

Assessing the effect of heavy metals on maize (*Zea mays* L.) growth and soil characteristics: plants-implications for phytoremediation

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Background. Heavy metal pollution has become a global environmental issue and is being raised due to different agricultural practices. Consequently, heavy metals are contaminating the agro-soils, growing crops, and vegetables through such practices. In the present study, besides the phytoremediation potential of maize, the role of chromium (Cr) and lead (Pb) on crop and soil health has been investigated. **Methods.** Two maize varieties Pak-Afgoi and Neelem were grown under varying concentrations of Cr (50-300 ppm) and Pb (30-300 ppm) and different growth parameters i.e. seed germination, leaf size/number, stem girth, plant height, biomass, chlorophyll content, relative growth rate (RGR), and net assimilation rate (NAR) were studied under Cr and Pb stress. Likewise, the effect of metals was also assessed on different soil characteristics including soil texture, pH, EC, soil organic matter, urease activity, soil respiration, and nutrients. **Results.** Studied plant attributes were adversely affected by the imposed heavy metals. Affected values of RGR and NAR showed a linear correlation with affected growth and dry matter yield of maize. Heavy metals impacted different soil parameters including soil microbial performance and revealed a declining trend as compared to control soil. Maize varieties showed a good phytoremediation potential i.e. uptake of Cr and Pb was 33.43% and 22% in Pak-Afgoi, while Neelem showed 37.51% and 24% at 300 ppm, respectively. Data about metal translocation factor (TF), bioaccumulation factor (ACF), and biomagnification ratio (BMR) significantly revealed the potential of maize varieties in the removal of Cr and Pb metals

from the soil. Cr accumulation was higher in shoots, and Pb accumulated in plant roots has shown a differential behavior of metal translocation and affinity with the varieties. These maize varieties may be recommended for general cultivation in the Cr and Pb-contaminated areas.

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potential of maize varieties in the removal of Cr and Pb metals from the soil. Cr accumulation was higher in shoots, and Pb accumulated in plant roots has shown a differential behavior of metal translocation and affinity with the varieties. These maize varieties may be recommended for general cultivation in the Cr and Pb-contaminated areas.

Keywords: metal pollution, plant growth, RGR, net assimilation rate, chlorophyll, phytoremediation, environmental contamination, and remediation

1. Introduction

Abiotic stresses including metal toxicity, salinity, temperature extremes, soil microplastic, and drought are enormous threats that are affecting agriculture and the natural environment (Wang *et al.*, 2003; Hasanuzzaman *et al.*, 2020; Li *et al.*, 2023). Heavy metals are one of the abiotic factors and are demarcated as metals with a density above 5 g/cm³ e.g., chromium (Cr), lead (Pb), nickel (Ni), cobalt (Co), arsenic (As), silver (Ag), etc. These heavy metals diverge in physical and chemical properties; and are taken as substantial environmental pollutants owing to their toxic interaction with soil properties, plants, animals, and humans (Hasan *et al.*, 2009; Das and Jayalekshmy, 2015; Zhang *et al.*, 2019; Karkush and Ali, 2020). An incessant boost of heavy metals in the agricultural soil system is over diverse agricultural practices including the use of industrial and sewage waste-waters as a crop irrigation source. Irrigation of vegetables and fodder crops with such a kind of wastewater through their discharge into freshwater bodies is a common practice (Khan *et al.*, 2003); and hence is the foremost source of heavy metal pollution for intact growing crops in the peri-urban areas (Mussarat *et al.*, 2007).

Chromium (Cr) is a toxic pollutant and is ranked as the 17th most toxic element among the hazardous substances (Wakeel *et al.*, 2020). Its density is 7.15g/cm³ and is ranged from 10 to 50 mg/kg of soil, naturally (Kousar and Khan, 2021). Cr is used in electroplating, textile dying, paint, metallurgy, pigment, and tanning industry. Similarly, sewage and fertilizers are also included as the main source of Cr (Amin *et al.*, 2013). Due to different oxidation states, Cr acts as a toxic element for organisms. However, the toxicity of Cr to plants depends on its uptake mechanisms, concentration, and focal plant species (Gardea-Torresdey *et al.*, 2005; Peternella *et al.*, 2021).

Lead (Pb) is ranked as the second most toxic metal on earth's crust and is toxic to humans and other living things including plants. Its density is 11.34 g/cm³ (WHO, 2010). Several industrial processes include Pb-use in their products like oil and paint, mines, agrochemicals, etc. Moreover,

Pb as salts or oxides is also being added to the environment through atmospheric dust, and automobile exhaust (IARC, 2012; Kumar *et al.*, 2019). In nature, Pb remains below 50 mg. kg⁻¹, but in some plants, Pb usually inhibits the growth mechanism when it is at a concentration of 30 mg/kg or more (Usman *et al.*, 2020), while some of the plant species can tolerate Pb stress up to 1000 mg. kg⁻¹ (Revees *et al.*, 2018). Both Cr and Pb have a strong effect on different growth attributes of exposed plants (wheat, maize, barley, sunflower, mustard, and soybean); and inhibit seed germination, plant height, root-shoot length, fresh-dry weight of seedlings, tolerance index, leaf number and photosynthesis (Orhue and Ekhomun, 2010; Naseem *et al.*, 2015; Akhtar and Iram, 2017; Kanwal *et al.*, 2020).

Heavy metals pretentiously affect different soil properties, particularly in clayey soils. Changes in soil properties depend upon the mobility and chemical activity of heavy metals in the soil predominantly when these metals exceed the accepted limits (Karkush *et al.*, 2013; Uddin, 2016; Karkush and Ali, 2020). Metal ions have acidification effects on the intact soil and lower the pH of the soil (Motuzova *et al.*, 2011). Soil pH is an important parameter that significantly affects the accessibility of soil nutrients available to the growing crops, affects their yield, and hence, acts as the key factor in sustainable agriculture (Ludwig *et al.*, 2001; Najafi and Jalali, 2016). Soil respiration (CO₂ evolution) is an indicator of the use of energy by soil microbes concerning their efficiency in degrading the soil organic material (Wardle and Ghani, 1995).

In agricultural management, microbes are taken as soil indicators for the affecting external abiotic stresses including heavy metals (Hassan *et al.*, 2013c), and are quite sensitive to such tresses. Microbes release important extracellular enzymes in the soil system, which are the key regulators of soil biochemical processes (Wang and Yanli, 2013). Soil urease is one of the most concerning extracellular enzymes released by microbes to hydrolyze soil urea into CO₂ and ammonia (Gulser and Erdogan, 2008). Similarly, soil enzymes act as biological catalysts and facilitate different soil reactions and metabolic processes of the biogeochemical cycles of soil nutrients to maintain soil fertility for growing crops (Moreno *et al.*, 2003).

As the heavy metals put adverse effects on soil properties, some efficient and cost-effective techniques are needed to restore the metal-affected agro-soils. Phytoremediation is a biological remediation technique that has received a lot of attention during the last few years. Nevertheless, plant efficiency for phytoremediation depends upon the type, availability, and concentration of heavy metals. Phytoremediation easily removes metal contaminants from the affected soil than

other remediation options (Marques *et al.*, 2009). It increases soil fertility through the release of different organic matter (from plant body) and hence, maintains the physical and biological properties of the soil (Aken *et al.*, 2009; Wuana and Okieimen, 2011; Jacob *et al.*, 2018). Plants used in the phytoremediation of heavy metals may be hyper-accumulator or Phyto-stabilizer. Family Brassicaceae (*Alyssum bertolonii* ; *Thlaspi caerulescens*) and Asteraceae (*Calendula officinalis* ; *Tagetes erecta*) have a greater hyper-accumulating ability (Glick, 2012). Similarly, the phytoextraction potential of soybean (*Glycine max* L.) and rice (*Oryza sativa* L.) for phytoextraction of cadmium-polluted lands has also been reported by Murakami *et al.*, (2007). Moreover, *Lolium perenne* (L.), *Panicum aquaticum*, *Typha species*, *Vetiveria zizanioides*, and *Paspalum fasciculatum* have also been documented as good phytoremediation tools for Cd, Cu, As, Zn, Cr, and Pb by Glick, (2012), Alvarenga *et al.*, (2009), Andra *et al.*, (2009), Dipu *et al.*, (2012) and Pires-Lira *et al.*, (2020).

Data were collected as previously described by Atta *et al.*, (2023) that due to excessive irrigation with wastewater, Cr and Pb have become the most frequent and health risk metals to the consumers of the study area (Dera Ghazi Khan, Punjab-Pakistan). Similar findings about heavy metal pollution in Dera Ghazi Khan have been reported by Rafique *et al.*, (2016). To our knowledge, the toxicity of Cr and Pb on soil physicochemical properties and soil enzyme activity under maize cultivation has not been documented adequately in the study area. Therefore, a pot experiment was set to understand the effect of heavy metals not only on plants (*Zea mays*: *Poaceae*) but also on different soil characteristics. Moreover, this study also uncovered the phytoremediation potential of maize to combat the heavy metal issue in the future under the particular environmental conditions of the area. The maize crop is exceptionally grown as a fodder crop in this study area; therefore maize has been selected for the current phytoremediation study.

2. Materials & Methods

2.1. Experimental Design, Germination and Growth Attributes

To evaluate the metal toxicity and phytoremediation potential of maize varieties, separate Petri plate and pot experiments were conducted in a block design during July-September 2018-20 (Temp. 35°C -32 °C, Humidity 56% -59%). Open-pollinated varieties (OPV) of maize (Pak-Afgoi & Neelem Desi) were used in the trial. Potassium dichromate and lead nitrate ($K_2Cr_2O_7$) &

129 $Pb(NO_3)_2$ were used and a stock solution was prepared *viz.* Cr (50, 100, 150, 250, 300 ppm) and
 130 Pb (30, 60, 100, 150, 300 ppm).
 131 Each treatment comprised of eight replicates followed by three maize plants per treatment.
 132 A seed germination test was performed in the laboratory at room temperature (30°C). To prevent
 133 fungal infection during the experiment, the selected seed material was thoroughly washed with 2%
 134 sodium hypo chloride for 5 minutes and then rinsed with distilled water (Kaya *et al.*, 2019). Seeds
 135 were imbibed in distilled water for 30 minutes and then were air-dried. For either variety, each
 136 Petri plate (10 cm in diameter) was employed with two filter papers and 10 seeds following four
 137 replicates per treatment. Each Petri plate was moistened with 10 ml of the metal solution while the
 138 control treatment continued with distilled water. Overall, the plates were observed daily for
 139 moisture/treatment requirements. Germinated seeds with 1 mm radicle were counted daily till the
 140 final germination day (day 10). Percent seed germination was determined following the study of
 141 Akinci and Akinci (2010) using the formula:

$$[\text{Germination (\%)} = \text{Total seeds germinated} / \text{total seeds arranged} \times 100]$$

142 Pre-washed, cleaned, dried, and labeled plastic pots of varying identifiable colors [dimension (cm):
 143 30.5 Diameter x 46 deep] were smoothly filled with 12 kg of the agro-soil (clay 63.1%, sand
 144 29.7%, saturation 54%, EC (mS/cm) 2.8, pH 7.6, SOM content 2.7%, available-P 8.2 ppm,
 145 available-K 182 ppm, and 2.8% N). For either variety, healthy seeds of uniform size were sown
 146 1-2 inches deep in the topsoil of the pots. After ten days of establishment, the seedlings were
 147 thinned by removing weak seedlings, and metal treatment was simulated for up to four weeks.
 148 Different growth attributes i.e. plant length, leaf area (Aliu *et al.*, 2010), leaf chlorophyll (by SPAD
 149 meter), plant fresh and dry weight, relative growth rate (RGR), and net assimilation rate (NAR)
 150 were assessed for the varieties at Harvest-1 and Harvest-2 i.e. 25th and 40th day of growth (Table
 151 1). RGR and NAR were assessed by using the method of Causton and Venus (1981) by the given
 152 formula:

$$RGR = \text{Loge } w_2 - \text{Loge } w_1 / t_2 - t_1$$

154 where, w_2 = plant dw at harvest time of 40 d (t_2), w_1 = plant dw at harvest time 25d (t_1)

$$NAR = 2(w_2 - w_1) / (LA_1 + LA_2)(t_2 - t_1)$$

156 where, w_2 = leaf dw at harvest time 40 d (t_2), w_1 = leaf dw at harvest time 25 d (t_1), LA_1 = leaf area
 157 measured at harvest time 25 d (t_1), LA_2 = leaf area measured at harvest time 40 d (t_2)

2.2. Determination of Soil Parameters

Soil texture (including clay 63.1%, sand 29.7%) was determined with the Bouyoucos hydrometer method by preparing a soil paste that was saturated with distilled water (Sheldrick and Wang, 1993). Soil pH (H₂O) and EC (mS/cm) were determined using a pH and EC meter, respectively. For this purpose, soil-water suspension 1:2.5 (w/v) was prepared, and the cathode of the meters was dipped into it (Hassan *et al.*, 2013c).

2.2.1. Determination of SOM

Soil organic matter content was determined by the method of Walkley and Black (1934). For this purpose, reduction of Cr ion by soil organic matter and an unreduced Cr₂O₇²⁻ was measured. 0.5 g ground and sieved soil mixed with 10 ml K₂Cr₂O₇ (1M) followed by the addition of 20 ml conc. H₂SO₄. The sample was well shaken for 30 minutes and the final volume was raised to 200 ml by distilled water. Afterward, soil material was titrated against acidified 0.5M ammonium ferrous sulfate. Reading of the sample was manipulated from blank upon the appearance of a green endpoint.

2.2.2. Determination of soil urease activity

Soil urease activity (UA) was determined by the method of Kandeler and Greber (1988) as described by Hassan *et al.*, (2013c). For this purpose, metal-treated 5 g soil was mixed with 10 ml of urea solution; and then 10 ml of buffer solution (citric acid, KOH, and NaOH) having pH 6.7 was also added to it. This solution was incubated at 37 °C for 24 hours. After filtration, the solution was mixed with reagents (phenol + NaOH); to this solution, sodium hypochlorite solution was also added. The absorbance of the appeared blue color was noted at 578 nm through a spectrophotometer.

2.2.3. Determination of soil respiration

For the determination of soil (microbial) respiration, a laboratory incubation experiment was performed to measure soil respiration under two different heavy metals following the method of Anderson (1982) as described by Devi and Yadava (2009). Soil samples were moistened with the five respective doses of either metal and were placed in closed jars provided with test tubes containing NaOH and distilled water test tubes. Evolved carbon dioxide over time was trapped by NaOH titrated with the acid of known normality.

$$[\text{formula: mg of CO}_2 = V \times N \times 22]$$

where V = volume of acid used against 10 ml NaOH N = normality of acid used; and the value 22 is a factor for CO_2 evolved during reaction.

2.2.1. Estimation of soil nutrients (N , P , K)

Soil K^+ was assessed by flame photometer taking a soil sample (2.5 g) by shaking with 33 ml of 1M KCl following Anderson and Ingram (1993). The excess K^+ in the soil sample was washed three times with 95% ethanol and the adsorbed K^+ was then extracted by addition of 33 ml of 1M NH_4OAc . The volume of this extract was raised to 100 ml and further added with 1M NH_4OAc to estimate K^+ in the extract. Similarly, soil phosphorus was assessed following the method described by Olsen and Sommers (1982). For available phosphorus, 5 g of soil was obtained in a 250 ml flask, and 0.5M NaHCO_3 (100 ml) was added to it. This solution was shaken for 30 minutes and then the filtrate was collected. Ten ml of the filtrate was shifted into a flask of 50 ml along with 1 ml of 5N H_2SO_4 (sulfuric acid) and the volume was increased up to 40 ml by adding distilled water. To this solution, 8 ml of ascorbic acid as a reagent was added to develop color; and transmittance was recorded at 880 nm using a spectrophotometer. Soil nitrogen was determined by Kjeldahl's method (Ahmad *et al.*, 2011) using the formula:

205

206
$$N (\%) = \frac{\text{acid used for sample} - \text{acid used for blank} \times \text{acid normality} \times 14.01 \times 10}{\text{volume of sample}}$$

207 $\times 100$

208

209

210 *2.3. Metal Detection in Soil and Plants*

211 For determination of soil metal content, maize plants were separated from contaminated pot soil
 212 was taken out from the respective pots. These samples were executed following the method of
 213 Welz and Sperling (1999) using an atomic absorption spectrometer (Perkin-Elmer). Hot acid
 214 digestion was used for a 1 g soil sample using 15 ml of the acid mixture in a 5:1:1 ratio (70%
 215 HNO₃, 70% H₂SO₄, and 65% HClO₄). After cooling, the transparent acidic solution was filtered
 216 (by Whatman no. 42) and diluted with distilled water. Metal analysis was carried out at analytical
 217 spectral lines i.e. Cr: 357.9 nm, and Pb: 283.3 nm. A similar digestion procedure was executed for
 218 plant metal detection. For metal accumulation and translocation study, different plant parts (root,
 219 stem, leaves, etc.) were used. In the treated plants, the bio-magnification ratio (BMR) and metal
 220 accumulation factor (ACF) was assessed by the method of Baker *et al.*, (1995) whilst the metal
 221 translocation factor (TF) was calculated according to Yanqun-Zu *et al.*, (2005) using the following
 222 equations:

223

224
$$BMR = PU/MA$$

225
$$ACF = PU/MT$$

226
$$TF = \text{Element (shoot)}/\text{Element (root)}$$

227 Where, PU = metal concentration in whole plant (µg g⁻¹), MA = available metal concentration in
 228 soil (µg g⁻¹), MT = total metal concentration in soil (µg g⁻¹)

229

230 *2.4. Quality control analysis and assurance*

231 Chemical analysis of samples was performed by AAS and spectrophotometer. High grade standard
 232 chemicals and glass ware were used (Merck-Germany). By using a calibration curve, calibration
 233 of instruments was executed with a series of standard solutions of varying concentrations. The
 234 chemical stock solution was prepared with double-deionized water. Glass ware was used after
 235 cleaning and rinsing with diluted HNO₃ to avoid some probable contamination. For quality results,

236 each sample was analyzed in a repeated way by following the standard reference procedure (Atta
237 *et al.*, 2023).

238

2.5. Data analysis

For comparison of the significance level of means under metal treatment, analysis of data was performed by calculating the F-value from ANOVA test using a statistical package IBM-SPSS (V. 20). While error graphs (LSD 5%) were prepared in MS-Excel.

3. Results

3.1. Effect of Cr and Pb on plant growth-related parameters

3.1.1. Seed germination

The Cr and Pb treatments suppressed the maize seed germination in a concentration-dependent manner (Table 1). The decrease in seed germination of both varieties was much more obvious at 150 ppm Cr and 100 ppm Pb treatment. At the highest concentration (300 ppm), Cr and Pb inhibited the seed germination of Pak-Afgoi by 67% and 64% and Neelem by 68% and 73%, respectively.

3.1.2. Green leaves and leaf area

Table 1 shows a minor suppressive effect of Cr and Pb at the early growth stage of maize varieties. Green leaf count was not significantly affected in Pak-Afgoi as compared to the variety Neelem. Cr rapidly decreased this agronomic trait at 150-300 ppm (Pak-Afgoi 5-10%; Neelem 5.9-29.41%) and 100-300 ppm Pb application (Pak-Afgoi 5-10%; Neelem 18-29%). A decline in leaf count was more at 300 ppm of Cr and Pb. Likewise, leaf area was also decreased along with the increasing metal doses in a more significant way, and decreased much at elevated levels of Cr and Pb (300 ppm), whereas Neelem declined more than Pak-Afgoi (29.9% and 31 %). Likewise, leaf area was also measured at 25th d (harvest-1) which decreased by 21-29% in Pak-Afgoi, while a decrease in Neelem was up to 32% under Cr and Pb stress.

3.1.3. Plant Height

Pb has more adverse effects on plant height than Cr. Comparatively, Neelem showed a pronounced decreasing trend for this agronomic trait. During the early growth stage, the maximum plant length for Pak-Afgoi and Neelem was recorded up to 68 cm and 54.1 cm, respectively. Plant height decreased much at higher metal concentrations (300 ppm). Plant height decreased in Pak-Afgoi under Cr and Pb by 22% and 23%, respectively. Plant height also decreased in Neelem by 32%

267 and 45%, respectively (Table 1). Both the varieties showed a tolerant behavior and were least
 268 affected at 50 and 30 ppm of Cr and Pb, whilst rapidly declining at 150 ppm Cr and 100 ppm Pb.
 269

3.1.4. Shoot girth

Both Cr and Pb affected the shoot girth of maize varieties in a declining and concentration-dependent pattern. During the early growth stage, the maximum shoot girth of Pak-Afgoi and Neelem was 6.2 cm and 5.5 cm in the control treatment, whilst the mean decrease in shoot girth of Pak-Afgoi viz. varying concentration of Cr and Pb metals was 11-11.3% and 13%, respectively. However, at 300 ppm of Cr and Pb; the mean decrease was more than 19.3% (Pak-Afgoi) and 27% (Neelem). Maize variety Neelem was less affected than Pak-Afgoi for this circumference trait (Table 1).

3.1.5. Plant biomass

Plant fresh weight and dry weight were assessed under two different metals that revealed metal-induced toxicity on plants. Statistical analysis has predicted a less significant effect of metals on the fresh weight of Pak-Afgoi than of Neelem. Comparative to the control value (46 g) and to the low concentrations of Cr and Pb, fresh weight decreased more up to 17% at 300 ppm. Fresh weight of Neelem in the control treatment was 26.6g later decreased up to 27% by Cr and 32% by Pb, likewise Pak-Afgoi (Table 1).

Plant dry weight also decreased both at harvest-1 and harvest-2 (Table 1). Pak-Afgoi attained a maximum of 16.2g dry weight that decreased up to 38.3% (Cr, Pb). Similarly, Neelem attained 14g dry weight as the control value and later underwent a significant decrease due to metal toxicity at 300 ppm of Cr (43%) and Pb (45%). Overall, data shows a rapid decrease in plant biomass parameters observed from 150 ppm (Cr) and 100 ppm (Pb) than at lower metal concentrations.

3.1.6. Relative growth rate (RGR) and net assimilation rate (NAR)

Data about RGR (g. d^{-1} increase in dry matter) revealed that both maize varieties continued growing viz. control and metal treatments and gained dry matter between the two harvests (H2-H1). However, a mean decrease in plant growth rate was noted by 28% & 31% (Pak-Afgoi), 31% & 37.5% (Neelem) under Cr and Pb, respectively. At maximum dose (300 ppm) of Cr and Pb, RGR decreased by 48.4% & 49% in Pak-Afgoi, while 53% & 56% in Neelem, respectively.

The decreasing trend of RGR and dry matter of plants strongly showed an affected accumulation of metabolites/ photosynthate between the two harvests due to metal stress. A similar decreasing trend was observed in the case of NAR. Metal treatment has revealed increased metal toxicity from H1-H2 (as the plant spent more under stressful conditions). A mean decrease in NAR under the Cr

effect in Pak-Afgoi and Neelem was 10% & 17%, whilst Pb affected this parameter by 21% & 22.2%, respectively. The decline in RGR and NAR was elevated at elevated metal concentrations (Table 1).

3.2. Effect of Cr and Pb on soil physicochemical properties

3.2.1. Soil pH

Soil pH of the control soil was 7.8, lowered up to 6.7 and 6.5 under Cr and Pb application at H2 (day 40), respectively. Results have shown a mild effect of metals on soil pH up to 100 ppm Cr and 60 ppm Pb during this incubation period. The effect of metals increased and lowered soil pH more at elevated metal doses (Table 2).

3.2.2. SOM content and urease activity

The control value of SOM at H2 was 2.8 percent, whilst a rapid decrease in SOM content was initiated at 150 ppm Cr and 100 ppm Pb. The mean cumulative decrease in SOM content was 17% & 20% under Cr (50-300 ppm) and Pb (30-300 ppm) toxicity. At 300 ppm Cr and Pb application, SOM content decreased by 43% and 46%, respectively (Table 2). Soil urease activity (UA) was found affected by different concentrations of the metals. At harvest time (day 40), the mean decrease in UA was up to 22% & 26% due to Cr and Pb, respectively. Table 2 shows that enzyme activity was much more affected at the elevated metal concentrations than at lower. Although lower metal doses had the least effect on UA, a clear decline in UA was initiated at 100 ppm level of the metals which turned to its peak at 300 ppm i.e. decreased under Cr (41%) and Pb (47%).

3.2.3. Soil respiration: (Evolution of CO₂)

Table 2 shows the impact of soil metals on the amount of soil CO₂ under cultivation of maize varieties. This parameter of the soil decreased from 176 mg to 124 mg. The mean effect of metal treatments (Cr 50-300 ppm; Pb 30-300ppm) showed metal toxicity following a significant decrease in soil respiration (SRP), compared to control. SRP decreased more by Cr (14%) than Pb (9.5%) showing much Cr-toxicity on SRP. However, differentiating the treatment effect, 300 ppm Cr and Pb level imposed drastic effects on this parameter by 29% & 23%, respectively.

3.2.4. Soil nutrients (NPK)

Both Cr and Pb treatments have toxic effects on available soil nutrients. Soil nitrogen (N), phosphorus (P), and potassium (K) contents were decreased gradually at lower metal concentrations than at maximum levels. Comparing the toxicity of Cr and Pb on NPK contents at harvest (day 40), the mean decrease in N, and P was more under Pb stress (31%, 7%) than Cr stress (25%, 6.8%). Likewise, K^+ was less affected under Pb stress (13%) than Cr (15%), respectively. The decreasing order of NPK at the maximum metal concentration of Cr and Pb was 42%, 17%, 32%, 47%, 22%, and 29%, respectively (Table 2).

3.2.5. Metal uptake from soil (bioaccumulation in plant tissues)

At harvest-2, plant parameters showed a substantial effect of metal toxicity along with the increasing metal concentrations. Cr accumulation was more in the stem than roots and leaves, whilst Pb accumulated more in the roots than stems and leaves of the test varieties (Figure 1). In Pak-Afgoi and Neelem, maximum Cr was accumulated in the stem (61.2 μ g/g & 68.5 μ g/g) at its highest concentration in the soil medium. Similarly, Pb accumulation in the stem part of Pak-Afgoi and Neelem was observed at 17.2 μ g/g and 19.2 μ g/g, respectively. However, Pb contents in the roots were 47.3 μ g/g in Pak-Afgoi and 51.2 μ g/g in Neelem. Although, metal accumulation in different plant parts was increasing way, however, a hasty metal up taken by plants occurred at 150 ppm (Cr) and 100 ppm of Pb (Figure 2).

The phytoremediation potential of maize varieties was assessed by calculating the metal accumulation factor (ACF), translocation factor (TF), and bio-magnification ratio (BMR). Results clearly showed significantly increased values of Cr and Pb metals for ACF, TF, and BMR by variety Neelem than Pak-Afgoi. However, both the varieties significantly removed Cr and Pb content from the soil and accumulated in different parts successfully (Figure 3).

4. Discussion

Results from the Petri plate experiment have shown that both the tested varieties undergo abiotic metal stress and seed germination decreased with the increasing concentration of Cr and Pb. Studies on seed germination characteristics showed its inhibition under metal (Pb) toxicity even at low or micromolar levels (Kopittke *et al.*, 2008). However, there are few reports about the progression of seed germination and inhibition of radical / hypocotyl length in *Elsholtzia argyi* (Islam *et al.*, 2008), but the same was not observed in the present course of the investigation. All

the concentrations of Cr and Pb were found to be inhibitory for maize germination and, inhibition exceeded along with the increasing metal concentrations in the medium. Arguments by Sengar *et al.*, (2008) revealed inhibition of germination due to interference of Cr and Pb with the essential enzymes for seed germination (amylase and protease). Moreover, Atici *et al.*, (2005) documented that inhibition of GA₃ (gibberellic acid) and activation of ABA (abscisic acid) during germination of *Cicer arietinum* (chickpea) were due to Zn, Pb, and Cd metals.

Heavy metals are considered the major environmental toxins that adversely affect all living organisms including plants (Ashraf *et al.*, 2018, Bargagli *et al.*, 2019). The toxic effects of metals on different growth attributes in plants are due to abnormal nutrient uptake from plant roots as metals become stuck in roots and oppose nutrient uptake from the soil (Singh *et al.*, 2016). Different agronomic parameters of rice plants i.e. plant length, tiller count, and dry weight biomass undergoes significant reduction due to Pb doses 0.6 mM - 1.2 mM. Observations highlighted less toxicity at a lower Pb dose of 0.6 mM than at 1.2 mM of Pb. A decline in the length of rice plants at the maximum Pb dose was 12.97% and dry weight decreased by 61% in cultivar Ilmi (Khan *et al.*, 2021). A similar observation was reported by Orhue and Ekhomum (2010). Cr affected plant height and dry matter in waterleaf after 100 mg Cr dose. Reduced plant length was due to Cr accumulation that suppressed mitotic activity in the affected plants. Decreases in plant fresh weight due to Cr-toxicity at the varying extent of Cr concentrations also have been reported in *Hibiscus esculentus* (Amin *et al.*, 2013), *Helianthus annuus* (Fozia *et al.*, 2008), and *Brassica oleracea* (Ozdener *et al.*, 2011). These studies also revealed that a decrease in growth and biomass parameters of the subsequent plants occurs due to increasing metal levels in the growth medium. In agreement with these earlier studies, the present study has also revealed the negative effect of increasing concentrations of Cr and Pb on maize growth (Figure 4).

Plant growth is attributed mainly to leaf characteristics and the photosynthetic performance of plants. Metals (Cr and Pb) are well-known abiotic stressors that inhibit the photosynthetic performance of intact plants and finally affect plant growth and biomass yield (Houry *et al.*, 2020). Altered values of RGR also predicted the affected plant growth whereas, NAR revealed the negative impact of metals on photosynthetic performance and product of photosynthesis i.e. dry matter content. A leaf is an important photosynthetic organ of plants that plays a key role in the growth of plants. Pb and Cr adversely affect the growth and development of leaves in *Lycopersicon esculantum* (L.), *Pisum sativum*, and *Zea mays* (Yoon *et al.*, 2006; Anjum *et al.*, 2016). Studies

showed the inhibitory role of heavy metals on leaf growth and development in rice plants through the generation of oxidative stress / ROS (Singh *et al.*, 2020). These studies are strappingly evident the findings of the present study that leaf number and leaf area in tested plants of maize significantly declined upon exposure to Cr and Pb doses.

Chlorophyll is one of the crucial molecules to facilitate photosynthetic activity in plants and is responsible for the electron transport chain to step forward the photosynthesis. However, heavy metals are responsible for altering the chloroplast structure and cause inhibition of the electron transport system by affecting its biosynthesis (Wakeel *et al.*, 2020) through increased activity of chlorophyllase reported reduced chlorophyll biosynthesis and affected activity of NADPH protochlorophyllide oxidoreductase enzymes under Hg-toxicity. In continuation, Singh *et al.*, (2020) investigated Cr⁺⁶-induced alterations up to 89% in the chlorophyll content of mung beans (*Vigna radiata* L.). A greater decline was at the highest concentration of 120µM than at lower doses of 60-90 µM. Studies on Cr and Pb stress in *Nicotiana tabacum* and *Cicer arietinum* by Bukhari *et al.*, (2016) and Singh *et al.*, (2020) have also supported the findings of the present study. Heavy metals cause land degradation through soil acidification that happens due to the leaching mechanism of toxic metal ions. Soils presenting a low pH profile make metals to be available for growing plants and thus reduce crop yield (Xu *et al.*, 2012). Although soils pose resistance to the pH change and act as a buffer (Curtin and Trolove, 2013); the long-term application of heavy metals put acidification effects on the subsequent soils. Heavy metals undergo hydrolysis in a solution of such soils, generate H⁺ ions, and lower the pH (Moutuzova *et al.*, 2011; Schwertfeger and Hendershot, 2012). Consequently, soil acidification results in nutrient depletion and affects crop plants (Najafi and Jalali, 2016).

Soil organic matter (SOM) is assumed as a potential source for microbial activity in the agricultural soils and releases nutrients into the soils through the degradation of soil organic components. Likewise, SOM also shows a large sorption affinity toward metals (Yin *et al.*, 2002). Likewise, microbes release certain enzymes of key value into the soil. These enzymes (urease, dehydrogenase, and phosphatase) are dynamic in the biochemical functionality of the soils including the decomposition of SOM. Hence, the soil enzymes are referred to as an indicator of soil quality, a good measure of soil microbial activity with the recycling of nutrients from the decomposed SOM (Puglisi *et al.*, 2006). Soil urease is a sensitive hydrolyzing enzyme and is a potential indicator of soil pollution and biological activities (Hinojosa *et al.*, 2004). Moreover,

despite the positive role of urease in soil chemistry; the addition of varying doses of Cu, Cd, Zn, Pb, and Cr decreases the activity of soil urease at large, in contrast depending on the incubation period (Malley *et al.*, 2005; Shen *et al.*, 2005). This might be correlated with the decomposition of SOM.

Soil respiration (CO₂ evolution) is another parameter to assess soil microbial performance with the decomposition of SOM in the subsequent soils (Nawaz *et al.*, 2015). Verma *et al.*, (2010) have reported a decreased rate of SOM decomposition under Cd, Cr⁺⁶, and Pb stress vide different incubation periods. Toxicity of all three metals was found to increase towards SOM-decomposition and soil respiration (CO₂ evolution) along with the increasing metal treatment and incubation period. Investigations by Algaidi *et al.*, (2013) have extended the Zn, Pb toxicity on aerobic bacteria. An elevated level of both metals significantly decreased the physiological activities of soil microbes and CO₂ production. Similar evidence has been provided by Mathe-Gaspar *et al.*, (2005) for Zn, Cu, and Cd metal ions.

Soil microbial activity and biodegradation of SOM content play a vital role in the soil fertilization process, cycling of nutrients, and hence increasing the soil fertility (Kumar *et al.*, 2019). Likewise, the soil urease enzyme has a potential role in soil N-cycle due to its hydrolytic properties. Consequently, the difference between pre and post-harvest soils revealed a remarkable decline in the available nitrogen under Cr and Pb stress is in agreement with the experimental outcomes of Orhue and Ekhomum (2010) i.e. increasing concentration of Cr⁺⁶ continues to decline the soil N by 38.46% at highest Cr dose 200 mg. A similar observation was reported for soil P and K availability in the present study, indicating the Cd-affected activity of dehydrogenase and phosphatase (Hassan *et al.*, 2013c). The present study also revealed similar effects of Cr and Pb on soil macronutrients and indicated the metal toxicity on mineral cycling with the affected SOM and enzymatic activity i.e. urease for N-cycling in the treated soil.

Crops grown on metal-contaminated soils have a greater accumulation of these metals than crops grown in uncontaminated soil (Sharma *et al.*, 2008). Plants have a natural capacity to absorb metal ions from the soil even in low concentrations through their root system. To attain efficient reclamation of metal-contaminated soil, plant roots form a rhizosphere ecosystem, absorb and accumulate the heavy metals and improve soil fertility (Jacob *et al.*, 2018). Hyper-accumulator plant species were found to be effective in the removal of metals. Plant species that have the potential of accumulating a major portion of metals from the soil are referred to as hyper-

451 accumulators; and are used in phytoremediation techniques to remove the pollutants (Clemens,
452 2006). However, phytoremediation potential exactly depends on the plant's capacity to extract
453 heavy metals from the intact environment and bio-accumulating them in various plant parts
454 without having adverse effects on soil structure, fertility, and biological activity (Yan *et al.*, 2020).
455 For instance, *Paspalum fasciculatum* showed the potential of accumulating Cd and Pb in declining
456 order of metal concentrations in roots > leaves > stem. Cd uptake was recorded more than Pb,
457 revealing this plant to be phytostabilizing as the maximum Cd amount was accumulated in roots
458 (Salas-Moreno and Marrugo-Negrete, 2020). Likewise, phytostabilization of ryegrass (*Lolium*
459 *perenne* L.) also showed to be potential for removal of Cd, Cu, As, Zn, and Cr (Alvarenga *et al.*,
460 2009). *Panicum* grass also exhibited a maximum accumulation of Pb in roots than in shoot i.e.
461 roots accumulate 96% more lead as compared to shoot (Pires-Lira *et al.*, 2020). In the present
462 study, uptake of Cr and Pb (Afgoi 33.43%, 22%; Neelem 37.51%, 24%) at 300 ppm by maize
463 varieties during EGS has uncovered the emergent potential i.e. hyper-accumulation and
464 phytostabilization of this crop cultivated under particulars soil and environmental conditions of
465 Dera Ghazi Khan. A higher portion of Cr metal was observed in stem tissues than in roots and
466 leaves. Likewise, Pb accumulation was more prominent in roots than stems and leaves of the
467 subsequent maize plants. The phytoaccumulation potential of maize variety Neelem was more
468 remarkable than Pak-Afgoi, indicating maize varieties as hyper-accumulators for Cr with
469 phytostabilization for Pb metal. These maize plants contain higher Cr and Pb levels than the
470 WHO/FAO permissible limits i.e. Cr 2.3 mg/kg and Pb: 0.3 mg/kg (Aderinola and Kusemiju,
471 2012). The contaminated maize plants are recommended as unsafe for health and be destroyed
472 systematically by burning in high-temperature cement kiln of *D.G. Khan Cement Company* which
473 is available in the study area. Moreover, in the future, screening of different native plant species
474 for phytoremediation purposes along with the focus on their biochemical responses, and tolerance
475 mechanisms is suggested. Application of phosphorus increases soil fertility through increased
476 microbial activity and improves soil nutrient status. The efficacy of soil enzymes (urease,
477 phosphatase) to recycle the nutrients turns high due to phosphorus implication in the contaminated
478 soil (Iqbal *et al.*, 2023). Similarly, the microbial role of bioremediation may be another choice to
479 reclaim contaminated soils. At present, to prevent further addition of heavy metals into agro-soils,
480 irrigation with municipal and industrial wastewater should be banned or if irrigation with the

wastewater is continued, it should be recycled through wastewater treatment plants (Atta *et al.*, 2023).

5. Conclusion

Both heavy metals are toxic to seed germination, plant height, leaf development, plant biomass, and chlorophyll content. Moreover, RGR and NAR values of both varieties also indicated the suppressive role of Cr and Pb in the soil medium. The affected plant growth mechanism showed its affinity with the affected soil characteristics under metal stress, which was much more on higher metal levels. Data has revealed an acidic effect of heavy metals on the soil and affected soil respiration by affecting soil microbial activity. Likewise, decreasing levels of soil enzymes have revealed an affected decomposition of SOM content and recycling of soil nutrients. However, besides the toxic effect of metals, maize plants showed great potential in accumulating/partitioning Cr and Pb from the subsequent rhizospheric pot soils. Therefore, it is suggested that maize test varieties (Pak-Afgoi & Neelem Desi) be grown as a tool of phytoremediation in the contaminated agro-soils of Dera Ghazi Khan District. However, such contaminated maize plants are recommended unsafe, and carcinogenic to use due to exceeding amounts of Cr and Pb metals than permissible limits, and be destroyed through cement kiln burning.

Conflicts of Interest: The authors confirm that there is no conflict of interest in this article.

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Figure 1

Figure 1. (A-D): Metal partitioning ($\mu\text{g.g}^{-1}$) in different plant parts of Pak-Afgoi & Neelem maize at 40 d

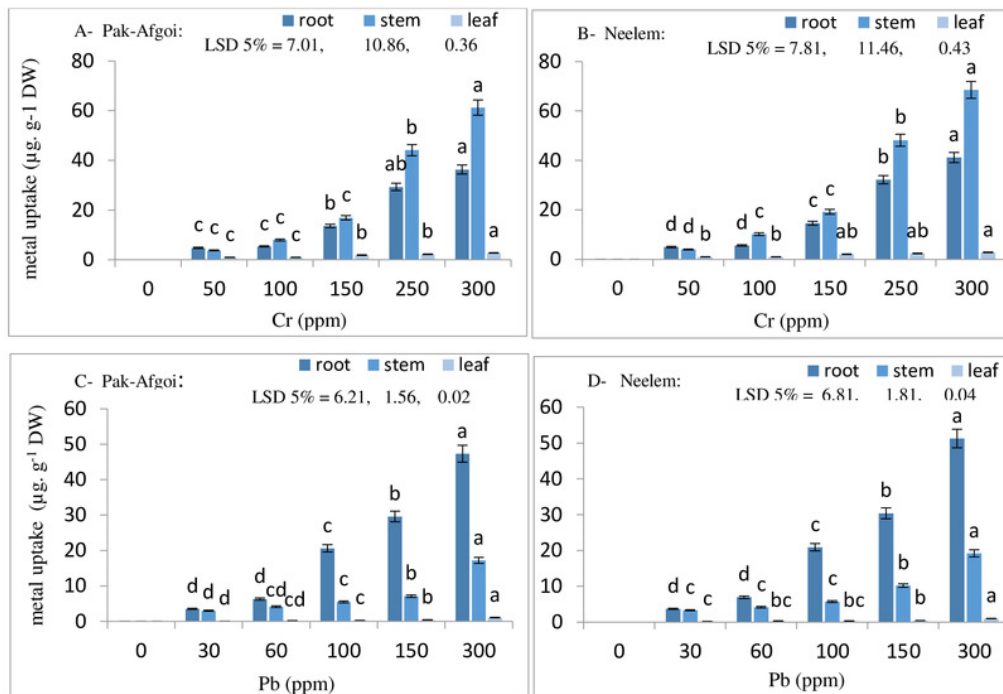


Figure 2

Figure 2. (E-H): Total metal uptake by plant (TPM) and soil metal retention ($\mu\text{g.g}^{-1}$) at 40 d

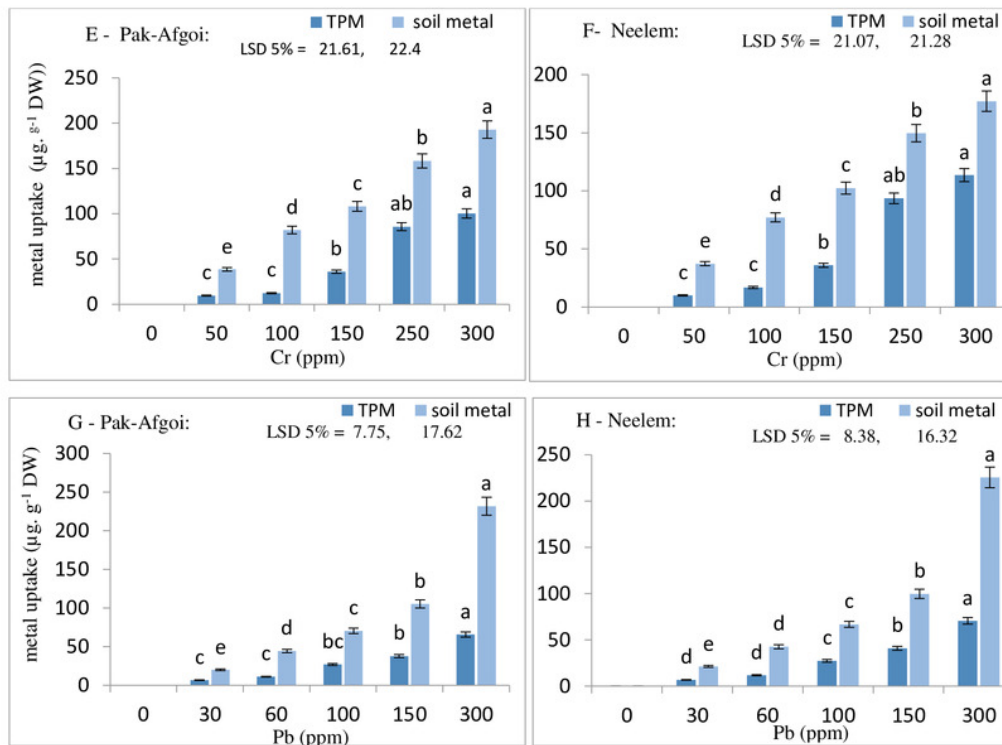


Figure 3

Figure 3 (A-D): Metal Bio-magnification ratio (BMR), metal accumulation factor (ACF) & translocation factor (TF) at day 40 in Pak-Afgoi & Neelem maize

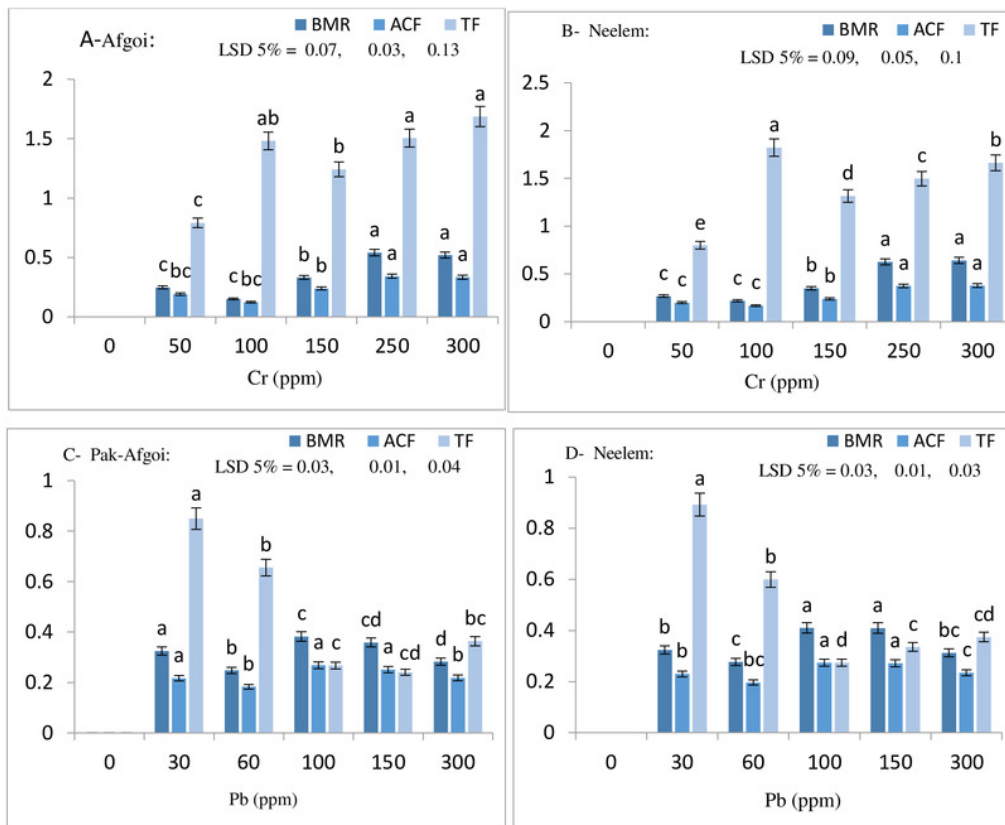


Figure 4

Figure 4: Comparison of growth/height of trialed maize plants from two varieties Pak-Afgoi & Neelem under Cr & Pb treatment (Source captured of picture: Author of this manuscript)



Table 1 (on next page)

Figure 4: Comparison of growth at harvest-2 in Pak-Afgoi and Neelem under Cr & Pb treatment

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SOV	Cr (ppm)						Pb (ppm)					F-value	LSD (5%)
Control	0	50	100	150	250	300	30	60	100	150	300		
Variety: Pak-Afgoi													
Germination(%)	97.5	97.5	86.5	75	62.6	31	90	82.5	62.5	52.5	35.8	41.8***	7.19
Plant height (cm)	68	68.4	66.4	62.1	58.9	53.3	69	66.2	61.7	57.3	49.7	4.86***	2.57
Leaf area (cm ³)	74	74	72	69.5	67.5	65.5	72	70	67.8	67.5	63.2	3.56**	0.79
Leaf area (cm ³): 25d	31.4	32	30.4	27.8	22.2	22	31	30	27.8	26.2	24.8	2.65*	1.45
Green leaf count	10	10	10	9	9	9	10	9.5	9.5	9	9	0.41 ^{NS}	0.17
Fresh weight (g)	46.2	45.8	45.5	43.7	41.4	40.4	45.9	44.8	42.6	39.9	38.4	2.7*	1.42
Dry weight (g)	17	17	14.5	13	12.2	10.5	17	14	12.8	12.1	10	3.46**	0.47
Dry weight (g): 25d	7.4	7.4	7.4	6.5	6.2	5.5	7.4	7.4	6.2	6.2	5.3	2.18*	0.23
RGR (g. day ⁻¹)	0.64	0.6	0.5	0.4	0.38	0.32	0.64	0.47	0.44	0.37	0.31	3.05*	0.03
NAR	0.29	0.29	0.28	0.24	0.24	0.23	0.31	0.27	0.22	0.18	0.15	2.84*	0.03
Leaf chlorophyll (SPAD value)	47	46.3	46	43.1	39.4	34	46	44.3	43	37.2	34.5	3.29**	2.51
Variety: Neelem Desi													
Germination (%)	95	85	78	62.5	50	27.5	85	70	62.5	47.5	27.5	38.9***	6.31
Plant height (cm)	54.1	53.9	51.2	47.7	43	36.	52.6	48.6	43	39	36.2	9.54***	2.78

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Leaf area (cm ³)	69.9	65.6	60.8	56.4	51	49	64	60.2	55.2	50.4	48.2	8.03***	2.83
Leaf area (cm ³): 25d	29.3	29	26.4	23.5	21	20	29.3	26.4	23.2	21.6	20	2.88*	2.03
Green leaf count	8	8	7	7	7	7	8	7.5	7	7	7	1.98*	0.17
Fresh weight (g)	36.6	36.4	35.7	33.4	29.5	25.9	36.6	35.5	32.6	28.8	24.8	2.11*	1.94
Dry weight (g)	14	13.2	11.5	10	9	8	13	11	9.8	9	7.7	13.8***	0.58
Dry weight (g): 25d	6.8	6.9	6.4	5.5	5	4.3	6.9	6.4	5.5	4.8	4.3	9.44**	0.35
RGR(g. day ⁻¹)	0.48	0.43	0.35	0.3	0.26	0.24	0.42	0.32	0.29	0.26	0.21	3.52**	0.02
NAR	0.18	0.16	0.15	0.14	0.14	0.13	0.15	0.14	0.14	0.13	0.11	3.73**	0.01
Leaf chlorophyll (SPAD value)	46.1	46	45	41.4	37.3	34.7	44.6	42.5	39.7	35.7	33.2	4.19***	1.97

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Table 2 (on next page)

Table 2: Assessment of some soil parameters at harvest-2 (40th day) under Cr & Pb stress

SOV	Cr (ppm)						Pb (ppm)					F-value	LSD (5%)	
	0	50	100	150	250	300	30	60	100	150	300			
pH	7.6	7.4	7.4	7.2	6.6	6.4	7.2	7	6.7	6.4	6.3	2.5*	0.1	
SOM (%)	2.8	2.6	2.46	2.31	2	1.8	2.7	2.51	2.36	2	1.8	19****	0.14	
Urease Activity (mg NH4-N kg ⁻¹ 24h ⁻¹)	14.6	14	13.2	11.1	9.7	8.5	14.2	12.5	10.7	9.2	7.6	15.3***	1.01	
CO ₂ evolution (mg): Soil nutrients	176	171	165	153.4	145	124	177	171.3	160.7	149.1	135	12.7**	6.54	
N (%)	2.82	2.5	2.3	2.1	1.9	1.62	2.4	2.2	2	1.6	1.45	22.1***	0.47	
P (ppm)	8.2	8.2	8	7.7	7.3	6.6	8.1	8	7.7	7.3	6.3	2.9*	0.18	
K (ppm)	182	178	172.4	155	142.1	124.1	179.2		177	160.2	143	128.6	9.6**	9.03