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Effect of chromium and lead on seedlings and yield contributing parameters of maize (*Zea mays* L.)

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Authors' Contribution **Junaid, J. A.**, conceived the idea, performed research, **H. Saleem** & **K. Jamshaid** performed data collection, **M. A. Khan**, **I. Sharif**, **U. S. Rana** and **S. Akram** reviewed and edited the paper.

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ABSTRACT

The development and output of maize plants are impacted by heavy metals in a number of ways, and they also hinder a number of physiological processes. Humans have major health issues when heavy metals are present in their diet. This study examined the impact of several heavy metals (both singly and in combination) on maize yield and numerous yield-related parameters. A replicated totally randomized factorial design was used to analyse eight parents—five lines, three testers, and their crosses. Data were recorded on days to 50% germination, fresh root length, fresh root weight, dry root weight, dry shoot weight, dry root to shoot weight ratio, chlorophyll content, and leaf temperature at seedling stage while yield contributing parameters like cobs per plant, grain rows per ear, grain yield per plant, and 100 grain weight were recorded after harvesting. These measurements were then analysed using line × tester analysis to determine the effects of combining ability and to evaluate the variations in heavy metals uptake in maize grain. With the exception of cobs per plant, all of the attributes in the combining ability analysis produced significant results. The genotype Sulltan and hybrid (K54TMS × Agatti 84) were found to only absorb a minimal quantity of the metals (Cr and Pb) both separately and in combination. This suggests that they should be used in breeding programs going forward to increase the genotypes' resistance to heavy metal absorption.

Keywords: Combining ability, heavy metals, morphological parameters, seedlings, yield, *Zea mays*

INTRODUCTION: Maize is an imposing and widely farmed crop of grass family that is a member of the Maydeae tribe. In comparison to other crops, maize, wheat, and rice are grown and produced in enormous amounts across the world; yet, maize has the highest potential for grain output per hectare. The maize is a third most important cereal after wheat and rice due to its multipurpose uses suc as cornmeal, grits, starch, flour, tortillas, snacks and breakfast cereals. Maize is a staple diet for humans and cattle alike in many nations throughout the world. It is crucial for nations like Pakistan, where a fast growing population has already depleted the existing food supply, as it is the highest yielding cereal crop in the world. In Pakistan, it is the fourth-largest crop grown crop after wheat, rice and cotton. It makes for 0.7% of the Gross domestic products and 3% of the value added. The area planted with maize for 2022–2023 was 1720 thousand hectares, and production was around 10.183 million tons (GOP, 2023). It is grown in the subtropical and temperate regions around the globe. It is a C4 plant and can endure high levels of oxidative stress and water scarcity. The leaf and seed might have greater concentrations of heavy metals (Shanker *et al.*, 2005). There are approximately 35 different types of metal in nature, of which 23 are categorized as heavy metals. Plants experience significant effects from heavy metal poisoning at various development stages. It occurs as a result of various metal ions in the soil and irrigation water being applied to the crop. Industrial wastes and untreated municipal effluents are utilized in underdeveloped nations to grow crops in and around metropolitan centres. Heavy metals, boron, salts, and other potentially harmful organic and inorganic compounds are present in it, which might have a negative impact on consumers, plants, and the soil (Mapanda *et al.*, 2005). Since many enzymes and proteins depend on different metals for normal plant growth and development, several of these metals are also necessary for human consumption. However, all metals can become toxic when they exceed their respective critical tissue concentrations. Humans have major health issues when heavy metals are present in their diet (Martin and Griswold, 2009). Lead and chromium have an impact on maize plants at various growth stages and reduce crop output. Chromium toxicity prevents seed germination, caused stunted seedling development, and inadequate formation of phytomass, all of which ultimately cause plant mortality. It also inhibits a number of metabolic activities (Zou *et al.*, 2009). Lead (Pb) is thought to have a comparatively low level of phytotoxicity due to its low availability and therefore low absorption from soils and soil solutions. Due to its adsorption to root surfaces and cell walls, translocation to shoot after root absorption is constrained.

OBJECTIVES: The objectives of the current study were : I) To evaluate the morphological traits of maize seedlings and yield contributing factors influenced by various heavy metals II) To assess the impacts of various heavy metal concentrations on various developmental stages of the crop.

MATERIALS AND METHODS: The experiment was carried out in the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan during 2021-22, located between 31.43° north and longitude 73.8° east and 184 m above sea level in central Punjab. The first step of experiment was to screen 20 inbred lines (F-219, F-216, F-289, 2P-735, T-02, F-01, F-275, EV 77, F-167, F-221, DTC-1, F-168, F-303, 2P-735, ML 22, L-5-1, 20 P2- 1, FH-735, K54TMS and 1335-2B) at seedling stage. Seeds of inbred lines were taken from the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. The inbred lines were sown in triplicated completely randomized design in polythene bags (8" × 4") filled with sand in the green house. Four treatments were applied at seedling stage to screen the inbred lines. The treatments were designated as: T_0 (Control), T_1 (5 mM of Chromium as $CrCl_3$), T_2 (5 mM of Lead as $PbCl_2$) and T_3 (2.5 mM of Chromium as $CrCl_3$ + 2.5 mM of Lead as $PbCl_2$). After 28 days of sowing, data were recorded for morphological parameters like Days to 50% germination, Fresh root length, Fresh root weight, Dry root weight, Dry shoot weight, Dry root to shoot weight ratio, Chlorophyll content and Leaf temperature. On the basis of recorded data, 5 tolerant inbred lines (F-167, F-168, 1335-2B, 2P-735 and K54TMS) were selected. In next season, selected lines from first phase of experiment were sown and crossed with open pollinated testers in a line × tester fashion. Each line was crossed with three testers (Agati-85, Sonneri and Sulltan) and also selfed (table 1). The seeds obtained through crossing and selfing of lines were used in the next experiment.

Sr. No.	Crosses	Sr. No.	Crosses	Parents
1	F-167 × Agatti 84	9	1335-2B × Sulltan	F-167 (Line)
2	F-167 × Sonneri	10	2P-735 × Agatti 84	F-168 (Line)
3	F-167 × Sulltan	11	2P-735 × Sonneri	1335-2B (Line)
4	F-168 × Agatti 84	12	2P-735 × Sulltan	2P-735 (Line)
5	F-168 × Sonneri	13	K54TMS × Agatti 84	K54TMS (Line)
6	F-168 × Sulltan	14	K54TMS × Sonneri	Agatti 84 (Tester)
7	1335-2B × Agatti 84	15	K54TMS × Sulltan	Sonneri (Tester)
8	1335-2B × Sonneri			Sultan (Tester)

Table 1: Crosses of selected inbred lines with testers in line × tester fashion.

The seeds of parents and their crosses were sown in duplicated randomized completely block design (factorial) and four treatments (Control, Chromium, Lead and Chromium + Lead) at a concentration of 5 mM were applied at the time of sowing. At maturity, seeds were harvested followed by the recording of yield parameters (Cobs per plant, Grains per ear row, Grain rows per ear, Grain yield per plant (calculated by accounting all the yield contributing factors, like number of cobs per plant, number of grain rows per ear, number of grains per ear row and 100 grain weight), Grains per ear, 100 grain weight and Heavy metals concentration in grain). Heavy metal toxicity (Cr and Pb) in the prepared samples were determined by using Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan). Calibrated standards were prepared from the commercially available stock solution (AppliChem®) in the form of an aqueous solution (1000 ppm). Highly purified de-ionized water was used for the preparation of working standards. All the glass apparatus used throughout the process of analytical work were immersed in 8N HNO_3 overnight and washed with several times of de-ionized water prior to use.

Statistical analysis: The data collected from seedlings were analysed by using Analysis of Variance (ANOVA) to assess difference among inbred lines (Steel *et al.*, 1960) and data collected from maturity experiment were subjected to Line × Tester analysis (Kempthorne, 1957) to compute the combining ability effects. The mean comparison of different genotypes for different traits was done through LSD.

RESULTS AND DISCUSSION: In first phase of experiment, data were recorded at seedling stage and inbred lines were evaluated for morphological parameters discussed below:

Days to 50% germination: The analysis of variance (ANOVA) for the days to 50% germination revealed significant variations across various genotypes. The results from the different treatments were highly significant. It was determined that the treatment and genotype interaction impact was not significant (table 2). The LSD readings showed a clear distinction between the control group and the various treatments. Under combined treatment with Cr + Pb, the greatest mean value was discovered to be 4.8000, while the lowest mean values were discovered under control, at 4.16. Non-significant differences were also found between the Cr, Pb, and combination treatments (Cr + Pb) (table 3). The time required to germinate the seed was significantly increased by the use of heavy metals. It slowed down germination by raising the content of heavy metals (Mahmood *et al.*, 2007; Datta *et al.*, 2011). Additionally, it lengthened the time it took for the seeds to sprout.

SOV	DF	DG-50%	FRL	FRW	DRW	DSW	DRSWR	CC	LT
Treatment	3	14.26**	6.37**	142.75**	36.97**	18.8**	28.57**	30.79**	29.87**
Genotype	19	2.52**	5.68**	17.04**	1.14^{NS}	12.48**	1.62^{NS}	2.01**	2.22**
Treatment*Genotype	57	1.34^{NS}	3.23**	15.09**	1.61**	15.43**	1.79**	2.51**	2.48**
Error	160	0.337	61.32	0.096	0.059	0.005	0.882	13.12	5.928

Table 2: Analysis of variance for various traits under the study at seedling stage.

Treatment	DG-50%	FRL	FRW	DRW	DSW	DRSWR	CC	LT
T_0	4.1667^B	53.655^A	2.9423^A	0.8150^A	0.3445^A	2.8333^A	25.397^A	36.765^A
T_1	4.6833^A	47.865^B	2.1418^C	0.3757^C	0.2662^C	1.4358^B	25.362^A	33.728^B
T_2	4.6833^A	49.255^B	1.8735^D	0.7058^B	0.315^B	2.4972^A	21.513^C	34.488^B
T_3	4.8000^A	48.957^B	2.6148^B	0.7075^B	0.2662^C	2.7665^A	23.987^B	32.725^C

Table 3: Mean pairwise comparisons of all treatments for various traits under the study at seedling stage.
(T_0= Control, T_1= 5 mM Chromium chloride, T_2= 5 mM Lead chloride, T_3= Chromium chloride + Lead chloride 2.5 mM of each) *= Significant at 5% probability level, **= Highly significant at 1% probability level, ^{NS}= Non-significant, DG-50%= Days to 50% germination, FRL= Fresh root length, FRW= Fresh root weight, DRW= Dry root weight, DSW= Dry shoot weight, DRSWR= Dry root to shoot weight ratio, CC= Chlorophyll content, LT= Leaf temperature.

Fresh root length: The ANOVA for fresh root length showed quite significant variations across various genotypes. It was also found that the effects of various treatments and their interactions with genotypes were highly significant (table 2). The control group had the greatest mean value (53.65 cm), whereas the Cr treatment group had the lowest mean value (47.86 cm). Between the control group and each of the other treatments (Pb, Cr, and Cr+Pb), there were substantial mean differences. The mean differences between the Cr, Pb, and combination treatments (Cr+Pb) were not statistically significant (table 3). As heavy metals were applied, the root length shrank. Pb was shown to be more harmful than Cr when applied. By applying certain heavy metals, the root length in wheat and rice decreased by up to 50% and 40%, respectively, in comparison to the control (Mahmood *et al.*, 2007; Atta *et al.*, 2014).

Fresh root weight: For fresh root weight, the analysis of variance indicated a significant difference across genotypes. The results of various treatments were also very significant. Additionally, a highly significant interaction between treatment and genotypes was identified (table 2). The Control group had the greatest mean value (2.94 g), whereas the Pb treatment group had the lowest mean value (1.87 g). All of the treatments had substantial mean changes (table 3). Root length is the key for plant to take up nutrients from the soil, also for good crop stands. Length of roots facilitates nutrient and minerals uptake from the deeper soil in water deficit stress. The lead has the adverse effect on the fresh root weight as compared to all other treatments (John *et al.*, 2009; Liang ChangCong *et al.*, 2009; Ghani, 2010; Hussain *et al.*, 2010).

Dry root weight: Dry root weight ANOVA showed no statistically significant changes between genotypes. The results of various treatments were very significant. Additionally, a highly significant interaction between treatment and genotypes was found (table 2). The LSD results showed a clear distinction between the control group and the various treatments. The control group had the greatest mean value (0.81 g), whereas the Cr treatment group had the lowest mean value (0.37 g). Between the Cr, combination (Cr+Pb), and Control treatments, there were statistically significant mean differences. Between Pb treatments and combination treatments (Cr+Pb), there were no statistically significant mean differences (table 3). As various metals are used, dry weight lowers. The decrease rate rises as concentration levels rise. Compared to Cd and other metals, Pb and Cr treatment resulted in a smaller decrease in dry root weight in maize (Hussain *et al.*, 2010; Malik *et al.*, 2010; Mukhtar *et al.*, 2010).

Dry shoot weight: Dry shoot weight analysis of variance demonstrated highly significant variations across genotypes. Highly significant results were observed for the impact of several treatments and the interaction between genotypes and treatments (table 2). The LSD results showed a clear distinction between the control group and the various treatments. The combined treatments (Cr+Pb) had the lowest mean value (0.26 g), whereas the control had the greatest mean value (0.34 g). The Pb, combination (Cr+Pb), and Control treatments showed the most significant mean differences. Between the Cr and combination treatments (Cr+Pb), there were no statistically significant mean differences (table 3). According to Ghani (2010) and Malik *et al.* (2010), Pb had a stronger negative impact on dry shoot weight than Cr.

Dry root to shoot weight ratio: The ANOVA exhibited no statistically significant variability between genotypes. Additionally, a highly significant interaction between treatment and genotypes was discovered (table 2). The LSD results showed a clear distinction between the control group and the various treatments. The control group had the greatest mean value (2.83), whereas the Cr treatment group had the lowest mean value (1.43). The Cr treatment group showed significant variation from all other treatments groups. The difference in mean between the control, Pb, and combination treatments (Cr+Pb) is not statistically significant (table 3). The standout treatment was chromium that effects the dry root to shoot weight ratio most as compared to all other treatments (Liang ChangCong *et al.*, 2009; Ghani, 2010; Hussain *et al.*, 2013).

Chlorophyll content: For Chlorophyll content, the analysis of variance showed highly significant variations among genotypes. The results of treatments were also highly significant. Additionally, a highly significant interaction between treatment and genotypes was found (table 2). The LSD results showed significant variations between the control group and the various treatments. The control group had the greatest mean value (25.39 mg/g), whereas the Pb treatment group had the lowest mean value (21.513 mg/g). Significant changes were found between the control, Pb, and combination (Cr+Pb) treatments. Between the control and Cr treatments, there were no statistically significant changes (table 3). In different treatments, Chlorophyll contents reduced as compared to control. The reduction in chlorophyll contents directly affects the performance of the maize especially photosynthetic activity. The lead treatment had more effect on chlorophyll contents but chromium did not affect the concentration of chlorophyll contents (Sengar *et al.*, 2008; Mukhtar *et al.*, 2010).

Leaf temperature: According to the ANOVA, highly significant differences were found among genotypes. It was also found that the effects of various treatments and their interactions with genotypes were extremely significant (table 2). The LSD data showed a clear distinction between the control group and the various treatments. The combination treatment (Cr+Pb) produced the lowest mean value (32.72 ^{0}C) and the greatest mean value (36.76 ^{0}C) under control. Significant changes were discovered between the Control, Cr, and combination (Cr + Pb) treatments. The treatments for Cr and Pb did not differ significantly from one another (table 3). In general, heavy metal treatments lower the leaf temperature as compared to control but individual treatments does not have any significant differences from each other (Malik *et al.*, 2010; Mukhtar *et al.*, 2010; Aliu *et al.*, 2013; Singh *et al.*, 2015).

Effect of heavy metals on yield and yield contributing parameters: In pots, the parents (Lines and Testers) and seeds obtained from the crosses made in line × tester fashion in previous season were planted together, and heavy metal treatments were applied. Data were gathered after harvesting for several yield and yield contributing factors. To determine the impacts of combining ability, the data from this experiment was subjected to line × tester analysis.

Cobs per plant: Under control and chromium treatment, the analysis of variance for treatment, parents, crosses, parent's versus crosses, testers, lines, and line tester interaction was found to be non-significant. Under lead treatment, the analysis of variance showed that treatment, crosses, lines, and the interaction between lines and testers were all very significant. The parents were the ones who got the most significant results. Parents vs crosses and testers showed non-significant findings. For the treatment, crosses, testers, lines, and line tester interaction under combined treatment, ANOVA revealed extremely significant results. The parent's vs. crosses comparison produced the significant findings. For parents, the results were non-significant (table 4). General combining ability (GCA) values were highest for 1335-2B (0.10) and lowest for F-167 (-0.067) under control; however, with chromium treatment, 1335-2B displayed the highest (0.133) and F-167 the lowest (-0.033) GCA values among lines (table 5). With lead treatment, the GCA values for the K54TMS were greatest (0.40) and lowest (-0.10) for F-167, while those for the F-167 were highest (0.233) and lowest (-0.10) for 2P-735 among the lines with combination treatment (Cr + Pb) (table 5). Sonneri (0.033) and Agatti 84 (-0.067) had the greatest general combining abilities (GCA) values under control, whereas Sulltan (0.067) and Sonneri (-0.033) had the lowest GCA values with chromium treatment. When exposed to lead, the Sulltan had the highest (0.100) and Agatti 84 the lowest (-0.10) GCA values, while Sonneri displayed the highest (0.100) and Agatti 84 the lowest (-0.100) GCA values in response to combined (Cr + Pb) treatments among testers (table 6). Under control conditions, K54TMS × Sonneri had the greatest specific combining ability (SCA) value (0.30), while 1335-2B × Sonneri had the lowest (-0.20), while for chromium treatment, 1335-2B × Sulltan had the best GCA value (0.267) and 1335-2B × Sonneri had the lowest (-0.133). With lead treatment, the K54TMS × Sulltan exhibited the greatest (0.40) and lowest (-0.40) GCA values recorded for K54TMS × Agatti 84, whereas the F-167 × Agatti 84 demonstrated the highest (0.56) and lowest (-0.33) GCA values for F-167 × Sulltan with combined treatment (Cr+Pb) (table 7).

Grain rows per ear: For the treatment, parents, crosses, parent's versus crosses, lines, and line × tester interaction under control, the analysis of variance was extremely significant. For testers, the results were non-significant. Under chromium treatment, the ANOVA showed that the treatment, parent vs. cross, and tester were all very significant. Crosses produced the findings with significance. Results for the parent's vs. crosses and line × tester interactions were not statistically significant. Under the lead treatment, the analysis of variance showed that the treatment, parents, parent's vs. crosses, crosses, and testers were all very significant. Results for lines and the interaction between lines and testers were not statistically significant. Under combined treatment (Cr+Pb), the analysis of variance for treatment and parents vs. crosses was extremely significant. Crosses, testers, and the interaction between lines and testers produced the most significant findings. The non-significant results were recorded for lines and parent's (table 4). Under control conditions, the general combining ability (GCA) value was found to be highest for F-167 (0.53) and lowest for 1335-2B (-0.8), but with chromium treatment, F-168 had the highest (0.53) and K54TMS lowest recorded with lowest (-0.46) GCA values. When exposed to lead, the F-168 displayed the greatest GCA value (0.4), while the 1335-2B displayed the lowest GCA value (-0.26), whereas the F-167 displayed the highest GCA value (0.33) and the F-168 shown the lowest GCA value (-0.33) for combined treatment (Cr+Pb) among the lines. Sulltan (0.26) and Sonneri (-0.13) had the highest and lowest general combining abilities (GCA) scores, respectively, under control conditions, whereas Agatti 84 (0.80) and Sulltan (-0.40) had the highest and lowest GCA values, respectively, with chromium treatment. The Agatti 84 recorded the highest (0.73) and Sulltan lowest (-0.66) GCA value under lead treatment while Sulltan exhibited highest (0.66) and Sonneri lowest (-0.33) GCA value under combined treatment (Cr+Pb) among testers (table 5 & 6). Pavan *et al.* (2011), and Haddadi *et al.* (2012) found the direct positive relation of grain rows per ear to yield of corn plants as our results are also in conformity with those studies. The SCA was found highest for F-167 × Sulltan (1.06) and lowest for F-168 × Sulltan (-0.60) under control conditions while F-168 × Agatti 84 exhibited highest (0.86) and F-168 × Sulltan recorded with the lowest (-0.93) GCA values under chromium treatment. The F-168 × Sulltan (1) and 2P-735 × Agatti 84 (-0.73) exhibited highest and lowest lowest GCA value respectively, for lead treatment while F-168 × Sulltan recorded highest (1.33) and F-168 × Agatti 84 lowest (-1.66) GCA value for combined treatment (Cr + Pb) (table 7). These results are according with the previous findings of Kanagarasu *et al.* (2010), Zare *et al.* (2011), Dawod *et al.* (2012), Ali *et al.* (2011) and Haddadi *et al.* (2012).

Grain yield per plant: The ANOVA for grain yield per plant indicated highly significant variations for treatment, parents, crosses, parent's vs crosses, lines and line × tester interaction under control. The statistically non-significant results were found for testers. For chromium, lead and combined treatment (Cr+Pb), ANOVA was found highly significant for treatment, parents, crosses, parent's vs crosses, testers, lines, and line × tester interaction (table 4). General combining ability (GCA) results presented in table 5 & 6. Under control, the lines F-167 (10.40) and 1335-2B (-7.66) recorded with highest and lowest GCA value respectively. The highest value was found for K54TMS (12.30) and 1335-2B (-13.41) showed lowest under chromium treatment. The F-168 exhibited highest (6.15) and K54TMS lowest (-3.73) GCA value for lead treatment while 2P-735 recorded highest (12.13) and K54TMS lowest (-11.58) GCA value under combined treatment (Cr+Pb). For testers, GCA value was recorded highest for Sulltan (4.38) and lowest for Sonneri (-2.43) under control while Sonneri exhibited highest (5.85) and Sulltan lowest (-4.93) for chromium treatment. The Sonneri has shown highest (4.45) and Sulltan lowest (-6.12) GCA value for lead treatment while Agatti 84 exhibited highest (2.60) and Sulltan lowest (-3.60) GCA value for combined treatment (Cr+Pb) (table 5 & 6). The cross 2P-735 × Sulltan (13.48) and K54TMS × Sulltan (-16.45) recorded with highest and lowest SCA value respectively for control while 2P-735 × Sulltan exhibited highest (12.89) and 2P-735 × Sonneri lowest (-18.73) SCA values for chromium treatment. The 2P-735 × Sulltan shown highest (9.99) and 2P-735 × Sonneri lowest (-10.28) SCA value for lead treatment while F-168 × Sulltan recorded highest (11.45) and F-168 × Sonneri lowest (-7.94) SCA value under combined treatment (Cr+Pb) (table 7).

Hundrad grain weight: For the control, chromium and lead treatment, the analysis of variance showed that the variables treatment, parents, crosses, parents vs. crosses, tester, lines, and line × tester interaction were all very significant. Under combined treatment (Cr+Pb), the ANOVA was very significant for the treatment, parents, crosses, lines, tester, and line × tester interaction. Parents vs. crosses yielded non-significant findings (table 4). The lines under control, chromium, lead, and combined treatment (Cr+Pb) showed the highest general combining ability (GCA) values at K54TMS (4.94), K54TMS (4.10), K54TMS (2.90), and F-168 (2.76) while the lines 1335-2B (-2.77), F-167 (-4.05), F-168 (-2.41), and K54TMS (-2.53) showed the lowest. Sulltan (5.59), Sulltan (2.68), Sulltan (0.46), and Agatti 84 (0.69) had the greatest general combining ability values, while Agatti 84 (-3.86) and Agatti 84 (-3.55), Agatti 84 (-0.4) and Sonneri (-0.60) had the lowest values under control, chromium, lead, and combination treatment (Cr+Pb) respectively (tables 5 and 6). The SCA value was found to be highest for F-167 × Sonneri (6.50), F-167 × Agatti 84 (4.19), F-168 × Sulltan (4.64), and F-168 × Sulltan (10.49), whereas F-167 × Sulltan (-4.68), F-167 × Sulltan (-4.60), F-168 × Agatti 84 (-3.35), and F-168 × Sonneri (-8.84) recorded lowest under control, chromium, lead and combined treatment (Cr+Pb) respectively (table 7).

S.O.V	DF	CPP				GRPE				GYP				100 GW				HMCG			
		T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
Replications	1	0.32NS	2.10NS	2.10NS	1.00NS	0.00NS	2.41NS	1.64NS	0.07NS	2.44NS	0.01NS	0.00NS	1.07NS	0.50NS	2.18NS	1.97NS	0.06NS		0.81NS	1.12NS	0.08NS
Treatments	22	0.88NS	1.00NS	2.90**	4.82**	7.33**	2.26**	4.07**	1.77**	34.59**	93.42**	256.09**	244.64**	43.27**	142.84**	72.56**	93.16**		31.09**	30.40**	640.89**
Parents	7	0.93NS	1.51NS	1.51*	0.00NS	2.36**	0.91NS	3.00**	0.94NS	35.91**	39.27**	181.26**	224.18**	15.94**	31.99**	102.77**	61.16**		11.96**	18.45**	1296.33**
Parents vs crosses	1	0.00NS	0.21NS	0.35NS	4.80*	117.91**	20.33**	42.60**	9.61**	1.01NS	320.60**	2201.17**	516.43**	25.62**	1339.96**	203.57**	3.61NS		23.54**	91.08**	2463.03**
Crosses	14	0.92NS	0.80NS	3.79**	7.23**	1.91**	1.65*	1.85**	1.62*	36.33**	104.27**	154.56**	235.46**	58.20**	112.75**	48.10**	115.55**	N/A	41.19**	32.04**	183.01**
Lines	4	0.74NS	0.80NS	7.23**	6.13**	3.02**	0.96NS	0.55NS	0.57NS	52.72**	150.15**	136.53**	589.35**	47.45**	136.69**	81.64**	77.82**		46.37**	6.63**	299.68**
Testers	2	0.50NS	0.80NS	2.41NS	4.60**	0.73NS	5.33**	5.83**	2.83*	16.18**	89.46**	380.81**	95.78**	215.59**	260.86**	6.05**	9.18**		70.92**	87.46**	4.06**
Lines x Testers	8	1.12NS	0.80NS	2.41**	8.43**	1.65*	1.07NS	1.50NS	1.84*	33.18**	85.03**	107.02**	93.43**	24.22**	63.75**	41.84**	161.01**		31.18**	30.88**	169.41**
Error	22	0.067	0.042	0.042	0.022	0.727	0.901	0.846	1.178	8.957	3.319	0.790	1.084	1.142	0.395	0.310	0.467		0.010	0.016	0.010

Table 4: Analysis of variance (Fcal) for yield and yield related traits at physiological maturity grown under different treatments.

Lines	CPP				GRPE				GYP				100 GW				HMCG			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
F-167	-0.067	-0.033	-0.100	0.233	0.533	0.200	0.067	0.333	10.407	0.523	-3.663	-9.847	-0.660	-4.050	0.607	2.233	0.000	-0.387	-0.057	-0.463
F-168	-0.067	-0.033	-0.100	0.067	0.200	0.533	0.400	-0.333	8.690	-0.193	6.437	3.137	0.357	-0.367	-2.410	2.767	0.000	0.180	-0.123	1.053
1335-2B	0.100	0.133	-0.100	-0.100	-0.800	-0.133	-0.267	0.333	-7.660	-13.410	-0.680	6.153	-2.777	1.300	0.357	-2.267	0.000	0.163	0.043	0.187
2P-735	-0.067	-0.033	-0.100	-0.100	-0.467	-0.133	-0.267	-0.333	-3.860	0.773	1.637	12.137	-1.860	-0.983	-1.460	-0.200	0.000	0.247	-0.073	0.070
K54TMS	0.100	-0.033	0.400	-0.100	0.533	-0.467	0.067	0.000	-7.577	12.307	-3.730	-11.580	4.940	4.100	2.907	-2.533	0.000	-0.203	0.210	-0.847

Table 5: General combining ability (GCA) of lines grown under different heavy metal treatments (Control, Chromium (Cr), Lead (Pb) and combined (Cr + Pb).

(T0= Control, T1= 5 mM Chromium chloride, T2= 5 mM Lead chloride, T3= Chromium chloride + Lead chloride 2.5 mM of each) *= Significant at 5% probability level, **= Highly significant at 1% probability level, NS= Non-significant, CPP=Cobs per plant, GRPE=Grain rows per ear, GYP= Grain yield per plant, 100GW= 100 Grains weight, HMC= Heavy metal concentration in grain

Testers	CPP				GRPE				GYP				100 GW				HMCG			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
Agatti 84	-0.067	-0.033	-0.100	0.100	-0.133	0.800	0.733	-0.333	-1.953	-0.920	1.673	2.607	-3.863	-3.557	-0.400	0.697	0.000	-0.297	0.040	0.067
Sonneri	0.033	-0.033	0.000	-0.100	-0.133	-0.400	-0.067	-0.333	-2.433	5.850	4.453	0.997	-1.733	0.873	-0.060	-0.603	0.000	0.113	-0.390	-0.063
Sulltan	0.033	0.067	0.100	0.000	0.267	-0.400	-0.667	0.667	4.387	-4.930	-6.127	-3.603	5.597	2.683	0.460	-0.093	0.000	0.183	0.350	-0.003

Table 6. General combining ability (GCA) of testers grown under different heavy metal treatments (Control, Chromium (Cr), Lead (Pb) and combined (Cr + Pb).

Crosses	CPP				GRPE				GYP				100 GW				HMCG			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_1	T_2	T_3	T4
F-167 × Agatti 84	0.067	0.033	0.100	0.567	-0.533	0.200	-0.067	0.667	-6.197	5.987	-3.207	-5.023	-1.820	4.190	1.933	1.687	0.000	-0.703	0.527	0.083
F-167 × Sonneri	-0.033	0.033	0.000	-0.233	-0.533	-0.600	0.733	-0.333	5.583	6.567	1.463	1.537	6.500	0.410	0.293	-1.413	0.000	0.537	-0.143	0.013
F-167 × Sulltan	-0.033	-0.067	-0.100	-0.333	1.067	0.400	-0.667	-0.333	0.613	-12.553	1.743	3.487	-4.680	-4.600	-2.227	-0.273	0.000	0.167	-0.383	-0.097
F-168 × Agatti 84	0.067	0.033	0.100	-0.267	-0.200	0.867	-0.400	-1.667	10.170	-7.947	-1.057	-3.507	-1.087	1.307	-3.350	-1.647	0.000	0.430	0.293	1.117
F-168 × Sonneri	-0.033	0.033	0.000	-0.067	0.800	0.067	-0.600	0.333	-0.600	5.283	0.813	-7.947	-2.767	-4.373	-1.290	-8.847	0.000	-0.280	-0.177	-0.503
F-168 × Sulltan	-0.033	-0.067	-0.100	0.333	-0.600	-0.933	1.000	1.333	-9.570	2.663	0.243	11.453	3.853	3.067	4.640	10.493	0.000	-0.150	-0.117	-0.613
1335-2B × Agatti 84	-0.100	-0.133	0.100	-0.100	-0.200	-0.467	0.267	0.667	-6.780	-4.430	2.360	0.177	1.747	-3.510	-0.067	-0.563	0.000	0.097	-0.173	-0.767
1335-2B × Sonneri	-0.200	-0.133	0.000	0.100	0.800	0.733	0.067	-0.333	-5.150	11.550	4.680	0.987	-2.583	2.660	1.343	1.937	0.000	-0.113	-0.443	1.263
1335-2B × Sulltan	0.300	0.267	-0.100	0.000	-0.600	-0.267	-0.333	-0.333	11.930	-7.120	-7.040	-1.163	0.837	0.850	-1.277	-1.373	0.000	0.017	0.617	-0.497
2P-735 × Agatti 84	0.067	0.033	0.100	-0.100	0.467	-0.467	-0.733	0.333	-8.730	5.837	0.293	6.143	1.080	0.673	-0.250	-1.530	0.000	0.063	-0.507	0.150
2P-735 × Sonneri	-0.033	0.033	0.000	0.100	-0.533	-0.267	0.067	-0.667	-4.750	-18.733	-10.287	1.403	-2.150	-0.357	-0.040	7.020	0.000	-0.047	0.273	-1.120
2P-735 × Sulltan	-0.033	-0.067	-0.100	0.000	0.067	0.733	0.667	0.333	13.480	12.897	9.993	-7.547	1.070	-0.317	0.290	-5.490	0.000	-0.017	0.233	0.970
K54TMS × Agatti 84	-0.100	0.033	-0.400	-0.100	0.467	-0.133	0.933	0.000	11.537	0.553	1.610	2.210	0.080	-2.660	1.733	2.053	0.000	0.113	-0.140	-0.583
K54TMS × Sonneri	0.300	0.033	0.000	0.100	-0.533	0.067	-0.267	1.000	4.917	-4.667	3.330	4.020	1.000	1.660	-0.307	1.303	0.000	-0.097	0.490	0.347
K54TMS × Sulltan	-0.200	-0.067	0.400	0.000	0.067	0.067	-0.667	-1.000	-16.453	4.113	-4.940	-6.230	-1.080	1.000	-1.427	-3.357	0.000	-0.017	-0.350	0.237

Table 7. Specific combining ability of yield and yield related parameters grown under different heavy metal treatments (Control, Chromium (Cr), Lead (Pb) and Combined (Cr + Pb).

(T_0= Control, T_1= 5 mM Chromium chloride, T_2= 5 mM Lead chloride, T_3= Chromium chloride + Lead chloride 2.5 mM of each) CPP=Cobs per plant, GRPE=Grain rows per ear, GYP= Grain yield per plant, 100GW= 100 Grains weight, HMC= Heavy metal concentration in grain

Heavy metals concentration in grain: For all of the treatments (Chromium, Lead, and Chromium+Lead), the ANOVA was very significant for treatment, parents, crosses, parents versus crosses, lines, tester, and line×tester interaction (table 4). The highest general combining abilities (GCA) values were recorded for 2P-735 (0.24), K54TMS (0.21), and F-168 (1.05), whereas lowest GCA values were found for F-167 (-0.38), F-168 (-0.12), and K54TMS (-0.84) when exposed to chromium, lead, and combination treatment (Cr + Pb). The Sulltan (0.18), Sulltan (0.35), and Agatti 84 (0.06) were found to have the highest general combining ability values, whereas Agatti 84 (-0.29), Sonneri (-0.39), and Sonneri (-0.06) showed the lowest values for chromium, lead, and combination treatment (Cr+Pb) respectively (tables 5 & 6). Based on these findings, it can be hypothesized that genotypes (K54TMS, 2P-735, and F-168) with high GCA values can be utilized to create synthetic varieties with high yields due to the existence of additive gene activity. The SCA was found to be greatest for F-167 × Sonneri (0.53), 1335-2B×Sulltan (0.61) and 1335-2B×Sonneri (1.26), while, F-167×Agatti 84 (-0.70), 2P-735×Agatti 84 (-0.50), and 2P-735×Sonneri (-1.12) showed the lowest specific combining ability (SCA) values under chromium, lead, and combined treatment (Cr+Pb), respectively (table 7). We may infer from these findings that the crosses (1335-2B×Sulltan, F-167×Sonneri, and 1335-2B×Sonneri) that exhibited the greatest SCA value can be utilized to create hybrids with the required features. The genotypes (Sulltan and K54TMS×Agatti 84) that take up heavy metals at the lowest concentrations can be suggested for commercial growth and employed in the future breeding program. Ghani, (2010), Mukhtar *et al.* (2010) and Ayesha Qaisar *et al.* (2014) all provided support for these findings.

CONCLUSIONS In conclusion, the genotype Sulltan and hybrid (K54TMS × Agatti 84) were found to only absorb a minimal quantity of the metals (Cr and Pb) both separately and in combination. This suggests that they should be used in breeding programs going forward to increase the genotypes' resistance to heavy metal absorption. Also, the genotypes with high GCA values can be utilized to create synthetic varieties with high yields due to the existence of additive gene activity whereas the crosses which shown highest SCA value can be used for future breeding program for the development of hybrids for desired traits.

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