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Organic amendments influence mustard (*Brassica juncea*) growth in chromium contaminated soils

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Abstract
Chromium is considered a serious environmental pollutant owing to its wide industrial use. Contamination of soil and water by chromium is of recent concern. Keeping these points in view an attempt was made to assess the chromium toxicity in the environment, especially in soil and provides new comprehensions about chromium toxicity in plants. Two sequential pot experiments were conducted in net house of Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during 2015-16 and 2016-17 to evaluate the effect of organic amendments on growth parameters (viz. Germination, Chlorophyll content, Plant height, number of leaves per plant and number of branches per plant) of mustard in chromium contaminated soils. Five levels of chromium viz. 0, 20, 40, 60 and 80 ppm with and without three organic amendments viz. Vermicompost @ 5 ton ha^{-1}, Farm Yard Manure @ 10 ton ha^{-1} and Sewage Sludge @ 20 ton ha^{-1} were taken in alluvial soil of Research Farm of IAS, BHU. Result showed that growth parameters (germination percentage, chlorophyll content (SPAD), plant height, number of leaves per plant and number of branches per plant) significantly decreased as level of chromium increased. Result also revealed that application of organic amendments significantly increased these growth parameters as compare to their respective chromium treatment. Maximum and significantly higher increment in growth parameters was found by the application of vermicompost @ 5 ton ha^{-1} followed by farm yard manure @ 10 ton ha^{-1}, followed by sewage sludge @ 20 ton ha^{-1}.

Keywords: chromium, contamination, growth, mustard and organic amendments

Introduction
Accumulation of non-essential elements in agricultural soils has mainly resulted due to anthropogenic activities (Nagajyoti *et al*., 2010; Adrees *et al*., 2015; Gill *et al*., 2015; Murtaza *et al*., 2015) [61, 3, 34, 16]. Among non-essential elements, chromium is of severe concern due to its toxic effects on plants and humans (Rehman *et al*., 2017) [70]. Chromium (Cr) is the 7th most abundant element in the Earth and the 21st on the Earth's crust (Ertani *et al*., 2017). It occurs naturally as chromite ($FeCr_2O_4$) in ultramafic and serpentine rocks or complexed with other metals like crocoite ($PbCrO_4$), bentorite Ca_6 (Cr, Al)$_2$ $(SO_4)_3$ and tarapacaite (K_2CrO_4), vauquelinite ($CuPb_2CrO_4PO_4OH$), among others (Babula *et al*., 2008). In the past decades, the increased use of chromium (Cr) in several anthropic activities (Gu *et al*., 2012; Wang *et al*., 2013a; Qiu *et al*., 2014) [36, 7, 68] caused soil and water contamination (Ertani *et al*., 2017). 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) [22]. 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). In the last few decades, Cr pollution has drastically increased. As a result, Cr toxicity is now a major threat to agricultural land and water bodies (Choudhary *et al*., 2012) [23]. Unrestrained discharge of Cr(VI) containing industrial effluents has pernicious effects on crops at concentration of 5–100 mg kg^{-1} within soil (Arshad *et al*., 2017) [15]. Chromium (VI) is toxic to agronomic plants at concentration of 0.5 to 5.0 mg mL^{-1} in nutrient solution and 5–100 mg kg^{-1} in soil (Ali *et al*., 2013; Chrysochoou *et al*., 2012) [24, 7]. Excess Cr contamination in soil and water results in accumulation of this toxic element in plants and subsequently into the food chain (Wang *et al*., 2013b). Chromium exists in several oxidation states (-2 to +6), but hexavalent chromate [Cr (VI)] and trivalent chromite [Cr (III)] forms are the most common and stable in the natural environment (Ashraf *et al*., 2017) [16]. Cr(VI) is considered to be more available due to its solubility, strong oxidizing properties and permeability through cellular membranes (Hu *et al*., 2016).

The mobility and toxicity of Cr^{6+} can be reduced by converting it to the reduced state of Cr^{3+} by means of organic matter and inorganic reducing agents in the, soil (Aceves *et al*., 2007) [2]. These Organic sources may be organic manures, green manure, rural wastes, crop residues, biofertilizers and vermicompost. The positive effect of vermicompost application on crop growth, yield and soil properties is well documented and established (Kumar *et al*., 2017a; Kumar *et al*., 2017b; Kumar *et al*., 2018) [49, 50, 51].

Several organic amendments can improve phytostabilization and the production of plant by decreasing the solubility, leaching and bioavailability of trace elements (Mench *et al*., 2010; Angelova *et al*., 2013) [58, 13]. The immobilizing effect of such amendments are thought to act through various complex processes *e.g.* formation of stable compounds with organic ligands, surface precipitation and ion exchange (Kumpiene *et al*., 2008; Ahmad *et al*., 2011a) [4, 52]. Organic amendments may enhance the soil fertility and microbial activity, leading to the amelioration of the soil quality as a whole. These overall modifications generally decrease the mobility and the bioavailability of trace elements, even if temporarily and thus promote the reestablishment of vegetation and increase plant growth (Branzini and Zubillaga, 2012) [20]. 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) [13]. However very few comparative studies have been performed so far and the choice of a particular organic amendment in assisted phytostabilization strategies often remain empirical (Hattab *et al*., 2015) [37]. Immobilization of metals in contaminated soils using amendments is a remediation technique that decreases mobility and phytoavailability of metals in the soils and their uptake by plants (Sabir *et al*., 2013; Rizwan *et al*., 2016; Rehman *et al*., 2017) [70, 73, 1].

In the present study, we investigated effect of sewage sludge (Sl), farmyard manure (FYM) and vermicompost (VC) applied at different levels on growth parameters in chromium contaminated soils.

Material and Methods

Pot experiment was conducted in the net house of the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during 2015-16 and 2016-17 to assess the effect of organic amendments on mustard growth in chromium contaminated soils. Bulk soil was collected from the Agricultural Research Farm of Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. For the analysis of initial soil physico-chemical properties soil was air dried gently and ground to pass through a 2 mm sieve, and results are depicted in table 1. Five levels of chromium viz. 0, 20, 40, 60 and 80 ppm with and without three organic amendments viz. vermicompost (VC) @ 5 ton ha^{-1}, farm yard manure (FYM) @ 10 ton ha^{-1} and sewage sludge (Sl) @ 20 ton ha^{-1} were taken in bulk soil samples and an aqueous solution of chromium ($K_2Cr_2O_7$) was added to soil and incubated in the net house for one month to maintain equilibrium before sowing. The treatments consists of T_1-Control, T_2-20 ppm Cr, T_3-40 ppm Cr, T_4-60 ppm Cr, T_5-80 ppm Cr, T_6- 0 ppm Cr + Sl, T_7-20 ppm Cr + Sl, T_8-40 ppm Cr + Sl, T_9-60 ppm Cr + Sl, T_{10}-80 ppm Cr + Sl, T_{11}- 0 ppm Cr + FYM, T_{12}-20 ppm Cr + FYM, T_{13}-40 ppm Cr + FYM, T_{14}-60 ppm Cr + FYM, T_{15}-80 ppm Cr + FYM, T_{16}- 0 ppm Cr + VC, T_{17}-20 ppm Cr + VC, T_{18}-40 ppm Cr + VC, T_{19}-60 ppm Cr + VC and T_{20}-80 ppm Cr + VC.

The number of seeds germinated in each treatment was counted after 7 days of sowing and germination percentage was calculated using the following formula:

$$\text{Germination percentage} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \text{X } 100$$

Plant height of the mustard was recorded with the help of meter scale from ground level to the tip of uppermost leaf of the plant before flowering and up to the tip of the flower after flowering and average height is calculated in centimeter. The numbers of leaves per plant, numbers of branches and Chlorophyll content (SPAD) were recorded at 30, 60, 90 DAS and at harvest from each pot and average numbers of leaves were calculated on per plant basis. For determining the significance between the treatment means and to draw valid conclusion, statistical analysis was made. The difference of the treatments mean was tested using critical difference (CD) at 1% level of probability by following the Complete Randomized Design (CRD) to draw the valid differences among the treatments.

Table 1: Physical and chemical properties of the initial soil, vermicompost, sewage sludge and farm yard manure

	2015-2016				2016-2017			
Parameter	**Initial Soil**	**Sewage Sludge**	**FYM**	**Vermicompost**	**Initial Soil**	**Sewage Sludge**	**FYM**	**Vermicompost**
pH_w (1:2.5)	7.94	6.43	6.54	7.07	7.86	6.55	6.74	7.17
EC_w (1:2.5) (dS/m)	0.11	2.32	3.48	6.06	0.13	2.48	3.73	6.18
Organic Carbon (%)	0.46	8.27	9.87	10.47	0.43	8.65	9.63	11.26
Available Nutrient Content (mg kg^{-1})								
N	72	1182	470	533	70	1195	476	537
P	12	1300	510	610	15	1210	490	550
K	100	4300	3300	1270	104	4322	3288	1258
S	14	56	24	410	15	54	28	415
Total Nutrient Content (%)								
N	-	1.63	0.45	1.78	-	1.54	0.48	1.88
P	-	1.18	0.22	0.95	-	1.26	0.24	1.04
K	-	0.73	0.41	1.66	-	0.84	0.46	1.82
S	-	0.91	0.02	0.38	-	0.98	0.03	0.42
DTPA Extractable metals (mg kg^{-1})								
Cr	0.48	11.62	1.37	1.56	0.39	9.94	1.31	1.43
Fe	39.55	73.35	112.42	154.63	42.65	70.65	118.53	159.42
Mn	13.66	28.82	83.60	110.88	14.96	31.21	80.21	116.22
Cu	1.92	21.26	7.67	11.24	1.74	23.08	6.83	13.16
Zn	1.18	27.64	12.68	18.12	1.38	25.14	14.22	16.12

Result and Discussion

Germination

Data pertaining to germination of mustard presented in table 2 indicated significantly different values of germination with application of organic amendments in chromium contaminated soils during both the years. Germination of mustard ranged from 88.87% to 56.67% and 88.27% to 56.31% in the year 2015-16 and 2016-17, respectively. Maximum germination 88.87% and 88.27% in 2015-16 and 2016-17, respectively was observed with the application of 20 ppm chromium + vermicompost @ 5 ton ha^{-1} (T_{17}) whereas the minimum germination 56.67% and 56.31% in 2015-16 and 2016-17, respectively was found with the application of 80 ppm chromium (T_5). 20 ppm chromium + vermicompost @ 5 ton ha^{-1} (T_{17}) found to be significantly higher with all other treatments except that of farm yard manure @ 10 ton ha^{-1} (t_{11}) and 20 ppm chromium + farm yard manure @ 10 ton ha^{-1} (T_{12}). Application of farm yard manure was on par with vermicompost at same level of chromium. Lower concentration of chromium had slightly stimulatory effect on germination of mustard but at higher concentration germination of mustard significantly decreased as dose of chromium increased. Lakshmi and Sundaramoorthy (2010) revealed that the germination enhanced at lower concentrations (2 and 5 ppm) of chromium. At and above 10 ppm chromium the germination never reached the level of control.

However, application of 20 ppm chromium + farm yard manure @ 10 ton ha^{-1} (T_{12}) was found next better treatment in germination (86.07% and 85.47% in 2015-16 and 2016-17) which remains on par with treatment T_{17} (20 ppm chromium + vermicompost @ 5 ton ha^{-1}). Furthermore, application of 20 ppm chromium + sewage sludge @ 20 ton ha^{-1} (T_7) recorded the significantly higher germination 83.67% and 83.29% in 2015-16 and 2016-17, respectively over rest of treatments. Among all the treatments the lowest germination percentage 56.67% and 56.31% in was observed in treatment T_5 (80 ppm chromium) in both the years. Delayed germination was also observed at and above 40 ppm chromium in all treatments. Fei *et al*. (2017) reported that Cr(VI) contamination greatly inhibited seed germination and root development of mustard. The germination rate of the selected seeds decreased with increasing Cr(VI) contamination. Farid *et al.* (2017) [31] reported that chromium application reduced seed germination of six cultivars of sunflower as compared to control treatment. The germination gradually decreased with increasing concentration of Cr. Malik *et al.* (2017) [8] found that germination percentage of wheat and barley decreased with the increasing concentrations of Cd and Ni in wheat and barley. Soni *et al.* (2016) found that no germination was recorded at 0.4 - 1.6 mM concentrations of chromium in *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia*. Kumar *et al.* (2016) stated that symptoms of Cr toxicity in plants are diverse and include decrease of seed germination. Jahan *et al.* (2015) [43] reported that chromium under 40 and 160 ppm concentrations was responsible for significant decline in germination parameters i.e. germination percentage, germination rate, seedling vigor index, shoot and root length, fresh weight and dry weight of seedlings in *Brassica napus* L. Nagarajan and Ganesh (2015) [62] found that 200 mg/L chromium concentration reduced the seed germination up to 50 to 60 per cent over control. No germination was recorded beyond 200 mg/L concentration of chromium. Amin *et al.* (2014) [11] found that high concentrations of chromium (50 and 100 mg kg^{-1}) can completely inhibit the seed germination. Datta *et al.* (2011) [26] observed that gradual increases in Cr (VI) concentration under various treatments significantly lead to inhibition of seed germination. Akinci and Akinci (2010) [6] showed that increase concentrations of chromium inhibited seed germination. Andaleeb *et al*. (2008) [12] showed that germination decreased with increase in Cr concentrations.

Chromium-induced toxicity to seed germination can be due to suppression in the activities of α and β amylase under Cr stress. Amylase hydrolysis of starch is vital for sugar supply to emergent embryos. Chromium toxicity reduces sugar availability to developing embryo and in turns decreases amylase activity, thereby inhibiting seed germination (Shahid *et al*., 2017) [14]. Arshad *et al*. (2017) [15] stated that at higher Cr(VI) concentration, reduction in germination percentage of wheat is linked with reduced α and β amylase activities. Amylase mediated hydrolysis of starch is essential for sugar supply to developing embryos. Decrease in amylase activity under Cr treatment decreases sugar availability to developing embryos which leads to inhibition of seed germination, and extended dormancy (Dey *et al*., 2009) [27]. Decline in seed germination under chromium stress could be because of harmful consequences of chromium on amylase action and result in the accumulation of sugars in the embryo axis. Similarly chromium is also involved in the enhancement of protease activity resulting in decline in proteins which are essential for germination leading to slow or loss of germination (Jahan *et al.*, 2015) [43]. The reduction in germination percentage, seedling length and dry weight of paddy seedlings at higher chromium concentrations may be attributed to the interference of metal ions, which may inhibit seed germination by exerting unfavorable effect on the activities of hydrolytic enzymes involved in the mobilization of major seed reservoirs such as starch, protein, RNA and phytin. The reduced germination of seeds under Cr stress would be due to the depressive effect of Cr on the subsequent transport of sugars to the embryo axis. Protease activity increases simultaneously with the chromium treatment which could also contribute to the reduction in germination of chromium treated seeds (Datta *et al*., 2011) [26]. Lower level of α and β amylase in maize plants treated with Cr was also reported by Islam *et al.* (2016) [39] and they admitted that it might be due to potential displacement of Ca, resulting in disparting the Ca from α and β amylase or changing its steric configuration. Jadia and Fulekar (2009) [42] reported that percentage of seed germination decreased as metal concentration increased in soil-vermicompost media. Delayed germination was also observed in all cases at higher i.e. 40 and 50 ppm concentrations.

From the results of germination it may also be described that application of organic amendments significantly increased the germination percentage of mustard as compared to their respective chromium treatment. Maximum increment in germination percentage of mustard was found with the application of 20 ppm chromium + vermicompost @ 5 ton ha^{-1} (T_{17}) followed by 20 ppm chromium + farm yard manure @ 10 ton ha^{-1} (T_{12}), followed by 20 ppm chromium + sewage sludge @ 20 ton ha^{-1} (T_7). Germination percentage with the application of vermicompost @ 5 ton ha^{-1}, farm yard manure @ 10 ton ha^{-1} and sewage sludge @ 20 ton ha^{-1} were on par at same level of chromium except that of application of 20 ppm chromium + vermicompost @ 5 ton ha^{-1} (T_{17}) and 80 ppm chromium + vermicompost @ 5 ton ha^{-1} (T_{20}) were significantly higher with the 20 ppm chromium + sewage sludge @ 20 ton ha^{-1} (T_7) and 80 ppm chromium + sewage sludge @ 20 ton ha^{-1} (T_{10}) in both the year. While application of vermicompost found to be superior over farm yard manure and sewage sludge in both the year where farm yard manure was next to vermicompost followed by sewage sludge.

Jadia and Fulekar (2008) [41] also observed higher seed germination in control treatment where only soil-vermicompost was used. Delayed germination was also observed with all metals above 40 ppm concentration. As an important developmental stage in the plant life cycle, the seed is highly protected against and sensitive to environmental stresses. Seed germination is amongst the most important physiological processes which would be indicative of a crop to tolerate a stress and germinate in the presence of that stress such as the ability of a crop plant to germinate in the presence of chromium would state its tolerance to chromium stress (Jahan *et al.* 2015) [43]. Since seed germination is the first physiological process affected by chromium, the ability of a seed to germinate in a medium containing chromium would be an indicative of its level of tolerance to this metal (Nagarajan and Ganesh 2015) [62].

Table 2: Effect of organic amendments on germination (%) of mustard in chromium contaminated soils

Treatment	2015-2016	2016-2017
T_1-Control	76.27	75.87
T_2-20 ppm Cr	77.87	77.09
T_3-40 ppm Cr	71.67	71.47
T_4-60 ppm Cr	65.67	64.27
T_5-80 ppm Cr	56.67	56.31
T_6- 0 ppm Cr + Sl	82.27	81.87
T_7-20 ppm Cr + Sl	83.67	83.29
T_8-40 ppm Cr + Sl	77.27	76.47
T_9-60 ppm Cr + Sl	71.27	70.67
T_{10}-80 ppm Cr + Sl	61.67	60.87
T_{11}- 0 ppm Cr + FYM	83.47	83.07
T_{12}-20 ppm Cr + FYM	86.07	85.47
T_{13}-40 ppm Cr + FYM	78.47	77.66
T_{14}-60 ppm Cr + FYM	73.67	73.07
T_{15}-80 ppm Cr + FYM	63.27	62.67
T_{16}- 0 ppm Cr + VC	85.47	84.87
T_{17}-20 ppm Cr + VC	88.87	88.27
T_{18}-40 ppm Cr + VC	80.07	79.31
T_{19}-60 ppm Cr + VC	74.87	75.07
T_{20}-80 ppm Cr + VC	66.67	66.87
SEm±	1.21	1.10
CD (P=0.01)	4.64	4.20

Cr = Chromium, Sl = Sewage Sludge, FYM = Farm Yard Manure, VC = Vermicompost,
CD = Critical Difference, SEm± = Standard error of mean

Chlorophyll content (SPAD)

The data of effects of organic amendments on chlorophyll content (SPAD) of mustard leaves in chromium contaminated soils are presented in table 3 exhibited significant differences in chlorophyll content (SPAD) of mustard leaves by the application of organic amendments in Cr contaminated soil during both the years. During 2015-16 chlorophyll content (SPAD) of mustard leaves ranged from 37.14 to 24.12, 45.47 to 29.70, 51.40 to 37.90 and 39.52 to 31.67 at 30, 60, 90 DAS and at harvest, respectively while during 2016-17 it ranged from 38.75 to 28.26, 46.57 to 30.00, 49.90 to 38.60 and 39.92 to 32.40 at 30, 60, 90 DAS and at harvest, respectively. It was also observed that chlorophyll content (SPAD) of mustard leaves increased from 30 DAS up to 90 DAS but at harvest it decreased during both the years. It was found that the application of vermicompost @ 5 ton ha^{-1} (T_{16}) recorded significantly highest chlorophyll content (SPAD) of mustard leaves 37.14 and 38.75, 45.47 and 46.57, 51.40 and 49.90, 39.52 and 39.92 at 30, 60, 90 DAS, at harvest in 2015-16 and 2016-17, respectively over remaining treatments. However, application of farm yard manure @ 10 ton ha^{-1} (T_{11}) was found next better treatment in chlorophyll content (SPAD) of mustard leaves in both the years. Furthermore, application of sewage sludge @ 20 ton ha^{-1} (T_6) recorded the higher chlorophyll content (SPAD) of mustard leaves in both the years over rest of treatments. The lowest chlorophyll content (SPAD) of mustard leaves was observed in treatment T_5 (80 ppm Chromium). Application of sewage sludge @ 20 ton ha^{-1} remains on par with farm yard manure as well as application of farm yard manure @ 10 ton ha^{-1} remains on par with vermicompost at all the growth stages and at the same level of chromium.

Further study of data demonstrated that chlorophyll content (SPAD) of mustard leaves significantly decreased as levels of chromium increased. However 20 ppm chromium was on par with their respective control in all the set of treatments. Farid *et al.* (2017) [31] observed that chlorophyll (a, b and total chlorophyll) and soil plant analysis development (SPAD) content significantly decreased in sunflower under increasing Cr concentration in soil. Mahmud *et al.* (2017) [55] found that chromium stress reduced chlorophyll (chl) content. Chlorophyll (a+b) as well as total chl content decreased gradually with the increase in Cr stress levels. Islam *et al.* (2016) [39] observed that exposure of maize plants to chromium contamination significantly decreased chlorophyll content compared to non-contaminated control. Kamran *et al.* (2016) [45] observed that chlorophyll contents decreased as Cr toxicity increased in *E. sativa* plants. Sheetal *et al.* (2016) [77] revealed that as the contamination level of nickel and chromium increased significant reductions were observed in chlorophyll a and b contents as compared to control plants. Islam *et al.* (2014b) [40] found that Cr stress significantly decreased chlorophyll content, chlorophyll florescence (Fv/Fm) and photosynthetic rate (Pn). Tandon and Vikram (2014) [80] reported that chlorophyll a, b and carotenoids contents were found to be decreased at increasing doses of chromium. Terzi and Yildiz (2014) [81] observed that chlorophyll content decreased significantly in canola cultivars with increasing Cr(VI) concentrations. Amin *et al.* (2014) [11] presented that photosynthetic pigment chlorophyll-a, chlorophyll-b and total chlorophyll of *G. max* were significantly decreased with increased Cr concentrations. Amin *et al.* (2013) [10] found that the toxicity of chromium metal to young seedling and their effects on chlorophyll content were increased with higher concentration of chromium in the soil system. Tiwari *et al.* (2013) [83] observed a significant decrease in chlorophyll contents as compared to control plants after 15 days of Cr exposure. Najafian *et al.* (2012) [63] showed significant reduction in total chlorophyll of leaves compared to control. Ahmad *et al.* (2011b) [5] studied that chlorophyll *a* and *b* and carotenoid contents were also reduced in Cr-treatment plants. Ali *et al.* (2011) reported that under Cr stress significant decrease in Chlorophyll content and chlorophyll fluorescence ratio (Fv/Fm) was observed in the two genotypes. Datta *et al.* (2011) [26] observed that the total chlorophyll content decreased with increasing concentration of Cr (VI) solutions in five wheat cultivars. Pandey (2008) [27] assessed that increased concentration of potassium chromate caused adverse effect on the vegetation as lesser formation of chlorophyll a, chlorophyll b and carotenoids.

The reason behind decreased chlorophyll content under Chromium stress is that the Chromium competes for Fe binding sites and interfere absorption of Fe that leads to the decrease of Fe accumulation required for the biosynthesis of chlorophyll and heme synthesis (Gopal *et al*., 2009; Islam et *al*., 2016) [39]. Sheetal *et al.* (2016) [77] stated that decrease in chlorophyll contents has been attributed to the direct inhibition of enzymes or competitive exclusion of some essential nutrients and binding of metals to protein sulfhydryl groups or even destruction of chlorophyll by formation of reactive oxygen radicals. The decrease in chlorophyll concentration may be result of an inhibited photosynthetic electron transport and decomposition of the chloroplast membrane. The adverse effects of excess heavy metals may be because of chromium interfering in transformation of chlorophyll either through the direct inhibition of an enzymatic step or through the induced iron deficiency (Tandon and Vikram, 2014) [80]. Chromium inhibits the activity of some chlorophyll synthesizing enzymes like delta amino levulinic acid dehydratase (ALAD) and proto chlorophilide reductase. Decrease in ALAD enzyme cause reduces in amount of porphobilinogen that is essential for synthesis of chlorophyll (Najafian *et al*., 2012) [63]. The formation of chlorophyll pigment depends on the adequate supply of iron as it is the main component of the protoporphyrin, a precursor of chlorophyll synthesis (Arshad *et al*., 2017) [15]. The formation of chlorophyll pigment depends on the adequate supply of iron as it is the main component of the protoporphyrin, a precursor of chlorophyll synthesis. An excessive supply of chromium seems to prevent the incorporation of iron into the protoporphyin molecule, resulting in the reduction of chlorophyll pigment (Datta *et al*., 2011) [26]. Cr-induced reduction in photosynthetic pigments has been attributed to both direct and indirect factors, such as alteration of chloroplast ultrastructure and inhibition of photosynthetic pigments (Gill *et al*., 2015) [34], inhibition of gas exchange parameters (Ali *et al*., 2011), and due to decomposition of chlorophyll by rise in chlorophyllase activity (Gill *et al*., 2015) [34]. Reduction in chlorophylls may also be due to the ROS generation in plants under metal stress (Ehsan *et al*., 2014; Ali *et al*., 2015) [29]. Chromium mostly in its hexavalent form can replace Mg ions from the active sites of many enzymes and deplete chlorophyll content (Revathi *et al*., 2011) [71]. Significant reduction in chlorophyll (a, b and total chlorophyll) and carotenoids, with increasing Cr concentration (5, 10, 20 mg kg^{-1}), was attributed to inhibited uptake and translocation of mineral nutrients (Atta *et al*., 2013; Kotschau *et al*., 2013). Similarly, the decrease in chlorophyll content in the present study is a common deleterious effect of Cr stress, which resulted in chlorosis of the mustard leaves (Mahmud *et al*., 2017) [55].

Result also revealed that application of organic amendments significantly increased the chlorophyll content (SPAD) of mustard leaves as compared to their respective chromium treatment. Maximum and significantly higher increment in chlorophyll content (SPAD) of mustard leaves was found with the application of vermicompost @ 5 ton ha^{-1} (T_{16}) and farm yard manure @ 10 ton ha^{-1} (T_{11}), followed by sewage sludge @ 20 ton ha^{-1} (T_6). However sewage sludge @ 20 ton ha^{-1} was on par with its chromium treatments and farm yard manure @ 10 ton ha^{-1}. Abbas *et al*. (2017) [1] showed that the chlorophyll a and b of wheat leaves significantly increased in the Cd contaminated BC amended soil. Arshad *et al*. (2017) [15] investigated that soil amendment with biochar and bacteria showed a significant improvement in chlorophyll content by improving physicochemical properties of soil and reducing Cr(VI) induced phytotoxicity and phytoavailability. Ali *et al*. (2015) observed that the application of Cr alone reduced the total chlorophyll and carotenoid concentrations as compared with control. Application of fulvic acid inhibited the Cr-induced toxicity to pigment contents. Dheeba *et al.* (2014) [28] was observed maximum reduction in *Chl* in test control group which received Cr alone. The levels of pigments have shown significant increase in fertilizer treated plants compared to test control. Revathi *et al*. (2011) [71] indicated that there is significant reduction of chlorophyll content of the plant with increased dosage of chromium. It is also observed that addition of vermicompost to the contaminated soil improves the chlorophyll content of the plants.

Increase in chlorophyll contents might due to the adsorption of free chromium ions to fulvic acid, which decreased the concentration of free metal ion in living cells (Ali *et al*., 2015). Moreover, increase in pigment concentration might also be due to decrease in Reactive oxygen species (ROS) production in leaves with fulvic acid (Shahid *et al*., 2012) [14]. However, Abbas *et al*. (2017) [1] delineated that the increase in chlorophyll contents might be due to the reversal of Cd induced toxicities in the plants with biochar (BC) application. Dheeba *et al.* (2014) [28] suggested that fertilizers, viz. Farm Yard Manure (FYM), NPK, Panchakavya (PK) and Vermicompost (VC) attenuate the damage caused by Cr to some extent and stimulates growth. Similarly, the decrease in chlorophyll content is a common deleterious effect of Cr stress, which resulted in chlorosis of the mustard leaves. Chlorophyll is the key chemical compound present in the plants (Ahmad *et al*., 2011b) [5]. Chlorophyll concentration (SPAD value) is commonly used as an important parameter to evaluate leaf health (Islam *et al*., 2014b) [39]. The increase in chlorophyll content in the present study might be due to supply of nitrogen and other nutrients and low chromium concentration in leaves and stems of mustard in organic amendments treatments.

Table 3: Effect of organic amendments on chlorophyll (SPAD) content of mustard in chromium contaminated soils

Treatment	30 DAS		60 DAS		90 DAS		At Harvest	
	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17
T_1-Control	33.13	35.12	40.13	41.13	43.67	44.28	34.97	34.57
T_2-20 ppm Cr	32.87	34.83	38.37	39.32	43.43	44.05	33.47	33.00
T_3-40 ppm Cr	30.13	31.94	34.53	35.33	40.70	41.36	34.50	34.03
T_4-60 ppm Cr	27.21	29.96	32.23	33.16	39.57	40.24	33.50	33.13
T_5-80 ppm Cr	24.12	28.26	29.70	30.00	37.90	38.60	31.67	32.40
T_6- 0 ppm Cr + Sl	34.53	36.60	41.53	42.54	47.60	46.92	37.57	37.93
T_7-20 ppm Cr + Sl	34.37	36.42	40.37	41.34	46.43	45.77	36.40	37.07
T_8-40 ppm Cr + Sl	31.00	36.35	39.47	40.42	45.87	45.21	35.50	35.27
T_9-60 ppm Cr + Sl	28.00	34.80	38.47	39.39	45.10	44.45	34.53	34.87
T_{10}-80 ppm Cr + Sl	25.17	30.98	37.60	38.51	42.90	42.29	35.00	34.60
T_{11}- 0 ppm Cr + FYM	36.19	38.24	43.67	44.72	49.17	49.25	39.20	38.60
T_{12}-20 ppm Cr + FYM	35.77	37.91	42.03	43.05	48.23	47.54	35.77	36.13
T_{13}-40 ppm Cr + FYM	33.20	35.19	39.80	40.76	46.77	46.09	35.23	35.67
T_{14}-60 ppm Cr + FYM	29.90	31.69	38.60	39.53	45.40	44.75	35.37	34.93
T_{15}-80 ppm Cr + FYM	28.09	31.33	37.30	38.20	43.43	42.81	33.77	33.27
T_{16}- 0 ppm Cr + VC	37.14	38.75	45.47	46.57	51.40	49.90	39.52	39.92
T_{17}-20 ppm Cr + VC	35.92	37.34	42.47	43.49	48.33	47.64	37.63	37.93
T_{18}-40 ppm Cr + VC	33.70	35.40	41.17	42.16	46.77	46.09	36.77	36.23
T_{19}-60 ppm Cr + VC	30.70	32.54	39.50	40.45	44.63	43.99	34.53	34.93
T_{20}-80 ppm Cr + VC	28.84	31.76	38.87	39.80	44.23	43.60	33.67	33.97
SEm±	0.66	0.82	0.84	0.92	1.26	1.26	0.86	0.93
CD (P=0.01)	2.54	3.15	3.20	3.53	4.82	4.81	3.27	3.54

Cr = Chromium, Sl = Sewage Sludge, FYM = Farm Yard Manure, VC = Vermicompost, CD = Critical Difference, SEm± = Standard error of mean

Plant height

The data related to the effects of organic amendments on plant height of mustard in chromium contaminated soils are presented in table 4 exhibited significant difference values of plant height of mustard with application of different organic amendments in chromium contaminated soil during both the years. During 2015-16 plant height of mustard ranged from 28.36 to 12.43 cm, 99.13 to 39.56 cm, 164.93 to 127.93 cm and 179.56 to 161.33 cm at 30, 60, 90 DAS and at harvest, respectively while during 2016-17 it ranged from 27.56 to 13.03 cm, 98.95 to 38.85 cm, 168.10 to 129.85 cm and 178.79 to 160.57 cm at 30, 60, 90 DAS and at harvest, respectively. It was found that at 30 DAS the application of vermicompost @ 5 ton ha^{-1} (T_{16}) recorded significantly highest plant height 28.36 and 27.56 cm of mustard in 2015-16 and 2016-17, respectively. At 60 DAS and 90 DAS the application of 20 ppm chromium + vermicompost @ 5 ton ha^{-1} (T_{17}) recorded highest plant height 99.13 and 98.95 cm of mustard in 2015-16 and 2016-17, respectively. At 90 DAS also the application of 20 ppm chromium + vermicompost @ 5 ton ha^{-1} (T_{17}) recorded highest plant height 164.93 and 168.10 cm of mustard in 2015-16 and 2016-17, respectively. At Harvest the application of 40 ppm chromium + vermicompost @ 5 ton ha^{-1} (T_{18}) recorded significantly highest plant height 179.56 and 178.79 cm of mustard in 2015-16 and 2016-17, respectively. However, application of farm yard manure and sewage sludge was found better treatment in plant height of mustard compared to their respective chromium treatments at all growth stages. Lowest plant height of mustard 12.43 and 13.03 cm, 39.56 and 39.85 cm, 127.93 and 129.85, 161.33 and 160.57 cm at 30, 60, 90 DAS, at harvest in 2015-16 and 2016-17, respectively was observed in treatment T_5 (80 ppm Chromium). Application of sewage sludge @ 20 ton ha^{-1}, farm yard manure @ 10 ton ha^{-1} and vermicompost @ 5 ton ha^{-1} remains on par with each other at all the growth stages and at the same level of chromium. It was also noted that at later stage of crop growth, chromium had no significant effect on plant height of mustard in all the treatments. It might be due to decline in phytoavailability of chromium by organic amendments at later growth stage and enhancement in ability of plants to tolerate the toxicity of chromium. Malik *et al.* (2017) [8] observed that after 10 DAT and 20 DAT root and shoot length of wheat and barley decreased with the increasing concentrations of lead and cadmium but at 30 DAT, seedling growth in all the treatments of lead and cadmium were comparable to control. Lower concentrations of heavy metals ranging from 5 to 20 ppm doses were observed to be stimulating the root and shoot length of the sunflower plant (Jadia and Fulekar, 2008).

Further study of data confirmed that plant height of mustard significantly decreased from 28.36 and 27.56 cm, 99.13 and 98.95 cm, 164.93 and 168.10 cm, 179.56 and 178.79 at 30, 60, 90 DAS, at harvest in T_1 (control) where chromium was not applied to 12.43 and 13.03 cm, 39.56 and 39.85 cm, 127.93 and 129.85, 161.33 and 160.57 cm at 30, 60, 90 DAS, at harvest in T_5 (80 ppm Cr) in 2015-16 and 2016-17, respectively as level of chromium increased. However no significant difference was observed up to 40 ppm chromium with their respective control in all the set of treatments. Coelho *et al.* (2017) demonstrated that plant height varied with Cr(III) concentration in a quadratic fashion, with plant height reaching an estimated maximum value of 97 cm at 0.005 mmol L^{-1} Cr(III). Mahmud *et al.* (2017) [55] found that plant height decreased under mild (0.15mM K_2CrO_4) and severe (0.3mM K_2CrO_4) stress, respectively, compared with control. Oladele *et al.* (2017) [65] showed that a negative relationship existed between the different metal concentrations in the soil and the stem height and root compared to control experiment. Islam *et al.* (2016) [39] observed that exposure of maize plants to Cr contamination significantly decreased plant height compared to non-contaminated control. Kamran *et al.* (2016) [45] observed that root and shoot length of *E. sativa* decreased as Cr contamination increased. Soni *et al.* (2016) showed that in *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* the length of entire plant was reduced due to chromium concentration in the treatment. Nagarajan and Ganesh (2015) [62] reported that root length and shoot length are gradually decreased with the increase in chromium concentrations. Islam *et al.* (2014b) [39] found that Cr at low level (100 mg kg^{-1}) did not significantly decrease plant height, root length and total biomass, but at high level (200-400 mg kg^{-1}) these parameters decreased significantly. Tandon and Vikram (2014) [80] observed that the plant height decreased significantly with the increase in chromium concentration. Tiwari *et al.* (2013) [83] evaluated that after 15 days of Cr exposure, a significant depression in height was observed as compared to controls. Akinci and Akinci (2010) [6] found that the elevated chromium at 2.5 to 70 mg L^{-1} induced a decrease in the hypocotyl height in melon seeds from 11.4 to 52.7%, compared with the control. Andaleeb *et al*. (2008) [12] showed that root and shoot lengths were decreased with increase in Cr concentrations. A gradual decrease was observed for plant height with increase in Cr levels.

Decreased growth in terms of root and shoot lengths and weight at increasing concentration of chromium in soil might be due to adverse effect of this metal on overall metabolism of plant (Ghani *et al*., 2017) [33]. Reduction in the plant height might be mainly due to the reduction in root growth and ensuing lesser nutrient and water transport to the above part of the plant. In addition to this, chromium transport to the aerial part of the plant can have a direct impact on cellular metabolism of shoots contributing to the reduction of plant height (Nematshahi *et al*., 2012; Tandon and Vikram 2014) [80, 64]. On the other hand it was indicated that the high concentrations of chromium, effects on the nitrate reductase enzyme activity, reduced nitrogen uptake and nitrate fixation. Therefore due to presence of nitrogen as an essential element in the structure of many biological molecules, its reduction prevents the plant growth. Therefore high concentrations of Cr^{+3} with a negative impact on the root system, as a result they cause plant height reduction (Nematshahi *et al*., 2012) [64]. Result also revealed that application of organic amendments increased the plant height of mustard as compared to their respective chromium treatment. Maximum and significantly higher increment in plant height of mustard was found with the application of vermicompost @ 5 ton ha^{-1} and farm yard manure @ 10 ton ha^{-1}, followed by sewage sludge @ 20 ton ha^{-1} at all level of chromium. However sewage sludge @ 20 ton ha^{-1} was on par with its chromium treatments, farm yard manure @ 10 ton ha^{-1} and vermicompost @ 5 ton ha^{-1}. Bashir *et al*. (2017) recorded that the plant height significantly decreased in soils contaminated with Cd, Cr, or the combination of both. It was noticed that the application of biochar to Cr- or Cd-contaminated soil significantly enhanced the plant height. Ali *et al.* (2015) reported that plant height significantly decreased as Cr concentration in culture media increased. In comparison with Cr treatments alone, application of FA significantly increased plant height compared with same treatments without fulvic acid. Laxman *et al*. (2014) [54] observed that height of *V. zizanioides* was significantly increased with the application of vermicompost in comparison to control. Rangasamy *et al*. (2013) showed that the growth parameters viz., shoot and root length of maize were significantly lower when grown on the chromium contaminated soil (control) without any amendments (T_1). The maize grown on soil amended with the vermicompost, with or without microbial strains and earthworms, had shown greater root and shoot growth. Molla *et al*. (2012) [59] found that in the manure-amended soil, there were no differences between the M-Cr(VI) and any other Cr added treatment in plant height of spinach.

Improvement in plant height may be due to improvement in physico-chemical properties of soil and reduction in Cr(VI) induced phytotoxicity and phytoavailability (Arshad *et al*., 2017) [15]. The increase in plant growth under fulvic acid application may be due to lower Cr uptake and translocation in wheat plants which reduced the toxic effects on plants and, as a result, enhanced wheat growth (Ali *et al*., 2015). Laxman *et al*. (2014) [54] has highlighted that vermicompost amendment could provide enough nutrients for the growth of vetiver

Table 4: Effect of organic amendments on plant height (cm) of mustard in chromium contaminated soils

Treatment	30 DAS		60 DAS		90 DAS		At Harvest	
	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17
T_1-Control	25.15	24.61	81.63	81.01	141.93	144.59	166.81	166.67
T_2-20 ppm Cr	24.72	24.08	81.41	82.52	143.93	146.94	167.94	167.60
T_3-40 ppm Cr	22.48	21.95	72.20	71.86	140.93	143.38	169.15	168.79
T_4-60 ppm Cr	17.03	16.49	64.27	63.99	134.93	137.75	167.67	166.90
T_5-80 ppm Cr	12.43	13.03	39.56	38.85	127.93	129.85	161.33	160.57
T_6- 0 ppm Cr + Sl	25.93	26.42	92.10	91.64	153.93	157.23	171.11	170.34
T_7-20 ppm Cr + Sl	26.97	26.48	94.16	93.35	157.93	160.69	173.22	172.46
T_8-40 ppm Cr + Sl	26.05	25.97	84.70	84.07	150.93	154.02	171.33	170.57
T_9-60 ppm Cr + Sl	23.85	23.23	73.91	73.38	146.93	149.62	171.11	170.34
T_{10}-80 ppm Cr + Sl	16.57	15.14	58.51	57.99	137.93	139.49	165.89	165.12
T_{11}- 0 ppm Cr + FYM	27.90	27.32	93.31	92.88	157.93	160.81	173.33	172.57
T_{12}-20 ppm Cr + FYM	27.09	26.63	95.73	95.10	160.93	164.36	177.78	177.01
T_{13}-40 ppm Cr + FYM	26.05	25.31	92.58	92.33	154.93	158.50	178.44	177.68
T_{14}-60 ppm Cr + FYM	18.31	18.06	75.97	75.85	149.93	153.08	174.11	173.34
T_{15}-80 ppm Cr + FYM	14.84	14.32	48.32	49.19	139.93	141.08	169.67	168.90
T_{16}- 0 ppm Cr + VC	28.36	27.56	96.95	96.48	159.93	163.16	177.00	176.00
T_{17}-20 ppm Cr + VC	26.74	26.74	99.13	98.95	164.93	168.10	179.33	178.57
T_{18}-40 ppm Cr + VC	26.16	25.21	96.34	95.79	157.93	161.12	179.56	178.79
T_{19}-60 ppm Cr + VC	22.98	22.22	78.64	77.85	151.93	154.70	177.44	176.68
T_{20}-80 ppm Cr + VC	14.96	15.67	50.50	50.13	141.93	145.10	171.72	170.96
SEm±	0.75	0.69	1.91	1.96	3.18	3.47	2.50	2.27
CD (P=0.01)	2.87	2.65	7.29	7.51	12.15	13.26	9.55	8.69

Cr = Chromium, Sl = Sewage Sludge, FYM = Farm Yard Manure, VC = Vermicompost, CD = Critical Difference, SEm± = Standard error of mean

Number of leaves per plant

The data associated to the effects of organic amendments on number of leaves per plant in chromium contaminated soils are depicted in table 5. Data showed that application of organic amendments significantly influenced number of leaves per plant in Cr contaminated soil during both the years. During 2015-16 number of leaves per plant varied from 9.00 to 5.55, 18.67 to 11.00, 65.78 to 30.78 and 56.22 to 26.89 at 30, 60, 90 DAS and at harvest, respectively while during 2016-17 it varied from 9.22 to 5.56, 20.22 to 11.22, 67.89 to 33.22 and 56.44 to 27.11 at 30, 60, 90 DAS and at harvest, respectively. It was also observed that number of leaves per plant increased from 30 DAS up to 90 DAS but at harvest it decreased during both the years. The reason of decrement of number of leaves per plant at harvest is shedding of leaves due to abscission. It was found that the application of 20 ppm chromium + vermicompost @ 5 ton ha^{-1} (T_{17}) recorded highest number of leaves per plant 9.00 and 9.22, 18.67 and 20.22, 65.78 and 67.89, 56.22 and 56.44 at 30, 60, 90 DAS, at harvest followed by application of 20 ppm chromium + farm yard manure @ 10 ton ha^{-1} (T_{12}) 8.66 and 9.00, 18.33 and 19.55, 63.22 and 65.11, 54.56 and 55.00 at 30, 60, 90 DAS, at harvest in 2015-16 and 2016-17, respectively. Furthermore, application of 20 ppm chromium + sewage sludge @ 20 ton ha^{-1} (T_7) recorded the higher number of leaves per plant 8.22 and 8.56, 18.00 and 19.22, 60.44 and 62.56, 50.89 and 51.45 at 30, 60, 90 DAS, at harvest in 2015-16 and 2016-17, respectively over rest of the treatments. Among all the treatments the lowest number of leaves per plant 5.55 and 5.56, 11.00 and 11.22, 30.78 and 33.22, 26.89 and 27.11 at 30, 60, 90 DAS, at harvest in 2015-16 and 2016-17, respectively was observed in treatment T_5 (80 ppm Cr). Application of sewage sludge @ 20 ton ha^{-1} was found on par with farm yard manure and farm yard manure @ 10 ton ha^{-1} was on par with vermicompost at all the growth stages and at the same level of chromium. However, number of leaves per plant increased with the application of sewage sludge @ 20 ton ha^{-1} as compared to chromium alone but the increment was not significant. It may be due to supply of excess heavy metal by sewage sludge.

Further study of data confirmed that number of leaves per plant significantly decreased from 7.89 and 8.00, 17.33 and 17.56, 56.11 and 59.22, 49.89 and 50.45 at 30, 60, 90 DAS, at harvest in 20 ppm chromium (T_2) to 5.55 and 5.56, 11.00 and 11.22, 30.78 and 33.22, 26.89 and 27.11 at 30, 60, 90 DAS, at harvest in 80 ppm chromium (T_5) in 2015-16 and 2016-17, respectively as level of chromium increased. However, 20 ppm chromium was found to be stimulatory effect on number of leaves per plant and was on par with their respective control in all the treatments. Anjum *et al.* (2017) [14] showed that Cr stress decreased number of leaves per plant in maize when compared to the control. Tandon and Vikram (2014) [80] observed that decrease in number of leaves was 10.86%, 19.56% and 26.08% at 0.25mM, 0.5mM and 1mM chromium respectively in rice plant.

The Cr-induced disruption in plant growth might be attributed to Cr transportation to the aboveground part of the plant that may have a direct effect on cellular metabolism resulting in growth inhibition. Restricted water availability for leaf expansion might be one of the possible reasons for reduced leaf area in the Cr-stressed plants. Moreover decreased cell volume reduced intracellular spaces and fluctuations in the size of areoles caused reductions in leaf area of the Cr-treated plants (Anjum *et al*., 2017) [14].

Decrease in number of leaves per plant might be due to abnormal transport of essential nutrients including zinc. As heavy metal interfere in iron metabolism and reduce the transport of essential nutrients like K, Fe to meristematic (foliar and bud) regions of plants. Absence of same essential nutrients in the meristematic regions of plants may also be a cause of reduced plant growth. Lack of growth might be due to the deficiency of zinc, which helps in the synthesis of auxin. Some essential nutrients are also known to be constituents of cytoplasm and enzymes (Tandon and Vikram, 2014) [80]. The decline in plant's physiological parameters was due to the rapid uptake of Cr, and the results were clear in the form of toxic symptoms on plant leaves (chlorosis and necrosis), lower plant height and reduced number of leaves (Junior *et al*., 2014; Farid *et al*., 2017) [31].

Result also revealed that application of organic amendments significantly increased the number of leaves per plant as compared to their respective chromium treatment. Maximum and significantly higher number of leaves per plant was found with the application of 20 ppm chromium + vermicompost @ 5 ton ha^{-1} (T_{17}) and 20 ppm chromium + farm yard manure @ 10 ton ha^{-1} (T_{12}), followed by 20 ppm chromium + sewage sludge @ 20 ton ha^{-1} (T_7). However, 20 ppm chromium + sewage sludge @ 20 ton ha^{-1} was on par with its chromium treatments and farm yard manure @ 10 ton ha^{-1} at all and same level of chromium.

In present study, the increased in number of leaves per plant might be due to the enhancement in the physico-chemical and biological processes in the soil with the addition of organic amendments (i.e. sewage sludge, farm yard manure and vermicompost) as they work as a reducing agent for Cr (VI) and substrate for microbes responsible for biological reduction of Cr (VI), which intimidated Cr toxicity. The rise in number of leaves per plant in the presence of organic amendments may also be ascribed to improved soil fertility/quality and by declining Cr toxicity through precipitation and removal of available Cr from soil.

Table 5: Effect of organic amendments on number of leaves per plant of mustard in chromium contaminated soils

Treatment	30 DAS		60 DAS		90 DAS		At Harvest	
	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17
T_1-Control	7.11	7.22	16.33	17.22	40.78	41.89	27.89	28.11
T_2-20 ppm Cr	7.89	8.00	17.33	17.56	56.11	59.22	49.89	50.45
T_3-40 ppm Cr	6.89	7.00	15.00	15.22	51.44	53.22	41.89	42.44
T_4-60 ppm Cr	5.89	6.00	14.00	14.22	45.11	46.22	43.89	44.11
T_5-80 ppm Cr	5.55	5.56	11.00	11.22	30.78	33.22	26.89	27.11
T_6- 0 ppm Cr + Sl	7.22	7.44	17.00	18.22	45.11	47.56	36.56	37.11
T_7-20 ppm Cr + Sl	8.22	8.56	18.00	19.22	60.44	62.56	50.89	51.45
T_8-40 ppm Cr + Sl	7.22	7.56	16.00	16.56	54.44	56.22	45.89	46.11
T_9-60 ppm Cr + Sl	6.22	6.56	14.67	15.56	47.45	48.22	43.56	44.11
T_{10}-80 ppm Cr + Sl	5.56	6.11	11.67	11.89	34.45	36.56	33.55	34.11

T_{11}- 0 ppm Cr + FYM	8.33	8.55	18.00	19.22	48.44	49.55	39.22	39.44
T_{12}-20 ppm Cr + FYM	8.66	9.00	18.33	19.55	63.22	65.11	54.56	55.00
T_{13}-40 ppm Cr + FYM	7.22	7.67	16.67	17.56	57.78	59.22	47.22	47.67
T_{14}-60 ppm Cr + FYM	6.56	7.11	15.33	16.22	52.00	53.22	48.56	49.11
T_{15}-80 ppm Cr + FYM	5.89	6.33	13.00	13.89	38.89	40.11	35.89	36.44
T_{16}- 0 ppm Cr + VC	8.78	8.89	18.67	19.89	50.44	51.89	41.55	42.11
T_{17}-20 ppm Cr + VC	9.00	9.22	18.67	20.22	65.78	67.89	56.22	56.44
T_{18}-40 ppm Cr + VC	8.00	8.33	17.00	18.22	60.44	62.55	52.22	52.78
T_{19}-60 ppm Cr + VC	7.22	7.56	15.67	16.56	55.11	57.89	49.89	50.44
T_{20}-80 ppm Cr + VC	6.67	7.00	13.67	14.55	40.89	42.56	38.44	39.56
SEm±	0.26	0.18	0.39	0.47	1.44	1.30	1.15	1.01
CD (P=0.01)	0.99	0.68	1.47	1.82	5.50	4.98	4.40	3.87

Cr = Chromium, Sl = Sewage Sludge, FYM = Farm Yard Manure, VC = Vermicompost, CD = Critical Difference, SEm± = Standard error of mean

Number of branches per plant

The data related to the effects of organic amendments on number of branches per plant in chromium contaminated soils are presented in table 6. Application of organic amendments significantly affected the number of branches per plant in Cr contaminated soil during both the years. During 2015-16 number of branches per plant varied from 15.78 to 7.78, 26.11 to 11.44 and 31.34 to 16.00 at 60, 90 DAS and at harvest, respectively while during 2016-17 it ranged from 16.00 to 7.55, 26.33 to 11.67 and 35.00 to 15.67 at 60, 90 DAS and at harvest, respectively. It was also observed that number of branches per plant increased from 60 DAS up to at harvest during both the years. It was found that at 60 DAS the application of vermicompost @ 5 ton ha^{-1} (T_{16}) recorded highest number of branches per plant 15.78 and 16.00 in 2015-16 and 2016-17, respectively, at 90 DAS the application of 60 ppm chromium + sewage sludge @ 20 ton ha^{-1} (T_9) recorded highest number of branches per plant 26.11 and 26.33 in 2015-16 and 2016-17, respectively and at harvest in 2015-16, the application of 80 ppm chromium + sewage sludge @ 20 ton ha^{-1} (T_{10}) recorded significantly highest number of branches per plant 31.34 while in 2016-17, the application of 60 ppm chromium + vermicompost @ 5 ton ha^{-1} (T_{19}) recorded highest number of branches per plant i.e. 35.00. The lowest number of branches per plant 7.78 and 7.55 at 60 DAS in 2015-16 and 2016-17, respectively were observed in treatment T_5 (80 ppm Cr), while at 90 DAS and at harvest the lowest number of branches per plant 11.44 and 11.67 and 16.00 and 15.67 in 2015-16 and 2016-17, respectively were observed with the application of 20 ppm chromium + sewage sludge @ 20 ton ha^{-1} (T_7). From the data of number of branches per plant it may be said that despite of showing significant difference between the treatments the numbers of branches per plant do not conferred any justifiable trend. Further study of data confirmed that number of branches per plant decreased as level of chromium increased. However, Tandon and Vikram (2014) [80] reported decrease in number of tillers in rice plants under chromium contamination. Tito *et al.* (2014) advocated that increasing levels of metals significantly influenced the number of branches, with the exception of the metal chromium, which showed no significant difference in crambe plant. Ali *et al.* (2011) studied that Al and Cr stress reduced number of tillers per plant in two cultivars of barley.

Result also revealed that the application of organic amendments increased the number of branches per plant in all the treatments as compared to their respective chromium treatment. Saengwilai *et al*. (2017) anticipated that MD (cow manure) treatment significantly increased the number of tillers in rice crop by 1.6 times. Ali *et al*. (2015) advocated that in comparison with Cr treatments alone, application of fulvic acid significantly increased number of tillers per plant compared with same treatments without fulvic acid in wheat plants. Khan *et al*. (2013) found that by using amendments of sewage sludge biochar (SSBC) number of tillers and the height of tillers all increased significantly in rice plant.

Adequate concentrations of essential nutrients were considered a key factor in supporting plant growth; in addition, organic amendments (sewage sludge, farm yard manure and vermicompost) served to dilute soil Cr, thereby decreasing Cr accumulation and phytoavailability to the mustard. Application of organic amendments (sewage sludge, farm yard manure and vermicompost) are known to significantly increase levels of essential nutrients, organic carbon content, and CEC of soil (Pillai *et al*., 2013) [67]. Organic amendments (sewage sludge, farm yard manure and vermicompost) have been applied to contaminated soil to support phytostabilization (Chaiyarat *et al*., 2011; Meeinkuirt *et al*., 2016) [21, 74].

Table 6: Effect of organic amendments on number of branches per plant of mustard in chromium contaminated soils

Treatment	60 DAS		90 DAS		At Harvest	
	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17
T_1-Control	11.78	11.00	16.11	16.33	20.67	18.00
T_2-20 ppm Cr	10.44	9.67	24.44	24.67	30.34	26.67
T_3-40 ppm Cr	9.78	9.34	25.00	25.33	30.67	31.00
T_4-60 ppm Cr	8.78	8.44	19.78	20.78	22.00	21.33
T_5-80 ppm Cr	7.78	7.55	14.45	14.67	18.67	16.33
T_6- 0 ppm Cr + Sl	13.11	11.67	17.78	18.00	22.33	20.67
T_7-20 ppm Cr + Sl	11.44	10.66	11.44	11.67	16.00	15.67
T_8-40 ppm Cr + Sl	9.78	9.56	16.78	17.00	26.33	28.67
T_9-60 ppm Cr + Sl	9.11	8.89	26.11	26.33	27.67	24.00
T_{10}-80 ppm Cr + Sl	8.44	8.00	23.11	23.33	31.34	31.33
T_{11}- 0 ppm Cr + FYM	15.12	14.66	23.11	23.33	23.33	22.33
T_{12}-20 ppm Cr + FYM	14.11	14.33	14.11	14.33	21.33	22.33

T_{13}-40 ppm Cr + FYM	12.11	11.34	22.11	22.33	27.00	25.67
T_{14}-60 ppm Cr + FYM	10.11	10.00	16.78	17.00	24.00	28.00
T_{15}-80 ppm Cr + FYM	9.11	9.22	21.11	21.33	25.00	23.67
T_{16}- 0 ppm Cr + VC	15.78	16.00	18.44	18.67	23.67	24.00
T_{17}-20 ppm Cr + VC	14.78	15.00	19.78	20.00	24.67	23.33
T_{18}-40 ppm Cr + VC	13.44	12.33	20.11	20.33	25.67	23.67
T_{19}-60 ppm Cr + VC	10.78	10.33	24.67	25.00	30.00	35.00
T_{20}-80 ppm Cr + VC	9.44	9.56	21.44	21.67	26.67	26.11
SEm±	0.29	0.32	0.48	0.57	0.77	0.57
CD (P=0.01)	1.12	1.21	1.81	2.19	2.94	2.19

Cr = Chromium, Sl = Sewage Sludge, FYM = Farm Yard Manure, VC = Vermicompost, CD = Critical Difference, SEm± = Standard error of mean

Conclusion

It was observed that chromium has negative impact on growth parameters. Mustard growth significantly decreased as level of chromium increased. Organic amendments found better option to mitigate chromium toxicities on growth parameters as they supply organic matter which reduces the toxicity of chromium by decreasing phytoavailability of chromium in soil. Among the organic amendments vermicompost exhibited superior efficacy in alleviating the effect of chromium.

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