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Effect of chelating compounds on growth of maize and mustard in chromium contaminated soil

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Abstract
Pot experiments were conducted in the net house of Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, B.H.U., Varanasi in alluvial soil during 2015-16 using maize-mustard cropping sequence to study the effect of chelating compounds on growth of maize and mustard in chromium contaminated soil. Five levels of chromium viz. 0, 5, 10, 20, and 30 ppm with and without five types of chelating compounds viz. EDTA, DTPA, Oxalic Acid, Citric Acid, and Humic Acid were applied. All the treatments of chelating compounds were applied to maize in *kharif* season and mustard was taken in *rabi* season as residual crop after harvesting of maize crop. Plant height (cm), number of leaves per plant, chlorophyll content (SPAD value) and dry matter accumulation were recorded at different growth stages starting from 30 days after showing (DAS), 60 DAS and at harvesting. Results indicated that growth parameters (plant height, number of leaves per plant, chlorophyll content and dry matter accumulation) of maize and mustard decreased with increasing Cr concentration (0, 5, 10, 20, and 30 ppm). The growth parameters significantly increased with the application of chelating compounds. There were no significant effects due to the application of oxalic acid and citric acid on the growth parameters of both the plant species; however humic acid, DTPA and EDTA significantly influence the growth of maize and mustard. At every level of chromium treatment with humic acid (1 g kg^{-1}) gave batter results followed by treatment with DTPA (10 mMole kg^{-1}).

Keywords: chelating compounds, chromium, growth, maize, mustard

Introduction

With the advances in science and introduction of new technologies, the number and/or the amount of harmful pollutants including metals and metalloids has continuously increased in the environment. Metal (loid)s are released into the environment through a number of industrial process, natural biogeochemical activities, agriculture, mining, etc. Metal (loid)s can accumulate in humans through the food chain and can lead to ailments due to their carcinogenic, mutagenic, and toxicological effects (Shukla *et al.*, 2018) [36]. The elevated concentration of these metal (loid)s can result in growth inhibition and toxicity symptoms, such as DNA damage, inhibition of cell division, protein denaturation, damage to membranous structure of the cell, and displacement of the essential micro- and macro-elements (Srivastava *et al.*, 2011 and Awasthi *et al.*, 2017) [37, 4]. Chromium is a heavy metal with risk to human health. Its presence in agricultural soils can be attributed to the use of industrial effluents for irrigation. Increase of world population has resulted in the pollution of the environment. Chromium is highly toxic non-essential element for microorganism and plants. The source of chromium in environment are both natural and anthropogenic, natural source include burning of oil and coal, petroleum from Ferro chromate refractory material, chromium steels, pigments oxidants, catalyst and fertilizers This element is also used in metal plating tanneries and oil well drilling (Ghani *et al.*, 2017) [14]. The contamination of the soil environment with chromium compounds is more and more frequently occurring problem throughout the world (Radziemska and Wyszkowski, 2017) [32]. Chromium pollution of soil and water is a serious environmental concern due to potential carcinogenicity of hexavalent chromium [Cr(VI)] when ingested (Choudhary *et al.*, 2017) [7]. Cr is widely used in industry as plating, alloying, tanning of animal hides, inhibition of water corrosion, textile dyes and mordants, pigments, ceramic glazes, refractory bricks, and pressure-treated lumber (Lukina *et al.*, 2016) [17]. With the development of industrial activities including chromate production, electroplating and leather tanning, hexavalent chromium (Cr(VI)) has been widely detected in soil (Su *et al.*, 2016; Lukina *et al.*, 2016) [17]. Chromium, due to its structural similarity with some essential elements, can affect mineral nutrition of plants in a complex way. Interactions of Cr with uptake and accumulation of other inorganic nutrients have received maximum attention by researchers (Kumar *et al.*, 2016) [24].

Organic and inorganic amendments are used for immobilization of metals in the soils with varying benefits but organic amendments could be better option due to improvement of physical, chemical, biological properties and fertility status of the soil (Park *et al.*, 2011). The mobility and toxicity of Cr6+ can be reduced by converting it to the reduced state of Cr3+ by means of organic matter and inorganic reducing agents in the soil (Kumar and Sharma, 2018) [22].

These Organic sources may be organic manures, green manure, rural wastes, crop residues, biofertilizers and vermicompost (Kumar *et al.*, 2018) [22]. Immobilization of metals in contaminated soils using amendments is a remediation technique that decreases mobility and phytoavailability of metals in the soils (Sabir *et al.*, 2013; Rizwan *et al.*, 2016; Rehman *et al.*, 2017) [35, 34, 33]. The effect of organic amendments on the mobility and the bioavailability of metal(loid)s depends on the nature of the organic matter itself, its microbial degradability, its effects on soil chemical and physical proprieties, as well as on the particular soil type and metal(loid)s concerned (Angelova *et al.*, 2013) [2].

Low-molecular weight organic acids (LMWOAs) such as citric, malic, succinic, and fumaric acid participate as crucial components in several cellular biochemical pathways such as energy production and amino acid synthesis. At the whole plant level, they play a role in metal tolerance, cope with nutrient deficiencies, and regulate rhizospheric plant–microbe interactions (Kaur *et al.*, 2017) [2]. Among LMWOAs, citric acid (CA), an important intermediate of the tricarboxylic cycle, plays a crucial role in respiratory and other biochemical pathways and its exogenous application reduces heavy metal toxicity and improves phytoextraction (Najeeb *et al.*, 2011) [30]. Also CA-enhanced phytoextraction of Cd is reported in *B. juncea* (Quartacci *et al.*, 2005) [31], *Solanum nigrum* (Gao *et al.*, 2010), and *Sedum alfredii* (Lu *et al.*, 2013) [26]. In addition to heavy metal tolerance, its role is also implicated in other abiotic stresses (Sun and Hong 2011; Hu *et al.*, 2016) [39, 13]. Although most of heavy metals have low bioavailability in soils, there is a need to meet stringent cleanup targets. To overcome the limitations of natural phytoextraction has led to several studies on different chelates that increase the bioavailability of heavy metals (Evangelou *et al.*, 2007) [10]. For this purpose, different synthetic and natural chelators are used to enhance the bioavailability of metals in contaminated medium. Among synthetic chelating agents, ethylene diamine tetra acetic acid (EDTA) and diethylene triamine pentaacetic acid (DTPA) are commonly used as they are efficient in complexing metals and increasing their concentration in the upper plant parts (Kanwal *et al.*, 2014) [19]. However, they are non-biodegradable and can cause ground water contamination due to uncontrolled leaching in the soil (Anwer *et al.*, 2012; Bareen, 2012) [3, 5]. Organic acids could be an interesting alternative to the persistent synthetic chelating agents described above. Organic chelating agents are low molecular-weight organic acids such as citric acid (CA) and can form complexes with heavy metals and have higher degree of biodegradability and less leaching hazard as compared to synthetic chelating agents (Bareen, 2012) [5]. Recently, it has been reported that citric acid (CA) significantly enhances metal solubility and uptake by plants (Yeh and Pan 2012; Freitas *et al.*, 2013) [41, 12]. To date, many studies have reported the effects of organic chelating agents on the extraction of heavy metals from the solution cultures (Gunawardana *et al.*, 2011; Das *et al.*, 2014; Ehsan *et al.*, 2014) [17, 8, 9]; there are few reports on the role of these chelating agents during phytoextraction of heavy metal from contaminated soils (Chigbo and Batty 2013) [6]. Thus, we need longer term (as compared to hydroponic cultures) and more realistic soil-based studies to better understand the practical implications of chelating agents mediated phytoextraction and tolerance of metals so that successful field experiments can be conducted (Afshan *et al.*, 2015) [1].

Chelating agents such as EDTA, DTPA, citric acid, oxalic acid and humic acid are added to soil to increase the bioavailability of heavy metals in soil for uptake by plants (Lai and Chen, 2004) [25].

Material and Methods

To conduct the pot experiment, the bulk of soil was collected from the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University; Varanasi U.P. Soil was air dried gently, ground to pass through 2 mm sieve and homogenized. Chemical properties of soil are depicted in table 1.

Treatment details

The pot experiments were conducted in FCRD with five levels of chromium and with and without five types chelating compounds

A.	**Chromium Levels (mg/kg soil)**	**Symbol**
1)	Control	Cr_0
2)	5 ppm	Cr_1
3)	10 ppm	Cr_2
4)	20 ppm	Cr_3
5)	30 ppm	Cr_4
B.	**Chelating compound**	**Symbol**
1)	Control	C_0
2)	EDTA (10 mMole kg^{-1})	C_1
3)	DTPA (10 mMole kg^{-1})	C_2
4)	Citric Acid (20 mMole kg^{-1})	C_3
5)	Oxalic Acid (20 mMole kg^{-1})	C_4
6)	Humic acid (1g kg^{-1})	C_5

Treatment combinations for pot experiment

Treatments	**Control C_0**	**EDTA (10 mMole kg $^{-1}$)**	**DTPA (10 mMole kg $^{-1}$)**	**Citric Acid (20 mMole kg $^{-1}$)**	**Oxalic Acid (20 m Mole kg^{-1})**	**Humic acid (1g kg^{-1})**
Cr_0 0.0 ppm	$Cr_0 C_0$ 1	$Cr_0 C_1$ 2	$Cr_0 C_2$ 3	$Cr_0 C_3$ 4	$Cr_0 C_4$ 5	$Cr_0 C_5$ 6
Cr_1 5.0 ppm	$Cr_1 C_0$ 7	$Cr_1 C_1$ 8	$Cr_1 C_2$ 9	$Cr_1 C_3$ 10	$Cr_1 C_4$ 11	$Cr_1 C_5$ 12
Cr_2 10.0 ppm	$Cr_2 C_0$ 13	$Cr_2 C_1$ 14	$Cr_2 C_2$ 15	$Cr_2 C_3$ 16	$Cr_2 C_4$ 17	$Cr_2 C_5$ 18
Cr_3 20.0 ppm	$Cr_3 C_0$ 19	$Cr_3 C_1$ 20	$Cr_3 C_2$ 21	$Cr_3 C_3$ 22	$Cr_3 C_4$ 23	$Cr_3 C_5$ 24
Cr_4 30.0 ppm	$Cr_4 C_0$ 25	$Cr_4 C_1$ 26	$Cr_4 C_2$ 27	$Cr_4 C_3$ 28	$Cr_4 C_4$ 29	$Cr_4 C_5$ 30

Observations recorded

The appropriate sampling technique implies proper balance in sampling to achieve maximum precision at minimum cost. Following this principal the various observations were recorded from each pot. Observation will be recorded at pertinent stages of maize and mustard crops.

Growth attributes
In order to assess the probable relationship between various growth attributes and crop yield, biometric observation on growth characters were recorded from the marked area of the pot at 30, 60 DAS and at maturity stage. The principle indices of observation during different growth period and at maturity in respect of maize and mustard are described here under

Plant height (cm)
Plant height was recorded with the help of meter scale from ground level to the tip of uppermost leaf of the plant. Plant height was recorded at 30, 60 DAS and at harvest from each pot.

Number of leaves/ plant
The numbers of leaves per plant were counted at 30, 60 DAS and at harvest from each pot and average numbers of leaves were calculated on per plant basis.

Chlorophyll content of the plant
Chlorophyll content of the plants was measured at 30, 60 DAS and at harvest by using SPAD meter.

Dry-matter accumulation/plant (g)
Plants from each pot were sun dried and later on transferred to hot air oven and dried at 65±2 °C to get constant dry weight of plants and weighed. The weight, thus obtained was recorded as dry weight per pot (g) after dividing the total dry weight by the total number of plants.

Statistical Analysis and Interpretation of Data
For determining the significance between the treatment means and to draw valid conclusion, statistical analysis was made.The raw data observed during the whole experiment, were subjected to statistical analysis by adopting appropriate method of "Analysis of Variance". The significance of the treatment effect was judged with the help of 'F' test (Variance ratio). The difference of the treatments mean was tested using critical difference (CD) at 1% level of probability (Gomez and Gomez, 1984) by following the Complete Randomized Design (CRD) to draw the valid differences among the treatments using SPSS software.

Results and Discussion
Data were collected on growth parameters *i.e.* plant height (cm), number of leaves per plant, chlorophyll content (SPAD value) and dry matter accumulation at different growth stages starting from 30 days after showing (DAS), 60 DAS and at harvesting. The plant height (cm), number of leaves per plant, chlorophyll content (SPAD value) and dry matter accumulation were recorded. The collected data were statistically analyzed and treatment means were compared by least significant difference test.

Effect on plant height
The data pertaining to effect of chelating agent on height of plants of both species at added varying level (0, 5, 10, 20 and 30 ppm) of chromium is presented in table 2. It is evident from the table that height of maize plants at 30 DAS varied from 90.8 to 125.7 cm. There is decrease in plants height with increasing level of chromium contamination starting from 0 to 30 ppm with all chelating agent (humic acid, citric acid, oxalic acid, EDTA and DTPA). For maize plants at 30 DAS control at all level of Cr (Cr_0 C_0, Cr_1 C_0, Cr_2 C_0, Cr_3 C_0, and Cr_4 C_0) produced minimum height (107, 104.5, 100.9, 97.2 and 90.8 cm) respectively and treatment with humic acid (Cr_0 C_5, Cr_1 C_5, Cr_2 C_5, Cr_3 C_5, and Cr_4 C_5) produced maximum height (125.7, 118, 114, 109.8 and 102.6 cm) respectively and were significantly higher over control at each level of chromium. Moreover, maximum height recorded at 30 DAS with Cr_0 C_5 (125.7 cm) followed by pot with chelating agent DTPA (Cr_0 C_2) and EDTA (Cr_0 C_1) @ 10 mMole kg^{-1} recorded height was 121.8 and 119.1 cm respectively and was significantly higher over control (Cr_5 C_0) with minimum height (90.8 cm) and also at each level (0, 5, 10, 20 and 30 ppm) of chromium contamination. It is evident from the data among all chelating agent humic acid (1 g kg^{-1}) effectively increase the number of leaves at 60 DAS and at harvest stage and was more effective as compared to other organic (CA and OA) and synthetic (EDTA and DTPA). Almost similar trend was noticed with the number of leaves recorded at 60 DAS and at harvesting.

For mustard plants height at 30 DAS varied from 14.64 to 28.89 cm. There is significant decrease in plants height with increasing level of chromium contamination starting from 0 to 30 ppm with all chelating agent (humic acid, citric acid, oxalic acid, EDTA and DTPA). For mustard plants at 30 DAS control (with no chelating agent) at all level of Cr (Cr_0 C_0, Cr_1 C_0, Cr_2 C_0, Cr_3 C_0, and Cr_4 C_0) produced minimum height (19.03, 17.95, 16.69, 15.58 and 14.64 cm) respectively and treatment with humic acid @ 1 g kg^{-1} (Cr_0 C_5, Cr_1 C_5, Cr_2 C_5, Cr_3 C_5, and Cr_4 C_5) produced maximum height (28.89, 27.28, 25.37, 23.69 and 22.26 cm) respectively and were significantly higher over control at each level of chromium. Moreover, maximum height recorded at 30 DAS with Cr_0 C_5 (28.89 cm) followed by pot with chelating agent DTPA (Cr_0 C_2) and EDTA (Cr_0 C_1) @ 10 mMole kg^{-1} recorded height was 26.67 and 23.61 cm respectively and was significantly higher over control (Cr_4 C_0) with minimum height (14.64 cm) and also at each level (0, 5, 10, 20 and 30 ppm) of chromium contamination. It is evident from the data among all chelating agent humic acid (1 g kg^{-1}) effectively increase the number of leaves at 60 DAS and at harvest stage and was more effective as compared to other organic (CA and OA) and synthetic (EDTA and DTPA). Almost similar trend was noticed with the number of leaves recorded at 60 DAS and at harvesting.

Comparison among both plant species at all stages of growth (30 DAS, 60 DAS and at harvesting) tallest plant of height 201.1 cm and 145.62 cm was observed in pot treated with Humic acid @ 1 g kg^{-1} (Cr_0 C_5) in maize and mustard respectively at harvesting stage. However, smallest plant of height 90.8 cm and 14.64 cm was observed in control pot (at 30 ppm) with no chelating agent in maize and mustard respectively at 30 DAS. Furthermore, there is decrease in height with increasing level of chromium at all growth stages. There were no significant effects from the application of oxalic acid on the height of plants of both the plant species but Humic acid, DTPA and EDTA have significant influences on plants height (Luo *et al.*, 2006). Addition of high molecular weight chelating agent leads to have significant effect on plant height due to high affinity toward metals. Moreover, increase in growth was observed with the addition of organic chelating agent due to high biodegradability (Anwer *et al.*, 2012) [3].

Table 1: Chemical properties of the initial soil

Parameter	Values	Parameter	Values
pH_w(1:2.5)	7.94	N (mg kg^{-1})	72.00
EC_w (1:2.5) (dS/m)	0.11	P (mg kg^{-1})	12.00
Organic Carbon (%)	0.46	K (mg kg^{-1})	100.00
CEC (C mole (p^+) kg^{-1})	20.10	Cr (mg kg^{-1})	0.48

Table 2: Effect of chelating compounds on plant height (cm) of maize and mustard in chromium contaminated soil

Treatment		Maize			Mustard		
		30 DAS	60 DAS	At Harvest	30 DAS	60 DAS	At Harvest
Control		107.2	150.0	171.5	19.03	57.09	95.91
Cr_0 + EDTA 10 mmol kg^{-1}		119.1	166.7	190.5	23.61	70.84	119.01
Cr_0 + DTPA 10 mmol kg^{-1}		121.8	170.6	194.9	26.67	80.01	134.41
Cr_0 + CA 20 mmol kg^{-1}		115.2	161.3	184.4	21.96	65.89	110.70
Cr_0 + OA 20 mmol kg^{-1}		109.0	152.6	174.4	20.39	61.16	102.75
Cr_0 + HA 1g kg^{-1}		125.7	175.9	201.1	28.89	86.68	145.62
5 ppm Cr		104.5	146.3	167.1	17.95	53.86	90.48
5 ppm Cr + EDTA 10 mmol kg^{-1}		113.3	158.6	181.2	22.31	66.92	112.42
5 ppm Cr + DTPA 10 mmol kg^{-1}		115.2	161.2	184.3	25.16	75.48	126.80
5 ppm Cr + CA 20 mmol kg^{-1}		110.1	154.1	176.2	20.72	62.15	104.41
5 ppm Cr + OA 20 mmol kg^{-1}		108.5	151.9	173.6	19.21	57.64	96.84
5 ppm Cr + HA 1g kg^{-1}		118.0	165.2	188.9	27.28	81.84	137.49
10 ppm Cr		100.9	141.3	161.5	16.69	50.08	84.13
10 ppm Cr + EDTA 10 mmol kg^{-1}		109.4	153.2	175.1	20.72	62.15	104.41
10 ppm Cr + DTPA 10 mmol kg^{-1}		111.3	155.8	178.0	23.39	70.18	117.90
10 ppm Cr + CA 20 mmol kg^{-1}		106.4	148.9	170.2	19.25	57.75	97.02
10 ppm Cr + OA 20 mmol kg^{-1}		104.8	146.8	167.7	17.89	53.68	90.18
10 ppm Cr + HA 1g kg^{-1}		114.0	159.7	182.5	25.37	76.12	127.88
20 ppm Cr		97.2	136.0	155.5	15.58	46.73	78.50
20 ppm Cr + EDTA 10 mmol kg^{-1}		105.4	147.5	168.6	19.35	58.04	97.50
20 ppm Cr + DTPA 10 mmol kg^{-1}		107.1	150.0	171.4	21.86	65.58	110.17
20 ppm Cr + CA 20 mmol kg^{-1}		102.4	143.4	163.9	17.98	53.93	90.60
20 ppm Cr + OA 20 mmol kg^{-1}		100.9	141.3	161.5	16.72	50.16	84.27
20 ppm Cr + HA 1g kg^{-1}		109.8	153.7	175.7	23.69	71.06	119.38
30 ppm Cr		90.8	127.2	145.3	14.64	43.92	73.78
30 ppm Cr + EDTA 10 mmol kg^{-1}		98.5	137.9	157.6	18.19	54.56	91.66
30 ppm Cr + DTPA 10 mmol kg^{-1}		100.1	140.2	160.2	20.51	61.54	103.39
30 ppm Cr + CA 20 mmol kg^{-1}		95.7	134.0	153.2	16.85	50.56	84.95
30 ppm Cr + OA 20 mmol kg^{-1}		94.3	132.1	151.0	15.70	47.10	79.13
30 ppm Cr + HA 1g kg^{-1}		102.6	143.7	164.2	22.26	66.77	112.17
SEm±	Chromium	0.67	0.94	1.08	0.18	0.55	0.92
	Amendments	0.74	1.03	1.18	0.20	0.60	1.00
	Interaction	NS	NS	NS	NS	NS	NS
CD (P=0.01)	Chromium	1.90	2.67	3.05	0.51	1.54	2.59
	Amendments	2.09	2.92	3.34	0.56	1.69	2.84
	Interaction	NS	NS	NS	NS	NS	NS

Cr= Chromium, EDTA= ethylene diamine tetra acetic acid, DTPA= diethylene triamine penta acetic acid, OA= Oxalic Acid, CA= Citric Acid, HA= Humic Acid, CD = Critical Difference, SEm± = Standard error of mean

Effect on number of leaves

A critical perusal of the data presented in Table 3 that a significant decrease was found in number of leaves per plant at 30 DAS, 60 DAS and at harvest with increasing concentration of chromium in pot. Furthermore, enhancing effect of chelating agent (Humic acid, DTPA and EDTA) on number of leaves per plant of both species at varying level (0, 5, 10, 20 and 30 ppm) of chromium was observed except Oxalic and Citric acid.

It is evident from the table that number of leaves per maize plant at 30 DAS varied from 4.6 to 9. There is decrease in number of leaves with increasing level of chromium contamination starting from 0 to 30 ppm with all chelating agent (humic acid, citric acid, oxalic acid, EDTA and DTPA). For maize plants at 30 DAS control at all level of Cr (Cr_0 C_0, Cr_1 C_0, Cr_2 C_0, Cr_3 C_0, and Cr_4 C_0) produced lowest number of leaves per plants (6.6, 6, 5.4, 5 and 4.6) respectively and treatment with humic acid (Cr_0 C_5, Cr_1 C_5, Cr_2 C_5, Cr_3 C_5, and Cr_4 C_5) produced maximum height (9, 9, 8.2, 7.5 and 6.9) respectively and were significantly higher over control at each level of chromium. Moreover, maximum leaves per plant recorded at 30 DAS with Cr_0 C_5 (9) followed by pot with chelating agent DTPA (Cr_0 C_2) and EDTA (Cr_0 C_1) @ 10 mMole kg^{-1} recorded leaves were 8.3 and 8.2 cm respectively and was significantly higher over control (Cr_4 C_0) with minimum leaves per plant (4.6) and also at each level (0, 5, 10, 20 and 30 ppm) of chromium contamination. It is evident from the data among all chelating agent humic acid (1 g kg^{-1}) effectively increase the number of leaves at 60 DAS and at harvest stage and was more effective as compared to other organic (CA and OA) and synthetic (EDTA and DTPA). Almost similar trend was noticed with the number of leaves recorded at 60 DAS and at harvesting.

For mustard plants number of leaves at 30 DAS varied from 4.28 to 8.20. There is also significant decrease in number of leaves with increasing level of chromium contamination starting from 0 to 40 ppm with all chelating agent (humic acid, citric acid, oxalic acid, EDTA and DTPA). For mustard plants at 30 DAS control (with no chelating agent) at all level of Cr (Cr_0 C_0, Cr_1 C_0, Cr_2 C_0, Cr_3 C_0, and Cr_4 C_0) produced lower number of leaves (5.43, 5.22, 4.83, 4.54 and 4.28) respectively and treatment with humic acid @ 1 g kg^{-1} (Cr_0 C_5, Cr_1 C_5, Cr_2 C_5, Cr_3 C_5, and Cr_4 C_5) produced maximum height (8.20, 789, 7.30, 6.86 and 6.46) respectively and were significantly higher over control at each level of chromium. Moreover, maximum number of leaves recorded at 30 DAS with Cr_0 C_5 (8.20) followed by pot with chelating agent DTPA (Cr_0 C_2) and EDTA (Cr_0 C_1) @ 10 mMole kg^{-1} recorded leaves was 7.39 and 7.16 respectively and was significantly higher over control (Cr_5 C_0) with minimum number of leaves per plant (4.28) and also at each level (0, 5, 10, 20 and 30 ppm) of chromium contamination. It is evident from the data among all chelating agent humic acid (1 g kg^{-1}) effectively increase the number of leaves at 60 DAS and at harvest stage and was more effective as compared to other organic (CA and OA) and synthetic (EDTA and DTPA). Almost similar trend was noticed with the number of leaves recorded at 60 DAS and at harvesting.

Comparison among both plant species at all stages of growth (30 DAS, 60 DAS and at harvesting) maximum leaves per plant 12.4 and 45.11 was observed in pot treated with Humic acid @ 1 g kg^{-1} (Cr_0 C_5) in maize and mustard respectively at harvesting stage. However, minimum leaves per plant 4.6 and 4.28 were observed in control pot (at 30 ppm) with no chelating agent in mustard and maize respectively at 30 DAS. Furthermore, there is decrease in leaves per plant with increasing level of chromium at all growth stages. There were no significant effects from the application of citric and oxalic acid on the number of leaves per plant of both the plant species but Humic acid, DTPA and EDTA have significant influences on leaves number. Khaled and Fawy (2011) [21] observed that soil application of humic substances increased the N uptake of corn while foliar application of humic acids increased the uptake of P and K.

Table 3: Effect of chelating compounds on number of leaves per plant of maize and mustard in chromium contaminated soil

Treatment		Maize			Mustard		
		30 DAS	60 DAS	At Harvest	30 DAS	60 DAS	At Harvest
Control		6.6	8.0	9.2	5.43	19.00	29.86
Cr_0 + EDTA 10 mmol kg^{-1}		8.2	9.7	11.2	7.16	25.07	39.39
Cr_0 + DTPA 10 mmol kg^{-1}		8.3	9.8	11.3	7.39	25.88	40.67
Cr_0 + CA 20 mmol kg^{-1}		8.0	9.5	10.9	6.12	21.43	33.67
Cr_0 + OA 20 mmol kg^{-1}		7.0	8.4	9.7	5.78	20.21	31.77
Cr_0 + HA 1g kg^{-1}		9.0	10.8	12.4	8.20	28.71	45.11
5 ppm Cr		6.0	7.2	8.3	5.22	18.27	28.71
5 ppm Cr + EDTA 10 mmol kg^{-1}		7.5	8.9	10.2	6.89	24.10	37.88
5 ppm Cr + DTPA 10 mmol kg^{-1}		8.2	8.9	10.3	7.11	24.89	39.11
5 ppm Cr + CA 20 mmol kg^{-1}		7.3	8.6	9.9	5.89	20.60	32.38
5 ppm Cr + OA 20 mmol kg^{-1}		6.4	7.6	8.8	5.55	19.44	30.54
5 ppm Cr + HA 1g kg^{-1}		9.0	9.8	11.3	7.89	27.60	43.38
10 ppm Cr		5.4	6.6	7.6	4.83	16.92	26.58
10 ppm Cr + EDTA 10 mmol kg^{-1}		6.8	8.1	9.3	6.38	22.32	35.07
10 ppm Cr + DTPA 10 mmol kg^{-1}		7.4	8.1	9.4	6.58	23.04	36.21
10 ppm Cr + CA 20 mmol kg^{-1}		6.6	7.8	9.0	5.45	19.08	29.98
10 ppm Cr + OA 20 mmol kg^{-1}		5.8	7.0	8.0	5.14	18.00	28.28
10 ppm Cr + HA 1g kg^{-1}		8.2	8.9	10.3	7.30	25.56	40.16
20 ppm Cr		5.0	6.0	6.9	4.54	15.89	24.97
20 ppm Cr + EDTA 10 mmol kg^{-1}		6.3	7.4	8.5	5.99	20.96	32.94
20 ppm Cr + DTPA 10 mmol kg^{-1}		6.8	7.5	8.6	6.18	21.64	34.00
20 ppm Cr + CA 20 mmol kg^{-1}		6.1	7.2	8.3	5.12	17.92	28.15
20 ppm Cr + OA 20 mmol kg^{-1}		5.3	6.4	7.3	4.83	16.90	26.56
20 ppm Cr + HA 1g kg^{-1}		7.5	8.2	9.4	6.86	24.00	37.72
30 ppm Cr		4.6	5.6	6.4	4.28	14.98	23.53
30 ppm Cr + EDTA 10 mmol kg^{-1}		5.8	6.8	7.8	5.64	19.76	31.05
30 ppm Cr + DTPA 10 mmol kg^{-1}		6.3	6.9	7.9	5.83	20.40	32.05
30 ppm Cr + CA 20 mmol kg^{-1}		5.6	6.6	7.6	4.83	16.89	26.54
30 ppm Cr + OA 20 mmol kg^{-1}		4.9	5.9	6.8	4.55	15.93	25.04
30 ppm Cr + HA 1g kg^{-1}		6.9	7.6	8.7	6.46	22.63	35.55
SEm±	Chromium	0.12	0.04	0.05	0.12	0.42	0.66
	Amendments	0.13	0.05	0.05	0.13	0.46	0.72
	Interaction	NS	NS	NS	NS	NS	NS
CD (P=0.01)	Chromium	0.33	0.12	0.14	0.34	1.19	1.87
	Amendments	0.36	0.13	0.15	0.37	1.30	2.05
	Interaction	NS	NS	NS	NS	NS	NS

Cr= Chromium, EDTA= ethylene diamine tetra acetic acid, DTPA= diethylene triamine penta acetic acid, OA= Oxalic Acid, CA= Citric Acid, HA= Humic Acid, CD = Critical Difference, SEm± = Standard error of mean

Effect on chlorophyll content (SPAD)

Chlorophyll content (SPAD) of both plant species varied significantly among different chelating agent with varying level of chromium (0, 5, 10, 20 and 30 ppm) in soil. Data on Chlorophyll content (SPAD) of plant of maize and mustard are given in the Table 4.

Chlorophyll content of maize plants were noted highest (44.50, 48.95 and 24.72) with Cr_0 C_5 (HA @ 1 g kg^{-1}) at 30 DAS, 60 DAS and at harvest respectively which was significantly higher over control Cr_4 C_0 (with no chelating agent + added chromium @ 30ppm) showed lowest SPAD (28.25, 31.07 and 15.69) at 30 DAS, 60 DAS and at harvest respectively. Followed by Cr_1 C_5 (Cr @ 5 ppm + HA @ 1 g kg^{-1}), Cr_2 C_5 (Cr @ 10 ppm + HA @ 1 g kg^{-1}), Cr_3 C_5 (Cr @ 20 ppm + HA @ 1 g kg^{-1}), and Cr_2 C_5 (Cr @ 30 ppm + HA @ 1 g kg^{-1}) etc.

Chlorophyll content of mustard plants were recorded highest (34.76, 53.87 and 39.49) with Cr_0 C_5 (HA @ 1 g kg^{-1}) at 30 DAS, 60 DAS and at harvest respectively which was significantly higher over control Cr_4 C_0 (with no chelating agent + added chromium @ 30ppm) showed lowest SPAD (17.92, 27.78 and 20.36) at 30 DAS, 60 DAS and at harvest respectively. Followed by Cr_1 C_5 (Cr @ 5 ppm + HA @ 1 g kg^{-1}), Cr_2 C_5 (Cr @ 10 ppm + HA @ 1 g kg^{-1}), Cr_3 C_5 (Cr @ 20 ppm + HA @ 1 g kg^{-1}), and Cr_2 C_5 (Cr @ 30 ppm + HA @ 1 g kg^{-1}) etc.

Chlorophyll content of both maize and mustard initially increase and then showing a decreasing trend toward harvesting as crop approaches maturity. Comparison among both plant species at all stages of growth (30 DAS, 60 DAS and at harvesting) the high SPAD (48.95 and 53.87) was observed in pot treated with Humic acid @ 1 g kg^{-1} (Cr_0 C_5) in maize and mustard respectively at 60 DAS. However, low SPAD (15.69 and 17.92) at 30 DAS and at harvesting respectively was observed in control pot (at 30 ppm level of Cr contamination) with no chelating agent. Furthermore, there is decrease in chlorophyll content with increasing level of chromium at all growth stages.

At all stages of growth at all level of chromium from 0 ppm to 30 ppm with humic acid as chelating agent gives higher SPAD value followed by treatment with DTPA (@10 mMole kg^{-1}) and EDTA (@10 mMole kg^{-1}) and were significantly higher over control with no chelating agent along different level of Cr contamination (0, 5, 10, 20 and 30ppm). While citric and oxalic acid have less influence on chlorophyll content of both the plant species and found at par among each other. However, treatment with no chelating agent (Cr_1 C_0, Cr_2 C_0, Cr_3 C_0, and Cr_4 C_0) gives lower SPAD at all growth stages. Farid *et al.*, (2017), depicted a clear decline in plant height, root length, leaf area, number of leaves and flowers per plant along with fresh and dry biomass of all parts of plant with increasing concentration of Cr in soil. Afsan *et al.* (2015), studied that the plant growth, biomass, chlorophyll contents, and carotenoid as well as soluble protein concentrations significantly decreased under Cr stress alone while these adverse effects were alleviated by application of CA due to biodegradable nature. While Luo *et al.* (2006) [28] observed much higher chlorophyll content and biomass with EDTA over LMWOA. Cr directly affect the biosynthesis of chlorophylls and photosynthetic pigments which result in reduced gas exchange activities and carbon assimilation (Mathur *et al.*, 2016) [29]

Table 4: Effect of chelating compounds on chlorophyll content (SPAD) of maize and mustard in chromium contaminated soil

Treatment		Maize			Mustard		
		30 DAS	**60 DAS**	**At Harvest**	**30 DAS**	**60 DAS**	**At Harvest**
Control		33.81	37.19	18.78	23.52	36.46	26.73
Cr_0 + EDTA 10 mmol kg^{-1}		42.07	46.28	23.37	31.62	49.01	35.93
Cr_0 + DTPA 10 mmol kg^{-1}		43.54	47.89	24.19	34.50	53.47	39.20
Cr_0 + CA 20 mmol kg^{-1}		39.38	43.31	21.88	28.60	44.32	32.49
Cr_0 + OA 20 mmol kg^{-1}		35.91	39.50	19.95	25.33	39.26	28.78
Cr_0 + HA 1g kg^{-1}		44.50	48.95	24.72	34.76	53.87	39.49
5 ppm Cr		32.20	35.42	17.89	22.40	34.72	25.45
5 ppm Cr + EDTA 10 mmol kg^{-1}		40.07	44.07	22.26	30.11	46.67	34.22
5 ppm Cr + DTPA 10 mmol kg^{-1}		41.47	45.61	23.04	32.86	50.93	37.34
5 ppm Cr + CA 20 mmol kg^{-1}		37.50	41.25	20.83	27.23	42.21	30.95
5 ppm Cr + OA 20 mmol kg^{-1}		34.20	37.62	19.00	24.12	37.39	27.41
5 ppm Cr + HA 1g kg^{-1}		42.40	46.64	23.56	33.10	51.31	37.61
10 ppm Cr		30.67	33.73	17.04	21.33	33.07	24.24
10 ppm Cr + EDTA 10 mmol kg^{-1}		38.16	41.97	21.20	28.68	44.45	32.59
10 ppm Cr + DTPA 10 mmol kg^{-1}		39.49	43.44	21.94	31.29	48.50	35.56
10 ppm Cr + CA 20 mmol kg^{-1}		35.71	39.29	19.84	25.94	40.20	29.47
10 ppm Cr + OA 20 mmol kg^{-1}		32.57	35.83	18.10	22.97	35.61	26.11
10 ppm Cr + HA 1g kg^{-1}		40.38	44.42	22.43	31.52	48.86	35.82
20 ppm Cr		29.27	32.20	16.26	19.82	30.73	22.53
20 ppm Cr + EDTA 10 mmol kg^{-1}		36.42	40.07	20.24	26.65	41.30	30.28
20 ppm Cr + DTPA 10 mmol kg^{-1}		37.70	41.47	20.94	29.08	45.07	33.04
20 ppm Cr + CA 20 mmol kg^{-1}		34.09	37.50	18.94	24.10	37.36	27.39
20 ppm Cr + OA 20 mmol kg^{-1}		31.09	34.20	17.27	21.35	33.09	24.26
20 ppm Cr + HA 1g kg^{-1}		38.55	42.40	21.41	29.29	45.40	33.29
30 ppm Cr		28.25	31.07	15.69	17.92	27.78	20.36
30 ppm Cr + EDTA 10 mmol kg^{-1}		35.15	38.66	19.53	24.09	37.34	27.37
30 ppm Cr + DTPA 10 mmol kg^{-1}		36.37	40.01	20.21	26.28	40.74	29.87
30 ppm Cr + CA 20 mmol kg^{-1}		32.89	36.18	18.27	21.79	33.77	24.76
30 ppm Cr + OA 20 mmol kg^{-1}		30.00	33.00	16.67	19.30	29.91	21.93
30 ppm Cr + HA 1g kg^{-1}		37.19	40.91	20.66	26.48	41.04	30.09
SEm±	Chromium	0.24	0.26	0.13	0.20	0.30	0.22
	Amendments	0.26	0.29	0.14	0.21	0.33	0.24
	Interaction	NS	NS	NS	NS	NS	NS
CD (P=0.01)	Chromium	0.67	0.74	0.37	0.55	0.86	0.63
	Amendments	0.73	0.81	0.41	0.61	0.94	0.69
	Interaction	NS	NS	NS	NS	NS	NS

Cr= Chromium, EDTA= ethylene diamine tetra acetic acid, DTPA= diethylene triamine penta acetic acid, OA= Oxalic Acid, CA= Citric Acid, HA= Humic Acid, CD = Critical Difference, SEm± = Standard error of mean

Effect on dry matter accumulation

The data obtained in relation to dry matter accumulation by maize and mustard crop influenced by different levels of chromium with organic and synthetic chelates presented in the Table 5.

In maize maximum plant dry weight (89, 185.12 and 233.18 g plant^{-1}) was recorded with Cr_0 C_5 (Humic acid @ 1 g kg^{-1}) at 30 DAS, 60 DAS and at harvest respectively which was significantly higher over control Cr_4 C_0 (with no chelating agent) with added chromium @ 30ppm showed minimum plant dry weight (50, 104.91 and 132.1 g plant^{-1}) at 30 DAS, 60 DAS and at harvest respectively. Followed by Cr_1 C_5 (Cr @ 5 ppm + HA @ 1 g kg^{-1}), Cr_2 C_5 (Cr @ 10 ppm + HA @ 1 g kg^{-1}), Cr_3 C_5 (Cr @ 20 ppm + HA @ 1 g kg^{-1}), and Cr_2 C_5 (Cr @ 30 ppm + HA @ 1 g kg^{-1}) etc.

In mustard maximum plant dry weight (5.32, 9.45 and 17.02 g plant^{-1}) was recorded with Cr_0 C_5 (Humic acid @ 1 g kg^{-1}) at 30 DAS, 60 DAS and at harvest respectively which was significantly higher over control Cr_4 C_0 (with no chelating agent + chromium @ 30ppm showed minimum plant dry weight (1.85, 3.28 and 5.91 g plant^{-1}) at 30 DAS, 60 DAS and at harvest respectively. Followed by Cr_1 C_5 (Cr @ 5 ppm + HA @ 1 g kg^{-1}), Cr_2 C_5 (Cr @ 10 ppm + HA @ 1 g kg^{-1}), Cr_3 C_5 (Cr @ 20 ppm + HA @ 1 g kg^{-1}), and Cr_2 C_5 (Cr @ 30 ppm + HA @ 1 g kg^{-1}) etc.

At all stages of growth at all level of chromium from 0 ppm to 30 ppm with humic acid as chelating agent produced maximum dry matter (g plant^{-1}) followed by treatment with DTPA (@10 mMole kg^{-1}) and EDTA (@10 mMole kg^{-1}) and were significantly higher control no chelating agent with different level of Cr contamination (0, 5, 10, 20 and 30ppm). However, treatment with no chelating agent (Cr_1 C_0, Cr_2 C_0, Cr_3 C_0, and Cr_4 C_0) produced minimum dry matter (g plant^{-1}) at all growth stages.

Comparison among both plant species at all stages of growth (30 DAS, 60 DAS and at harvesting) high dry matter accumulation (233.18 and 17.02) was observed in pot treated with Humic acid @ 1 g kg^{-1} (Cr_0 C_5) in maize and mustard respectively at harvesting stage. However, low dry matter (50.44 and 1.85) at 30 DAS was observed in control pot (at 30 ppm level of Cr contamination) with no chelating agent in mustard and maize respectively. Furthermore, there is decrease in dry matter accumulation with increasing level of chromium at all growth stages.

While citric and oxalic acid were less effective in increasing dry matter (g plant^{-1}) of both the plant species but Humic acid, DTPA and EDTA have significant influences on dry matter (g plant^{-1}). Application of OC (organic chelates) to heavy metal contaminated soils significantly decreased dry matter yield of both species, and plants showed significant decrease when OCA addition levels were higher than 2.5 mMol kg^{-1} (Turan and Angin, 2004) [40]. However, treatment with high molecular weight organic and synthetic compound increased dry matter production (Afshan *et al.*, 2015) [1].

Table 5: Effect of chelating compounds on dry matter accumulation (g plant^{-1}) of maize and mustard in chromium contaminated soil

Treatment		Maize			Mustard		
		30 DAS	60 DAS	At Harvest	30 DAS	60 DAS	At Harvest
Control		64.94	135.08	170.15	2.49	4.43	7.98
Cr_0 + EDTA 10 mmol kg^{-1}		79.02	164.35	207.02	3.91	6.95	12.51
Cr_0 + DTPA 10 mmol kg^{-1}		83.27	173.20	218.17	4.58	8.15	14.66
Cr_0 + CA 20 mmol kg^{-1}		74.10	154.13	194.15	3.36	5.98	10.76
Cr_0 + OA 20 mmol kg^{-1}		69.93	145.46	183.23	3.08	5.47	9.85
Cr_0 + HA 1g kg^{-1}		89.00	185.12	233.18	5.32	9.45	17.02
5 ppm Cr		63.05	131.14	165.19	2.35	4.18	7.53
5 ppm Cr + EDTA 10 mmol kg^{-1}		76.71	159.57	200.99	3.69	6.56	11.80
5 ppm Cr + DTPA 10 mmol kg^{-1}		80.84	168.16	211.81	4.32	7.68	13.83
5 ppm Cr + CA 20 mmol kg^{-1}		71.94	149.65	188.50	3.17	5.64	10.15
5 ppm Cr + OA 20 mmol kg^{-1}		67.90	141.22	177.89	2.90	5.16	9.30
5 ppm Cr + HA 1g kg^{-1}		86.41	179.73	226.39	5.02	8.92	16.06
10 ppm Cr		60.62	126.10	158.84	2.17	3.86	6.94
10 ppm Cr + EDTA 10 mmol kg^{-1}		73.76	153.43	193.26	3.40	6.04	10.88
10 ppm Cr + DTPA 10 mmol kg^{-1}		77.73	161.69	203.67	3.98	7.08	12.75
10 ppm Cr + CA 20 mmol kg^{-1}		69.18	143.89	181.25	2.92	5.20	9.35
10 ppm Cr + OA 20 mmol kg^{-1}		65.29	135.79	171.05	2.68	4.76	8.57
10 ppm Cr + HA 1g kg^{-1}		83.08	172.82	217.68	4.62	8.22	14.80
20 ppm Cr		56.29	117.09	147.49	2.00	3.55	6.39
20 ppm Cr + EDTA 10 mmol kg^{-1}		68.50	142.47	179.46	3.13	5.56	10.01
20 ppm Cr + DTPA 10 mmol kg^{-1}		72.18	150.14	189.12	3.67	6.52	11.73
20 ppm Cr + CA 20 mmol kg^{-1}		64.24	133.61	168.30	2.69	4.78	8.60
20 ppm Cr + OA 20 mmol kg^{-1}		60.62	126.09	158.83	2.46	4.38	7.88
20 ppm Cr + HA 1g kg^{-1}		77.15	160.47	202.13	4.25	7.56	13.62
30 ppm Cr		50.44	104.91	132.15	1.85	3.28	5.91
30 ppm Cr + EDTA 10 mmol kg^{-1}		61.37	127.65	160.79	2.90	5.15	9.26
30 ppm Cr + DTPA 10 mmol kg^{-1}		64.68	134.52	169.45	3.39	6.03	10.86
30 ppm Cr + CA 20 mmol kg^{-1}		57.56	119.72	150.80	2.49	4.43	7.97
30 ppm Cr + OA 20 mmol kg^{-1}		54.32	112.98	142.31	2.28	4.05	7.30
30 ppm Cr + HA 1g kg^{-1}		69.13	143.78	181.11	3.94	7.00	12.61
SEm±	Chromium	0.48	0.99	1.25	0.04	0.07	0.13
	Amendments	0.52	1.09	1.37	0.04	0.08	0.14
	Interaction	NS	NS	NS	NS	NS	NS
CD (P=0.01)	Chromium	1.35	2.81	3.54	0.11	0.20	0.37
	Amendments	1.48	3.08	3.87	0.13	0.22	0.40
	Interaction	NS	NS	NS	NS	NS	NS

Cr= Chromium, EDTA= ethylene diamine tetra acetic acid, DTPA= diethylene triamine penta acetic acid, OA= Oxalic Acid, CA= Citric Acid, HA= Humic Acid, CD = Critical Difference, SEm± = Standard error of mean

Conclusion

The growth parameters (plant height and number of leaves) at all stages of growth were significantly decreased with increasing rate of chromium contamination by the application of humic acid. Among the organic chelating compounds the highest values were recorded with treatment Cr_0C_5 (0 ppm Cr + 1 g kg^{-1} HA) and treatment with DTPA, Cr_0C_2 (0 ppm Cr + 10 mMole kg^{-1}) among synthetic chelating compounds in maize. Except at harvest number of leaves was highest in mustard with same treatment. However, at every level of contamination treatments with humic acid (1 g kg^{-1}) performed supremely followed by treatment with DTPA (10 mMole kg^{-1}).

At all stages of growth dry matter production and chlorophyll content were significantly decreased with increasing rate of chromium contamination with the application of humic acid. At every level of contamination treatments with humic acid (1 g kg^{-1}) gave batter results followed by treatment with DTPA (10 mMole kg^{-1}). Among the organic chelating compounds the highest DM were recorded with treatment Cr_0C_5 (0 ppm Cr + 1 g kg^{-1}HA) and treatment with DTPA, Cr_0C_2 (0 ppm Cr + 10 mMole kg^{-1}) among synthetic chelating compounds in maize. Lowest DM production was recorded with Cr_4C_0 (30 ppm Cr with no chelating agent)

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