterizing with EC 1.73 ds/cm, pH 7.21, and O.M 4.12 percent. Six seeds of either variety were sown one inch deep in the soil. Sunflower plants were thinned after attaining six inches in size, while two healthy seedlings per pot were left as harvest. At 90 DAS (days after sowing), the following growth parameters were determined.

- Total plant height (cm)
- Leaf area (cm^2) was determined by method of De Swart *et al.,* (2004) using following formula:
  Leaf area (LA) = Length × Width × 0.68 (calculated factor)
- Leaf count per plant per treatment
- Plant fresh and dry weight (g) was estimated by method of Ahmad & Wahid, (2011).

2.3. Photosynthetic Parameters of Plants:

Post flowering (90 DAS) physiological data regarding different photosynthesis parameters was conceded by a infrared gas analyzer (IRGA, ADC-LCA4/ Analytical Development Company, England) following Vernay *et al.,* (2008) and Diwan *et al.,* (2012). IRGA readings were carried out during full sunny mornings for three young similar aged leaves. Following different parameters were selected for physiological evaluation of sunflower plants:

- Internal carbon use or assimilation (Ci = Vpm)
- Rate of photosynthesis ($A = \mu M\ m^{-2}\ s^{-1}$)
- Transpiration rate ($E = m.\ mol\ m^{-2}\ s^{-1}$)
- Stomatal conductance ($Gs = mol\ m^{-2}\ s^{-1}$)
- Water utilizing efficiency of plants (WUE) as described by (Ahmad *et al.,* 2008).

2.4. Post Flowering Biochemical Analysis of Plants:

2.4.1. Chlorophyll Value:

Total chlorophyll content for selected leaves was estimated by using a digital chlorophyll meter SPAD - 502 (Konica-Japan) having SPAD-unit accuracy ± 1.

2.4.2. Determination of Protein and Proline Content:

Leaf protein content was estimated by Bradford (1976) at 595 nm whereas; proline content was estimated by the method of Bates *et al.* (1973) as described by at 520 nm absorbance using spectrophotometer.

2.4.3. Estimation NPK and Mg^{2++} Content of Sunflower Leaves:

Nitrogen content of plant material was estimated by Kjeldahl method as described by Ahmad *et al.,* (2011) with following formula:

$$N\,(\%) = \frac{\text{Acid used for sample} - \text{Acid used for blank} \times \text{Acid normality} \times 14.01 \times 10 \times 100}{\text{Volume of Sample}}$$

Likewise, percent leaf content of K^+ and Mg^{++} were determined as oxides by using XRF (PW 1660-Philips) following Aftab and Ali *et al.,* (2011). The leaves were converted into ash in an electric furnace (Veckstar-Germany) at 450 ºC for 2 hours, then cooled, powdered and pelletized at a by Herzog press machine. The pellet was then used for elemental analysis through XRF. Leaf phosphorous (mg) was determined by method described by Ryan *et al.,* (2001).

2.5. Metal Analysis of Plants:

Cr heavy metal in sunflower tested plants was estimated by combustion & wet digestion method of Panichev *et al.,* (2005) using acid mixture of dilute HNO_3 and HCl. After complete digestion, the volume of solution was raised up to 25ml using distilled water. Chromium metal was estimated at 357.9 nm using a spectrometer.

2.5.1. Metal Accumulation Factor:

Transfer of metal content from soil to the growing plants was estimated by the method of Raskin *et al.,* (1994) as described by Atta *et al.,* (2023) using the following formula:

ACF = PU=MT

Where, PU = metal content in whole plant ($mg.\ kg^{-1}$), MT = total metal content in soil ($mg.\ kg^{-1}$).

2.6. Determination of Yield and Yield Components:

Yield and its components were determined for treated plants. Head diameter (cm), kernel count per plant, kernel weight per plant, and weight of 100 random seeds per plant were determined following Andaleeb *et al.,* (2008). Harvest index (H.I %) was calculated by the method of Nanja *et al.,* (2003) by taking ratio of seed count with total plant dry weight.

2.7. Statistical Analysis:

Data was analyzed with ANOVA ($p < 0.05$) and least significant difference (LSD 5%) using

SPSS V. 20. Error graphs were prepared in MS-Excel.

3. Results:

3.1. Seed Germination, Growth and Biomass Attributes:

In present study, petri-plate experiment was conducted to test the rate of seed germination against various concentrations of hexavalent chromium. Germination in sunflower seeds was not affected and a booming germination was observed in all plates. However, a delay in germination time was observed along the increasing Cr concentration i.e. 50-500 mg /L. Metal free seeds were completely germinated in three days while germination extended to be delayed along the increased Cr doses (Table 1). Chromium treatment also affected the length of radicle (RL) significantly. It declined 2-41% in sunflower variety-SF1 at 50-150 mg/L Cr and 48 -72% at elevated Cr level of 250-500 mg/L. Likewise, RL decreased in variety- SF2 for 3-19.5% and 33.9-71%, respectively (Table 1).

Both sunflower varieties have showed negative impact of Cr on plant height (root and shoot length) characteristic that decreased significantly from 148.5 cm to 130 cm in SF1, and 149 cm to 134.1 cm. Comparative to control and lower Cr doses, TPH found to be rapidly decreased at 250-500 mg.kg dose by 8-13% and 5-10% in SF2. Root length was most probably affected by Cr application than stem length. Both varieties showed no effect of Cr on TPH at 50 mg/kg Cr dose. Likewise, number of leaves plant-1 and leaf area plant-1 (leaf size) were also decreased in response to Cr application contrasting the control treatment. At lower Cr doses (100-150 mg/kg) outcomes were consistent i.e. 1.4 - 8.1% and 0.5 - 1.1% than at higher doses. Both varieties presented a significant decrease in leaf count and leaf size by 15 - 34% and 3.1 - 5.2%, respectively at 250-500mg/kg of Cr.

Sunflower plants grown in control treatment were quite vigorous. A rapid decline in plant fresh and dry weight biomass was observed at higher Cr doses than lower doses. Plant fresh weight dropped from 173.3g to 150g, and dry weight reduced 65.1g to 54.8g (Table 1). Both varieties were much affected at elevated Cr doses (250-500 mg/kg) than at lower doses (50-150 mg/kg). Cr application has negatively affected the plant growth and morphological characteristics, but responses of two varieties at 50 mg/kg of Cr dose was adaptive like control treatment.

Table 1: Assessment of growth attributes in sunflower plants under Cr treatment

SOV	variety	T0	T1	T2	T3	T4	T5	T6	T7	*P*-value
Germination time (days):										
	SF1	3	4	6	7	7	7	8	8	10.18***
	SF2	3	3	5	6	7	7	7	8	12.26***
Radical length (mm):										
	SF1	20.3	20	16.6	12	10	7	6.4	6	170***
	SF2	21	20	18.4	17	13	10	8.5	6	248***
Plant height (cm):										
	SF1	148.5	150	148.1	147	140	137.1	136	132	387.8***
	SF2	149	149	146.4	144	140	137	134	132.4	27.8**
Leaf area (cm^2):										
	SF1	62	62	61.2	61	59.3	59	59	58.4	33.51***
	SF2	62	61.9	61.5	61	60	59.2	59.8	59	133.1***
Leaf count per plant:										
	SF1	20	21	20	19	16	17	15.5	13	55.54***
	SF2	21.2	21.5	20.6	19.4	17.5	17.2	16	14.5	18.93***
Plant fresh weight (g. plant^{-1}):										
	SF1	169.3	169.4	168	166.5	161.5	160.6	157	150.5	331.8***
	SF2	174	174	173	169.4	165.4	163	161	155.3	41.5***
Plant dry weight (g. plant^{-1}):										
	SF1	65.2	65	62	62	59.1	58.3	57	54.2	215.8***
	SF2	67	67	66.4	64	62.5	61	59.3	56.9	62.3***

3.2. Photosynthetic Parameters of Plants:

Figure 1 (A-C) shows the significant effect of Cr on sunflower plants that decreased the rate of photosynthesis (*A*) by reducing internal carbon assimilation *(Ci)*, stomatal conductance (Gs) and rate of transpiration (*E*), as compared to control treatment. The maximum decrease in these physiological attributes for both sunflower varieties was at 500 mg/kg of Cr dose, while toxicity increased from lower to elevated Cr doses (50-500 mg/kg of Cr). In SF1, these physiological attributes were more aggressively affected at 250-500 mg/kg (12.1 - 25%, 25

- 37%, 29 - 43.1% and 32 - 57%) as compared to 50 - 150 mg/kg of Cr application and control treatment as well. A similar decline pattern was observed in SF2 i.e., 13.3 - 23.2%, 22 - 37%, 28 - 43% and 25- 65.2%, respectively. Sunflower plants showed a struggling behavior for water use efficiency (WUE) that increased along the metal concentrations.

3.3. Biochemical Analysis of Plants:

Figure 2 (D, E) shows a significant ($P \leq 0.05$) decrease in foliar chlorophyll and protein contents with an increased Cr application. For sunflower test varieties SF1 & SF2, rate of chlorophyll reduction was 7-11.4% and 19-31.01% at lower to higher Cr application in the soil (100 - 150 mg/kg & 250 - 500 mg/kg), respectively. Likewise, leaf soluble protein content reduced at elevated Cr doses (up to 20-41.1% at 250-500 mg/kg of Cr) than at lower Cr application (up to 6-12% at 100-150 mg/kg). Besides the decrease in foliar protein under Cr stress, leaf proline content was significantly increased up to 46.2 percent. SF2 was more tolerant for biochemical traits as compared to SF1 (Fig. 2-F). However, no alteration was observed in these biochemical traits at 50 mg/kg of Cr dose.

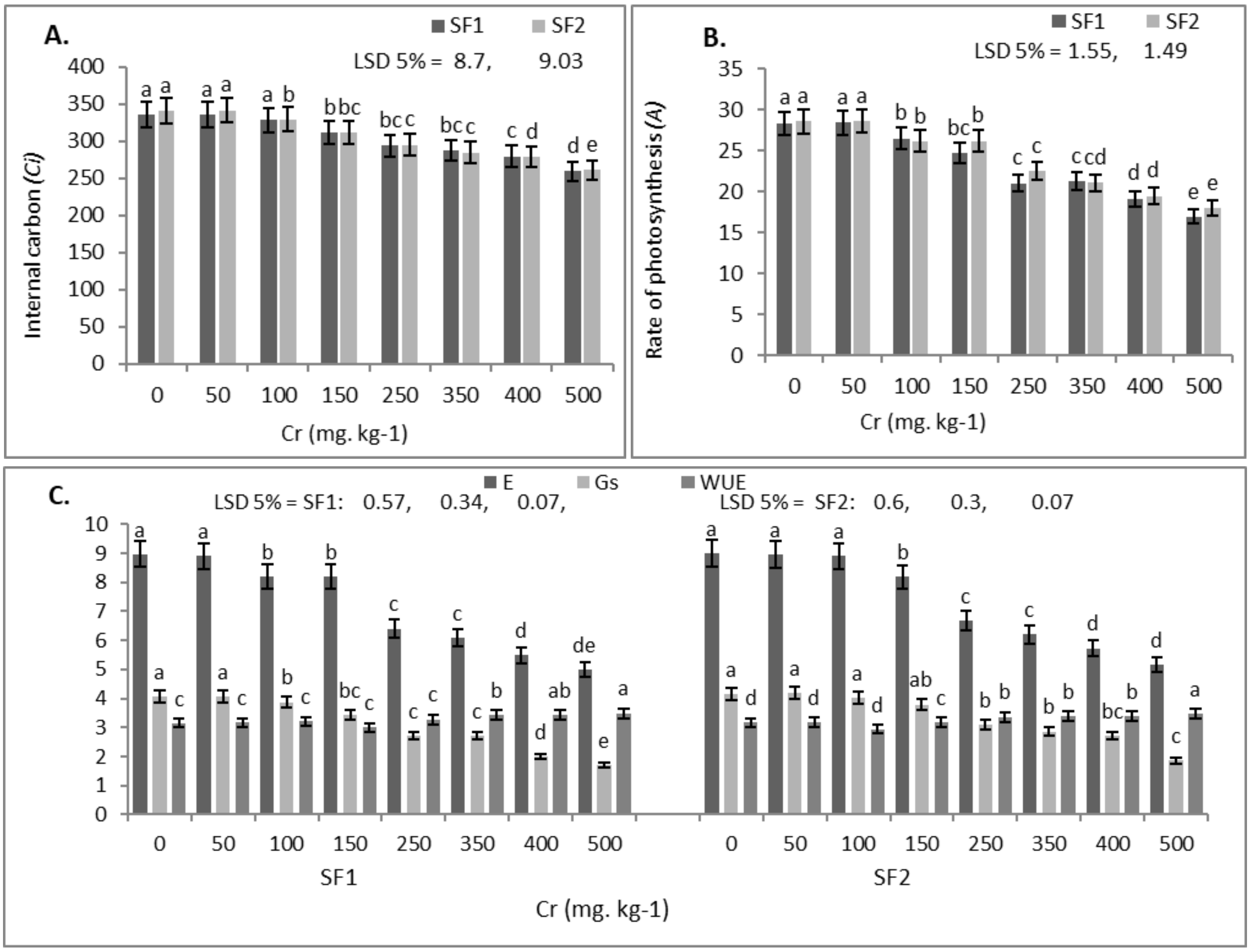

Figure 1: Evaluation of Cr effect on photosynthetic parameters in sunflower varieties

3.4. Effect on Leaf Mineral Content:

3.4.1. NPK and Mg:

Figure 2 (G, H) has shown significant effect of Cr toxicity on foliar mineral composition in the subsequent sunflower varieties. Leaf content of nitrogen, phosphorus, potassium and magnesium were decreased with the increased dose of chromium in the soil, as compared to control treatment. In brief, by comparing control and lower metal treatment (50-150 mg/kg), the declining trend in NPK content was observed maximum at 250-500 mg/kg of Cr dose i.e., 13 - 35%, 11.2 - 28% and 3.4 - 5.3%, respectively.

Likewise, maximum decrease in Mg2++ ions was up to 26.3 percent at 500 mg/kg of Cr dose. Sunflower variety SF2 showed less decline rate for foliar mineral content and was more tolerant for this trait than SF1.

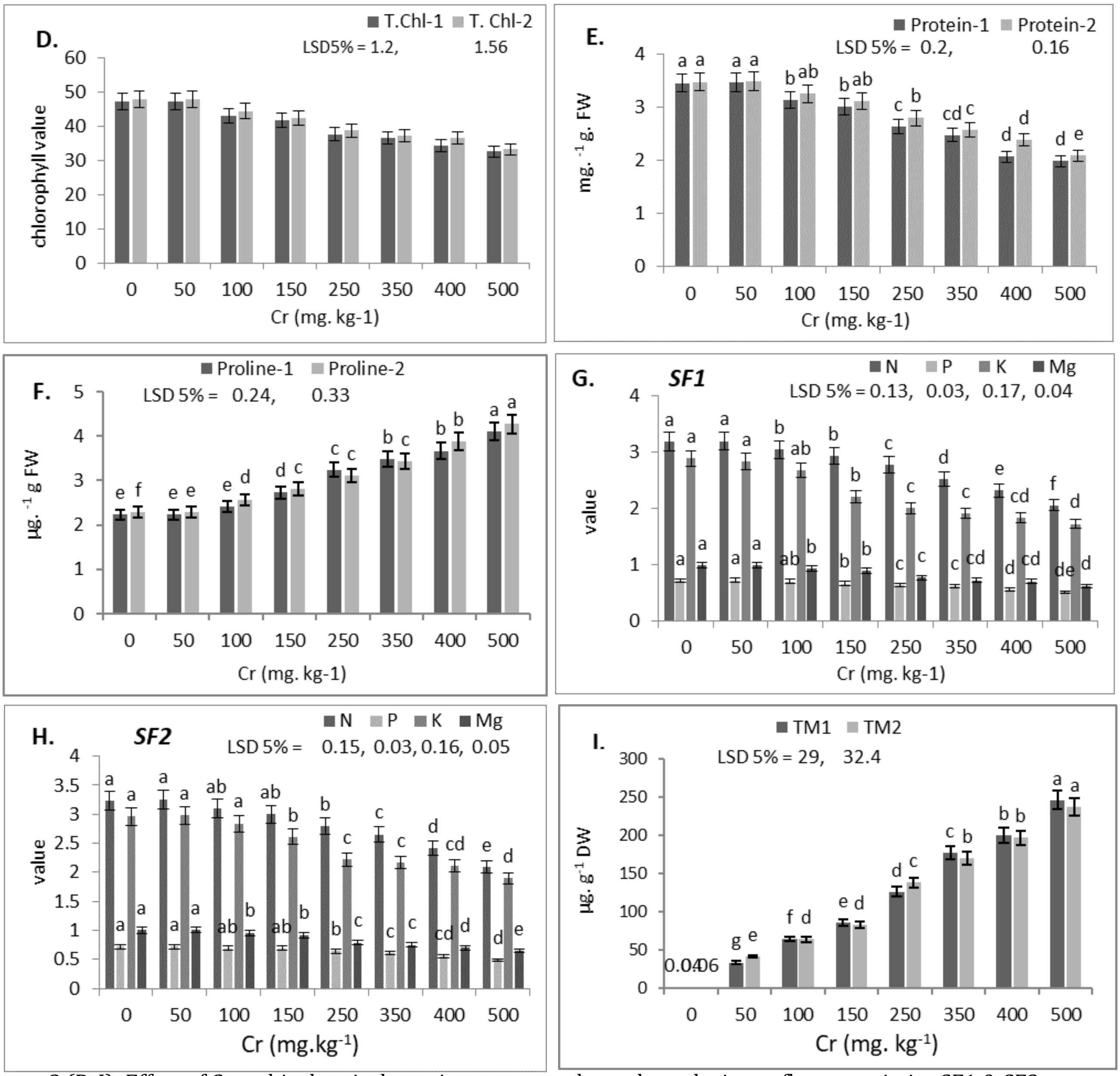

Figure 2 (D-I): Effect of Cr on biochemical, nutrient status and metal uptake in sunflower varieties SF1 & SF2

3.5. Effect of Cr on Yield and Yield Components:

Different yield attributes were assessed under Cr metal stress for two sunflower varieties. Head (capitulum) diameter, seed count per plant, seed weight and harvest index (H.I %) were decreased due to Cr toxicity significantly ($P \leq 0.05$). In SF1 & SF2, decreasing trend for yield components was up to 29.5 - 31.7%, 34 - 37.3%, 27.5 - 29.4% and 11.4-16%, respectively (Table 2). Data revealed SF2 to be more tolerant with a low reduction rate in yield parameters as compared to SF1.

Table 2: Effect of Cr on some yield components and harvest index (H.I) in sunflower plants

SOV	variety	T0	T1	T2	T3	T4	T5	T6	T7	*p*-value
Capitulum / head diameter (cm):										
	SF1	8.5	8.5	8.2	7.8	6.8	6.5	6.3	5.8	141***
	SF2	8.8	8.8	8.5	8.1	7.3	7.1	6.58	6.2	78.4***
Number of seeds per plant:										
	SF1	329.8	334	318.8	305	264	250	215	206	417.9***
	SF2	345.3	345.8	331.4	316	284	269.3	236.7	228.3	24.5***
Seed weight per plant (g. plant 1):										
	SF1	17.7	17.7	17	16	15.8	15.2	14.4	12.6	136.5***
	SF2	18.1	18.2	18	17.1	16.1	15.4	14.9	13.4	163.5***
100 seeds weight (g):										
	SF1	6.74	6.76	6.2	6.1	5.8	5.6	5.3	5.1	188***
	SF2	6.9	6.89	6.51	6.3	5.9	5.6	5.5	5.3	117.6***
H.I (%)										
	SF1	27.2	27	27	26.7	26	25.9	25.1	22	31.8***
	SF2	27	27.2	27	26.4	25.5	25.3	25.2	23.8	20.3***

3.6. Chromium Metal Uptake and Metal Accumulation Factor:

Cr heavy metal uptake from soil to plant was estimated at yielding stage of sunflower varieties and was more 49.3% in SF1 than 47.5% in SF2 (Fig. 2-I). Partitioning the Cr metal in different plant parts, Cr accumulation was highest in roots than in shoots and leaves. An increasing trend for Cr accumulation in plant organs was due to increased Cr doses i.e., at Cr dose 50-500 mg/kg. Moreover, chromium accumulation factor (ACF) was calculated to be significant up to 250 mg/kg level (0.82 - 0.5) that decreased to 500 mg/kg (0.47) showing the efficacy of sunflower plants for metal uptake from the contaminated pot soil.

4. Discussion:

Heavy metals are the toxic substances that adversely affect growth and development of crop plants (Atta *et al.,* 2023). Seed germination is regarded as a crucial developmental stage that determines establishment of a future healthy crop pertaining to its vigor, growth, and higher yield (Pavlova *et al.,* 2018). Chromium inhibited 49% seed germination in *Hibiscus esculentus* (Amin *et al.,* 2013), and its toxicity bowed extreme along increasing Cr level to suppress seed germination in metal treated sunflower plants (Atta *et al.,* 2014). Plant-hormones play a key role in progressive seed germination i.e., accumulation of GA (gibberellic acid) found affected and quantified to be low during chickpea germination under metal stress (Zn, Pb, and Cd) with elevated level of abscisic acid (ABA) content in contrast to the control treatment (Atici *et al.,* 2005). Metal toxicity in plants caused a setback in the development of embryo (*Accacia*) by reducing radicle size. Moreover, histological data revealed that delay in embryo development occurred due to mitotic inhibition under metal toxicity (Zerkout *et al.,* 2018). Similar investigation by Atta *et al.,* (2014) also exposed the role of chromium and lead metals that suppressed the embryonic growth with arrested mitotic activity in different sunflower varieties. In present study, Cr toxicity has affected delay in germination time without affecting percent seed germination in all treatments. However, length of embryonic axis has shown an inhibitory effect of Cr toxicity. Results of present study regarding percent seed germination are also supported by Akinci & Akinci (2010).

The increasing Cr level in the subsequent growth medium caused adverse decrease in the morpho-physiological characteristics of mungbean plants. Phyto-toxic effects of chromium decreased growth and biomass production in *Vigna radiate* (Rout *et al.,* 1997; Imran & Rehim, 2017), *Brassica oleracea* (Ahmad *et al.,* 2020), *Solanum lycoperscium* (Javed *et al.,* 2021), and *Brassica parachinensis* (Kamran *et al.,* 2021). In present course of investigation, Cr has affected plant height, fresh and dry weight biomass in both the sunflower varieties, and this inhibitory effect increased with the increased Cr doses (Table 1). Studies also revealed Cr induced decrease in root length that could be due to damaged root tip cell due to Cr translocation into intact root cells, whereas the decrease in shoot length may be due to affected cell division and some ultra-structural damage in the intact shoot developing cells (Jasmine *et al.,* 2020). Likewise, Aliu *et al.,* (2013) reported metal (Cd, Hg, Pb) induced reduction in leaf area. Similar results were also reported in *Hordeum vulgare* (Ali *et al.,* 2013), *Oryza sativa* (Ma *et al.,* 2016; Chen *et al.,* 2017), *Brassica juncea* (Handa *et al.,* 2018), *Arabidopsis thaliana* (Wakeel *et al.,* 2019) and *Cicer arietinum* (Singh *et al.,* 2020). Our results have proved that sunflower plants could not withstand Cr stress and the Cr-induced decrease in different growth traits was more in SF1 than in SF2 (Table 1).

Photosynthesis is a crucial phenomenon for plant growth and development. However, plant exposure to metals like chromium and cadmium caused a decrease in the rate of photosynthesis, stomatal conductance, rate of transpiration etc. (Chen *et al.,* 2011) and *Zea mays* (Mohammed *et al.,* 2021). Chromium found to be an interfering trace element with plant biochemistry and photosynthetic machinery (*Zea mays*) that reduced chlorophyll content and adversely affected the photosynthetic activity by inhibiting photosystem I & II (Sytar *et al.,* 2013; Anjum *et al.,* 2016; Gul *et al.,* 2016). Moreover, protein degradation through suppression of proteases under metal toxicity was also reported by Joshi, *et al.,* (2018). However, accumulation of proline as proteinogenic amino acid and being an osmolyte under metal toxicity had showed it an adaptive strategy of plants against metal induced ROS/reactive oxygen species (Liang *et al.,* 2013). Plant minerals status has an important role in the physiological and growth potential of growing plant and is negatively affected by metal toxicity in the growing medium. In rice plants, content of NPK found to be decreased under varying concentration of Cr up to 82%, 37% and 42%, respectively (Ahmad *et al.,* 2011). Decrease in NPK and Mg^{2++} content in *Helianthus annuus* (L.) has been reported by Andaleeb *et al.,* (2008) due to chromium toxicity. Heavy metals (such as Cr, Cd, Pb) inhibited uptake of minerals from soil to plant through transport system and caused deficiency in accumulation of these minerals (del Real *et al.,* 2013; Ali *et al.,* 2014). Results of present investigation are supported by these earlier findings. Both varieties have revealed an affected photosynthetic performance following degradation of protein content. Decreased NPK and Mg content in sunflower leaves also correlate with plant growth. Moreover, affected N and Mg content can also be correlated with decreased chlorophyll content (Fig. 2: D, G, H).

Heavy metals are the substances that react with the active sites of enzymes and affect plant yielding potential (Xiong *et al.,* 2014). Anjum *et al.,* (2016) reported abnormal grain yield and harvest index in maize plants due to different toxic Cr levels. Cr had a strong negative effect on sunflower plants even at 60 mg/kg level and reduced the achene number, head diameter and achene weight as well (Andaleeb *et al.,* 2008). Similar phytotoxic effects of Pb metal on different yield parameters were documented for *Lycopersicon esculentum* (Mill.) and *Triticum aestivum* (L.) by Zhao *et al.,* (2011) and Ghanghro *et al.,* (2022). Results from present study are also agreed by these investigations. Both sunflower varieties SF1 and SF2 have shown decreased seed yield and harvest index due to Cr toxicity. Yield attributes were more affected at elevated Cr level than at lower Cr application. It revealed metal tolerance potential of sunflower plants up to 150 mg/kg (Table 2).

Despite metal toxicity due to their accumulation in agro soils, these contaminated soils can be revived into metal free useable lands by growing some crop plants having potential to accumulate heavy metals from such soils (Atta *et al.,* 2023). Hyper-accumulators are the plant species that accumulate a major proportion of metals from the medium and are the good tools to remove heavy metals (Ozay & Mammadov, 2013; da Conceicao *et al.,* 2016). In our present investigation, chromium uptake study has uncovered the potential of sunflower plants to be a good green tool for phytoremediation of Cr metal in the soil medium. Chromium transfer from soil to plant was significant. Metal accumulation factor showed progressive up to lower metal application but decreased afterward. In overall, at 500 mg/kg of Cr, metal uptake was 49% in SF1 followed by 47.5% by SF2 (Fig. 2-I).

5. Conclusion:

Chromium is a toxic environmental pollutant. Its application to sunflower plants was found to be phytotoxic. Cr has affected germination time by inhibiting embryonic induction, affected plant growth and development. Plant biomass was also found to be affected by Cr toxicity. Moreover, affected internal carbon and rate of photosynthesis have predicted Cr toxicity for photosynthetic machinery of sunflower plants. Biochemical profile of plants has revealed Cr toxic effects on protein and plant mineral status as compared to control treatment. On the other hand, sunflower plants increased proline level showing their osmolyte adaptive strategy against possible ROS due to Cr metal. Affected plant growth can be correlated with the affected seed yield in the sunflower varieties. The harvest index also decreased under Cr stress and has predicted the affected yielding potential of crop under Cr application. The present study has also uncovered the phytoremediation potential of sunflower test varieties against Cr contamination in the soil. Therefore, these varieties are strongly recommended for the growers as green tools to clean the contaminated agricultural soil.

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