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Effect of chromium on growth attributes in sunflower (*Helianthus annuus* L.)

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Abstract

Heavy metal soil pollution takes place when the metal concentration of soil exceeds natural background level and causes ecological destruction and deterioration of the environment. In the present study, a pot experiment was conducted to evaluate the effect of chromium-contaminated soil in sunflower (*Helianthus annuus* L.) growth attributes. Three different levels of chromium (Cr) i.e., 20, 40, and 60 mg/kg were applied to three varieties of sunflower (G-3, G-9, and G-59). The results of morphological, chemical, and yield parameters were recorded at crop maturity. The result showed that germination, root, and shoot lengths were decreased with increase in Cr concentrations. A gradual decrease was observed for various morphological parameters like root fresh and dry weights, shoot fresh and dry weights, and plant height with increase in Cr levels. A comparison among Cr treatments obtained a significant decrease in yield parameters as achenes/capitulum, achenes/plant, and 100 achenes weight in three varieties. Cr was significantly absorbed by roots but its transport to other parts of plants was slow, and uptake in seeds was much lower than in roots and shoots.

Key words: *Helianthus annuus* L.; soil; chromium; growth attributes

Introduction

Sunflower (*Helianthus annuus* L.) belonging to the family Asteraceae is the world's fourth largest oil-seed crop. The genus *Helianthus* comprises 9 species and 19 subspecies with 12 annual and 37 perennial species (Schilling and Heiser, 1981). Seeds of sunflower are used as food and dried stalk as fuel. Sunflower has also been used as an ornamental plant and for ceremonies. In addition, parts of this plant are used in making dyes for textile industry, body painting, and other decorations. Sunflower meal is a potential source of protein for human consumption due to its high nutritional value and lack of anti-nutritional factors (Chimenti *et al*., 2002).

Chromium (Cr) is considered as a serious environmental pollutant, due to its wide industrial applications. Contamination of soil and water by Cr^{2+} is of recent concern. Toxic effects of Cr^{2+} on plant growth and development include alterations in the germination process as well as in the growth of roots, stems, and leaves, which may affect dry matter production and yield (Shanker *et al*., 2005). Cr is found in all phases of the environment, including air, water, and soil with Cr^{2+} ranged from 10 to 50 mg/kg depending on the parental material. In ultramafic soils (serpentine), it can reach up to 125 g/kg (Adriano, 1986). In fresh water, Cr^{2+} concentrations generally range from 0.1 to 117 μg/L, and range from 0.2 to 50 μg/L for sea water (Nriago, 1990).

Leather industry is one of the major sources of pollution in Pakistan, which releases Cr during tanning process with various levels of impacts on plants, animals and human life. The chemicals used in the leather industry during the tanning operation include chromium sulfate, formic acid, sulfuric acid, sodium chloride, sodium bicarbonate, calcium hydroxide, magnesium sulfate, dyes, fat liquors etc. Similarly, textile industry is also a major consumer of chemicals, i.e., soda ash, sulfuric acid, caustic soda, and metals containing alloys, and so on (Tahira, 1999, Nriago, 1990).

The Cr^{6+} compounds are used in industry for metal plating, cooling water treatment, hide tanning, and until recently, wood preservation. These anthropogenic activities have led to the wide spread contamination that Cr shows in the environment and have increased its bioavailability and biomobility (Kotas and Stasicka, 2000).

The present study was conducted to determine the sunflower response and the capacity of removing Cr in the Cr contaminated soil.

1 Materials and methods

The present study was conducted at Environmental Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad,

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Pakistan to assess the influence of Cr on growth of three sunflower (*Helianthus annuus* L.) hybrid varieties V1, V2, and V3 (named as G-3, G-9, and G-59, respectively). The sunflowers procured from Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan, were grown in Cr contaminated soil. This research work was conducted in two phases as laboratory experiment and pot experiment.

1.1 Sowing of seeds for laboratory experiment

A total of 36 Petri dishes were used during this study, which were washed thoroughly and sterilized in an autoclave. A single layer of filter paper was placed in each Petri dish. Various concentrations of Cr, i.e., 20, 40, and 60 mg/kg were applied triplicate along with control (without any treatment). Five seeds were placed in each Petri dish and were soaked for 2 h in distilled water. The Petri plates were incubated at 37°C for 72 h. After germination, the data were collected daily for 7 d.

1.2 Pot experiment

1.2.1 Preparation of chromium spiked soil

The soil used for the experiment was taken from the experimental area of NIBGE. Earthern pots (30 cm i.d.) lined with polythene bags were used, containing 8 kg of homogeneously mixed dried soil. The soil was treated for Cr with adding 3.47 g $CrSO_4$ into 1 kg of soil (1,000 mg/kg Cr). This stock was diluted with normal soil to make up the Cr concentrations as 20, 40, and 60 mg/kg. Each of the above dilution including control was performed in triplicate.

For the analysis of soil, the method introduced by US Salinity Lab (1954) was applied. The classification/soil texture used in this study was clay loam. The physical parameters of soil like EC, pH, and saturation percentage were 6.75 mS/cm, 6.72, and 37.5%, respectively. Chemical properties without carbonate traces were detected, the concentrations of HCO_3^-, Na^+, K^+, and Mg^{2+} were 2, 1.7, 5.1, and 12.6 mg/L, respectively. The Cr contents of the soil were found to be 2.88 mg/kg.

Sowing of seeds was done on February 10, 2005 at NIBGE. Ten holes with about 2 cm deep at equal distance were made in each pot. One seed was put in the hole and covered with a small amount of soil. Germination was started since day 6 after sowing. After 20 d of germination, the plants were thinned to maintain 5 seedlings in each pot. The plants were irrigated with enough water on alternate days. Two plants from each pot at mid of crop growth and three plants at maturity were observed for morphological, yield, and chemical parameters.

1.3 Morphological parameters

Plant height (cm) was measured from the stem base up to the tip of main stem using a meter rod and mean values were calculated. Roots of the two plants were removed carefully to estimate the root fresh and dry weights after dried in an oven at 70°C for 72 h (Shanker *et al*., 2005)

1.4 Yield parameters

Total number of achenes per capitulum was counted from each replicate of the treatments. Achene yield/plant, noted as achenes from each plant of a treatment were collected with a estimation of 100 achene weight for each plant of a treatment. The weight of 10 seeds was taken and the average was calculated (Shanker *et al*., 2005).

1.5 Chemical analysis

EC (mS/cm) of soil saturation extracts were determined by conductivity meter (WQC-20A, TOA, Japan) at 30°C. The dried materials of roots and shoots were chopped into small pieces and 0.3 g of them were digested with H_2SO_4 and H_2O_2 (Wolf, 1982) to determine Na, K, P, N, and Cr contents.

The Na^+ and K^+ were determined using a flame photometer (PFP-7, Jenway, UK). The value of Na^+ and K^+ from flame photometer was compared with standard curve and total quantities were computed (Rump and Krist, 1992). Phosphorous was determined using the ascorbic acid method (Rump and Krist, 1992). N was estimated as distilled plant samples by Kjeldahl distillation apparatus (UDK 126 A, VELP, Italy) unit using the method of Keeney and Nelson (1982). Cr^{2+} was determined with atomic absorption spectrophotometer (AAS-5000, Perkin-Elmer, Germany). A graded series of standards (1–5 mg/L) were used for preparation of standard curves and values of Cr^{2+} were compared with standard curve and final values were calculated (Rump and Krist, 1992).

1.6 Statistical analysis

Data for various morphological, yield and chemical parameters were analyzed statistically. Means were compared by applying Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1984).

2 Results and discussion

Heavy metals influence the growth and tissue ionic concentration. According to Paiva *et al*. (2000), dry weight of shoot and root showed a significant reduction with increase in heavy metals level. Such reduction becomes more damaging at high levels of heavy metals, therefore, plants show greater variation ranging from morphological characters to physiochemical characters.

In the present study, growth rate, increase in plant height and fresh weight were significantly reduced. Although there were remarkable reductions in various growth parameters, the effect was more pronounced with high Cr levels, which inhibit growth more than control. It was noticeable that the fresh and dry weight parameters were better in control than that under Cr stress. This is probably due to the reason that toxicity of heavy metals significantly inhibited root vitality, preventing plant from absorbing inorganic nutrients and leading to inhibit plant growth (Shu *et al*., 1997).

2.1 Germination percentage

Ability of a seed to germinate in Cr contaminated medium would indicate its tolerance level of Cr, because seed germination is the first physiological process affected by Cr (Peralta *et al*., 2001). It was shown that germination percentage was maximum (100%) in control (without any treatment), and reduced to 40% with increasing Cr 60 mg/kg (Table 1). The results are in agreement with Rout *et al*. (2000), where seed germination of the weed *Echinochloa colona* was reduced to 25% with 200 mol/L Cr. Peralta *et al*. (2001) found that 40 mg/L of Cr reduced by 23% the ability of seeds of lucerne (*Medicago sativa* cv. Malone) in contaminated medium.

2.2 Root length

The increase in root length showed decreased values during the 1st harvest in the three cultivars (Table 2). The maximum (1.46 cm/d) and minimum (1.23 cm/d) values for root length were observed in control of G-59 and 60 mg/kg of Cr in G-3, respectively. It indicated that with the increase in Cr concentration, root length was decreased. However, in 6th interval, root length/growth was much lesser than in preceding intervals (Table 2). This may be due to inhibition of root cell division/root elongation or to the extension of cell cycle in the roots (Barcelo *et al*., 1986). Statistical analysis showed that data about harvest and treatments are highly significant while non-significant results were obtained between varieties.

The decrease in root length was due to heavy metals in trees and crops (Shanker *et al*., 2005; Chen *et al*., 2001). Cr toxicity inhibits root cell division/root elongation or the extension of cell cycle in the roots, thereby inhibits root growth while roots directly contact with Cr in the medium, causing a collapse and subsequent inability of the roots to absorb water from the medium (Barcelo *et al*., 1986).

2.3 Shoot length

The greatest increase in shoot length was observed during the 3rd and 4th interval in all the three varieties. In the 3rd harvest, maximum relative increase was observed in control and minimum was found at highest Cr concentration. In the 4th harvest, the same trend was found. However, in the 5th and 6th harvest, toxic effect of Cr was pronounced compared to the preceding intervals (Table 3). It was shown by statistical analysis that data regarding to harvest and treatments are highly significant while the difference between varieties is non-significant.

According to Rout *et al*. (1997), there were adverse effects of Cr on shoot length and plant height. The same results were obtained in this experiment as shoot length and plant height was reduced with increase Cr content.

Table 1 Effect of different concentrations of Cr on germination percentage of various sunflower varieties

Sunflower	Treatment (mg/kg)	Total number of seeds	Number of seeds germinated	Germination percentage (%)
G-3	Control	5	5	100
	20	5	4	80
	40	5	3	60
	60	5	2	40
G-9	Control	5	5	100
	20	5	4	80
	40	5	3	60
	60	5	2	40
G-59	Control	5	5	100
	20	5	3	60
	40	5	3	60
	60	5	2	40

Table 3 Effect of Cr on the increase of shoot length in various sunflower varieties

Sunflower	Treatment (mg/kg)	Shoot length (cm/d)			
		3rd harvest	4th harvest	5th harvest	6th harvest
G-3	Control	0.69	0.65	0.61	0.58
	20	0.55	0.48	0.32	0.26
	40	0.48	0.40	0.32	0.21
	60	0.36	0.30	0.22	0.19
G-9	Control	0.68	0.65	0.62	0.55
	20	0.58	0.49	0.36	0.29
	40	0.46	0.39	0.31	0.24
	60	0.33	0.28	0.20	0.17
G-59	Control	0.67	0.64	0.60	0.55
	20	0.55	0.47	0.34	0.27
	40	0.45	0.37	0.29	0.21
	60	0.35	0.30	0.19	0.19

Table 2 Effect of Cr on the increase in root length in various sunflower varieties

Sunflower	Treatments (mg/kg)	Root length (cm/d)					
		1st harvest	2nd harvest	3rd harvest	4th harvest	5th harvest	6th harvest
G-3	Control	1.46	1.28	1.18	0.97	0.63	0.54
	20	1.25	1.09	0.84	0.76	0.53	0.41
	40	1.21	1.02	0.74	0.56	0.37	0.26
	60	1.23	1.20	0.89	0.53	0.26	0.17
G-9	Control	1.45	1.25	0.17	0.95	0.60	0.52
	20	1.24	1.06	0.79	0.74	0.51	0.42
	40	1.22	1.01	0.71	0.55	0.39	0.29
	60	1.21	1.21	0.85	0.52	0.23	0.13
G-59	Control	1.46	1.27	1.17	0.96	0.61	0.53
	20	1.25	1.0	0.28	0.76	0.52	0.42
	40	1.20	1.03	0.72	0.49	0.34	0.24
	60	1.24	1.21	0.83	0.54	0.24	0.14

1st harvest: day 2–3 after sowing; 2nd harvest: day 3–4 after sowing; 3rd harvest: day 4–5 after sowing; 4th harvest: day 5–6 after sowing; 5th harvest: day 6–7 after sowing; 6th harvest: day 7–8 after sowing.

2.4 Morphological parameters

2.4.1 Plant height

There were adverse effects of Cr on plant height (Rout *et al*., 1997), such as *Curcumas sativus*, *Lactuca sativa*, and *Panicum miliaceum* (Joseph *et al*., 1995). Because Cr transporting to the aerial part of the plant can have a direct impact on cellular metabolism, which may contribute to the reduction.

Plant height decreased in response to increase Cr levels (Table 4). For all the three varieties, during the highest increase (0.040 cm/d) in control was observed in the 1st–2nd harvest, while the lowest (0.020 cm/d) was in 60 mg/kg Cr content. During 2nd–3rd harvest interval the increase in plant height was the highest compare to 1st–2nd and 3rd–4th harvest intervals. Plant height reflected that harvests, treatments, and varieties are highly significant when compared through ANOVA.

2.4.2 Root fresh and dry weight

The prerequisite for higher yields in plants is an increase in biomass production in terms of fresh and dry matter/weights (Bishnoi *et al*., 1993). The data in Table 5 represent the fresh and dry weight of roots in response to different Cr levels. Maximum growth was recorded in 1st harvest in all the three varieties, followed by 2nd and then 3rd harvests. All the three varieties showed the highest growth rate in control and decreased by increasing Cr levels in all three harvests.

Table 4 Effect of chromium on plant height in various sunflower varieties

Sunflower	Treatment (mg/kg)	Plant height (cm/d)		
		1st–2nd harvest	2nd–3rd harvest	3rd–4th harvest
G-3	Control	0.040	0.096	0.054
	20	0.038	0.085	0.049
	40	0.026	0.079	0.033
	60	0.020	0.071	0.028
G-9	Control	0.038	0.089	0.048
	20	0.035	0.076	0.043
	40	0.029	0.079	0.041
	60	0.024	0.065	0.038
G-59	Control	0.035	0.087	0.044
	20	0.030	0.076	0.038
	40	0.026	0.067	0.028
	60	0.023	0.064	0.024

In all the three harvests, the root dry weight was maximum in control and minimum at the highest concentration 60 mg/kg Cr treatment. There was a significant decrease in fresh and dry weights of roots. It is in agreement with Kocik and Ilavsky (1994), where the effect of Cr was showed on quality and quantity of biomass in sunflower and observed that dry matter production and uptake of Cr into plant organs was positively correlated with the contents in the soil.

2.4.3 Shoot fresh and dry weight

The shoot fresh weight was maximum in control in all the three varieties, while this rate gradually decreased with increasing levels of Cr at every harvest intervals (Table 6). The weight of fresh and dry shoot was maximum in 1st–2nd harvest. It can be explained by that the higher source size and increased photosynthetic process were found to be the basis for the building up of organic substances and dry matter production under heavy-metal stress in general and Cr in particular (Bishnoi *et al*., 1993).

2.5 Yield parameters

Data pertaining to number of achenes/capitulum as influenced by different levels of Cr showed the maximum number of achenes 104, 100, and 98 in G-3, G-9, and G-59, respectively. A marked difference was observed in achene yield/plant (g) between different treatments. In control, the achene yield observed was 18.9 g/plant, but this amount reduced to 8.93 g/plant at maximum Cr level (60 mg/kg) in G-3, the same trend was followed in other two varieties. It was concluded that yield and productivity was affected by Cr (Barcelo *et al*., 1993). Moreover, Sharma and Mehrotra (1993) also observed that achene dry weight yield was 2.11 g/plant without Cr and 0.39 and 0.16 g/plant with 20 and 200 mg/kg of Cr, respectively. The difference of our studies might be due to the reason that Cr uptake by seeds is very low as compared to other parts of the plant.

2.6 Chemical analysis

The Cr compounds are highly toxic to plants and are detrimental to their growth and development. Interactions of Cr with uptake and accumulation of other inorganic nutrients have received maximum attention by researchers. There was a decreasing trend in Na, K, P, and N contents of roots and shoots by increasing Cr level in all the three

Table 5 Effect of Cr on root fresh weight (fw) and dry weight (dw) of various sunflower varieties

Sunflower	Treatment (mg/kg)	1st harvest		2nd harvest		3rd harvest	
		fw (g/d)	dw (g/d)	fw (g/d)	dw (g/d)	fw (g/d)	dw (g/d)
G-3	Control	0.89	0.058	0.65	0.032	0.47	0.023
	20	0.63	0.031	0.45	0.018	0.23	0.009
	40	0.49	0.028	0.23	0.015	0.19	0.006
	60	0.38	0.022	0.19	0.014	0.12	0.004
G-9	Control	0.87	0.057	0.60	0.032	0.44	0.020
	20	0.56	0.033	0.34	0.018	0.20	0.010
	40	0.47	0.026	0.29	0.013	0.18	0.004
	60	0.43	0.019	0.22	0.007	0.14	0.001
G-59	Control	0.87	0.057	0.62	0.033	0.46	0.022
	20	0.52	0.032	0.34	0.015	0.22	0.007
	40	0.42	0.022	0.24	0.012	0.16	0.005
	60	0.34	0.020	0.17	0.006	0.09	0.002

Table 6 Effect of Cr on shoot fresh and dry weight in various sunflower varieties

Sunflower	Treatment (mg/kg)	1st–2nd harvest		2nd–3rd harvest		3rd–4th harvest	
		fw (g/d)	dw (g/d)	fw (g/d)	dw (g/d)	fw (g/d)	dw (g/d)
G-3	Control	1.18	0.064	0.93	0.038	0.071	0.018
	20	1.05	0.046	0.85	0.029	0.65	0.013
	40	1.02	0.021	0.78	0.013	0.52	0.009
	60	0.98	0.016	0.63	0.011	0.45	0.007
G-9	Control	1.13	0.062	0.91	0.031	0.75	0.014
	20	1.05	0.049	0.75	0.024	0.50	0.009
	40	1.03	0.024	0.73	0.014	0.49	0.007
	60	0.99	0.016	0.69	0.012	0.43	0.006
G-59	Control	1.12	0.058	0.91	0.030	0.71	0.013
	20	1.05	0.045	0.73	0.027	0.58	0.011
	40	1.02	0.022	0.68	0.012	0.44	0.007
	60	0.98	0.014	0.56	0.008	0.31	0.004

cultivars, and accumulation of these elements in seeds was minimum. Such findings are in line with Sujatha and Gupta (1996) who reported that tannery effluent irrigation caused micronutrient deficiencies in several agricultural crops. In soil grown rye grass, the influence of Cr on mineral nutrition was highly variable (Ottabbong, 1989). Cr induced decrease in Ca, Na, K, Mg, P, N, and Cu concentrations in soil-grown soybean was observed, but Fe, Mn, and Zn uptake was not affected (Turner and Rust, 1971). The nutrient elements N, P, K, Na, Ca, and Mg concentrations in stems and branches were significantly affected by the Cr treatments (50 and 100 mg/L) in tomato (Moral *et al*., 1995). Total P in sunflower hull was the highest with Cr (0.5 mg/L) (Gupta *et al*., 2000).

Table 7 Estimation of Cr in various parts in sunflower varieties

Sun-flower	Treatment (mg/kg)	1st harvest		2nd harvest	
		Roots (mg/kg)	Shoots (mg/kg)	Roots (mg/kg)	Shoots (mg/kg)
G-3	Control	2.73	0.04	2.84	0.11
	20	5.63	2.16	8.98	3.89
	40	10.65	3.78	18.86	5.91
	60	13.23	3.63	22	5.93
G-9	Control	2.69	0.06	2.82	0.11
	20	5.62	2.23	8.98	3.84
	40	10.43	3.81	18.81	6.01
	60	13.53	3.72	22.29	6.03
G-59	Control	2.78	0.04	2.90	0.09
	20	5.31	2.13	8.26	3.76
	40	10.51	3.73	18.72	5.84
	60	3.32	3.68	22.31	5.99

Cr is thought to alter plant membrane systems, but the physiological and metabolic responses are not well understood. It has been suggested that Cr is retained in vacuoles and cell walls of roots, but mechanisms of chromium hyperaccumulation are not well known (Vazquez *et al*., 1987). As shown in Table 7, Cr was highly accumulated in roots, in all varieties of sunflower. At control level the plants almost accumulated all the Cr in the soil, during the first harvest. During the second and final harvest the three varieties G-3, G-9, and G-59 accumulated 8.98, 8.98, and 8.26 mg/kg Cr^{2+} in their roots at 20 mg/kg Cr^{2+} level, and the same trend was found at other two levels. It was revealed that harvests and varieties are highly significant while comparison among treatments show non-significant results.

During 1st harvest, the shoots of three varieties have minimum levels of Cr in control. In shoot, the G-9 variety accumulated maximum Cr, followed by G-3 and G-59 at three Cr levels. During the 2nd harvest, G-3 and G-9 accumulated 0.11 mg/kg Cr, while G-59 accumulated 0.09 mg/kg of Cr in control. At 20 mg/kg Cr level, maximum amounts were recorded in G-3 followed by G-9 and G-59, while the trend was different at 60 mg/kg Cr level. Data and its statistical interpretation showed highly significant results in case of varieties, harvests and treatments. It was observed that in control, the three cultivars.

In case of level, there was a decreasing trend by increasing level of Cr (Table 8). It was concluded from analysis of variance that treatments and varieties were highly significant. It was observed that all varieties behave

Table 8 Chemical analysis in various sunflower varieties

Sunflower	Treatment (mg/kg)	Na (mg/g)		K (mg/g)		P (mg/g)		N (mg/g)	
		Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots
G-3	Control	5.68	4.18	14.29	14.12	2.19	5.98	15.40	33.50
	20	5.04	3.52	12.65	11.11	2.03	4.49	12.86	17.43
	40	3.84	2.81	10.85	9.98	1.98	4.10	10.63	15.26
	60	2.02	2.16	8.75	7.64	1.52	3.89	9.69	12.64
G-9	Control	5.13	3.78	11.60	13.89	2.11	5.58	15.60	34.10
	20	4.09	3.21	12.23	10.83	1.99	4.48	12.92	17.31
	40	3.24	2.49	10.63	9.24	1.74	3.98	10.69	15.19
	60	1.18	1.86	8.61	7.02	1.25	3.78	9.71	12.59
G-59	Control	5.32	4.08	13.24	12.43	2.05	4.58	15.20	33.90
	20	4.71	3.46	12.12	10.23	1.75	4.28	12.49	17.23
	40	3.21	2.53	10.39	8.84	1.65	3.61	10.54	14.89
	60	1.9	2.09	8.03	6.54	1.13	2.53	8.56	12.46

independently from each other.

The Na, K, N, and P contents recorded were decreased with an increasing Cr in roots and shoots for three varieties. Maximum accumulations of Na, K, N, and P in were observed in control (except K in control in shoots). The analysis of variance showed the highly significant results for both varieties and treatments. All the interactions were highly significant.

3 Conclusions

The experimental results clearly showed that Cr is toxic at different degrees at different stages of plant growth and development and also that the toxicity is concentration and medium dependent.

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