# COMPARATIVE STUDY OF SIX MAIZE (ZEA MAYS L.) CULTIVARS CONCERNING CADMIUM UPTAKE, PARTITIONING AND TOLERANCE

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**Abstract.** This experiment was conducted to determine Cd accumulation, partitioning, translocation and their ultimate consequences on growth and biochemical traits of six maize cultivars to screen out Cd tolerant one. A significant decrease in growth and biochemical traits as well as in Cd tolerance index was found, especially at higher Cd level. Moreover, the varieties and Cd treatments were found significant for the growth and biochemical traits. Uptake of Cd, Ca, K and P in the different plant tissues were significantly affected by the varieties and Cd treatments. Translocation, bioconcentration and bioaccumulation factors were found non-significant for varieties and Cd treatments. A significant positive correlation was found between growth and biochemical traits while, among plant total Cd, Ca, K and P, total plant dry; and fresh weight and plant height were significantly negative. Furthermore, the PC1 was loaded by growth traits; Ca, K and P while PC2 by the Cd in different plant tissues. Hence, a strong negative correlation was observed between the PC1 and PC2 parameters. The maximum Cd accumulation was in the roots rather than the crown and leaf tissues of maize cultivars. The EV-1098 was to be more tolerant to Cd stress than other cultivars.

**Keywords:** heavy metal toxicity, growth and biochemical traits, varietal differences, tolerance index, bioconcentration factor

#### Introduction

Life and biodiversity are significantly affected by the accumulation of heavy metals in soils either by natural processes or by human activities. The toxicity of heavy metals is one of the major reasons of environmental and ecological issues (Roy et al., 2018). The rise of heavy metal contamination in agricultural fields and soils caused primarily by anthropogenic activities resulted in their incorporation into the grown crops and ultimately into the food chain (Siebers et al., 2014; Retamal-Salgado et al., 2017). A large number of industries produce wastewater containing significant concentrations of heavy metals which is discharged into drains and rivers. These wastewater effluents contain a large number of heavy metals such as Cr, Cd, Cu, Pb, Zn, and Ni that have a major impact on crops and their yields (Najam et al., 2015; Ali et al., 2020). The long-term irrigation with industrial wastewater allows heavy metals to persist in the soil, which increases their absorption and accumulation by the plants which ultimately enter into the food chain (Najam et al., 2015). Several non-essential heavy metals even in traces induce toxicity in plants and affect the growth of several crops (Marquez et al., 2018). Heavy metals such as Cd, Ni, Pb, Cu, Cr and Zn not only negatively affect the growth and development of plants but also disturb the micro-flora of the soils (Abdu et al., 2017). To achieve the goals of a sustainable environment and agriculture it is necessary to control heavy metal pollution on a priority basis (Naveed et al., 2020).

Cd is a poisonous, non-degradable and non-essential heavy metal that naturally exists in most of the soil types (Naveed et al., 2020). Its naturally occurring levels are usually < 1 mg/kg, but these levels have increased to 1000 mg/kg in some geographical regions because of haphazard urbanization/ industrialization in the last two centuries (Coakley et al., 2019). Cd exposures can be toxic or even lethal to plants in certain cases even at concentrations as low as 2.5 mg/kg (Henson et al., 2013). The anthropogenic activities increasing Cd concentration in the environment are manure (sewage sludge), combustion of fossil fuel, iron foundries, electroplating, smelting, waste disposal of water and usage of synthetic industrial products such as paints, pesticides and sludge (Anjum et al., 2015; Rizwan et al., 2017; Abedi and Mojiri, 2020). Interestingly, physiochemical properties of Cd are similar to other micronutrients such as Zn, which enable it to be taken up readily by plants and crops (Coakley et al., 2019). Cd salts are comparatively more soluble than others in the soil, consequently, Cd can readily be incorporated into the natural environment and easily accumulated in the roots and other edible parts of the plants (Naveed et al., 2020). Plants can easily transport Cd to different parts through the vascular tissues (xylem and phloem) (Ismael et al., 2019). The Cd has a greater absorption and accumulation rate than other heavy metals like Cu and Zn (Liao et al., 2019). It alters the physiological functions by disturbing many metabolic processes including nitrogen metabolism (Roy et al., 2016; Abedi and Mojiri, 2020). Cd absorption and accumulation depends upon the age of plants (Godinho et al., 2018) and its deposition gradually increases with time, affecting consumers directly or indirectly (Abedi and Mojiri, 2020). Daily consumption of 1 mg/kg (body weight) Cd is considered harmful to humans (Retamal-Salgado et al., 2017). The long-term exposure and ingestion of Cd-contaminated food is toxic to the food chain and poses a severe health risk to human. It is highly imperative that Cd-polluted soils be remedied to preserve and restore the ecological functionality of these soils (Naveed et al., 2020).

Accumulation of Cd in edible parts of the plant is more harmful than the overall plant intake. While certain techniques are being used to reduce the Cd in the food chain (Rai et al., 2019). Cd toxicity can negatively affect biomass, chlorophyll contents, number of leaves/flowers/fruits, leaf area, crop yield of plants and its ability to uptake essential nutrients (Ghani, 2010; Coakley et al., 2019). Cd accumulation is directly related to its applied concentration and exposure time (Rolli et al., 2010). Cd affects the lateral roots in maize, changes the root color and roots become stiff. It also decreases the coleoptiles size, causes chlorosis, and ultimately necrosis (Shafi et al., 2010; Kaznina and Titov, 2014). The increased Cd concentration amplified the Cd absorption in the shoots of maize (Nguyen et al., 2016) while roots accumulate more Cd than shoots (Stritsis and

Claassen, 2013). Cd translocation from roots to shoots is limited by the plants of the Poaceae family in order to maintain the nutritional balance that is disturbed by the Cd induction, e.g., by the suberization of endoderm cells and lignification of root cortex cells (Kaznina and Titov, 2014). The main symptoms of Cd toxicity including stunted growth, enzyme activation or deactivation, photosynthetic activity and plant-water relationship disturbance (Raza et al., 2020). The plant species and varieties vary for Cd accumulation and translocation as well as Cd partitioning into their different tissues (Yang et al., 2014; Coakley et al., 2019), such as apple (Zhou et al., 2017), maize (Shah et al., 2016; Rizwan et al., 2017), mungbean (Ghani, 2010), mustard and oats (Boros-Lajszner et al., 2020), pea (Naveed et al., 2020), rice (Fahad et al., 2015; Marquez et al., 2018), sorghum (Roy et al., 2016) and wheat (Shafi et al., 2010; Abedi and Mojiri, 2020). The varied distribution of Cd at the cellular/subcellular level in different organs was attributed to the high accumulation capacity of plants (Coakley et al., 2019). Plants have adapted different mechanisms for Cd tolerance and to mitigate its toxic effects, such as maize compartmentalizes Cd as a mechanism of tolerance in the cell wall of stems (Akhter et al., 2014).

Maize (Zea mays L.) is a cereal crop grown worldwide belonging to grasses (Poaceae). It is known as a heavy metal tolerant and accumulator because of its survival ability in the metal-polluted soils and metal accumulation capacity. Some maize genotypes show tolerance to heavy metal toxicity (Rizwan et al., 2017) and Cd contaminated soils (Van Slycken et al., 2013). Due to this property maize is potentially used plant for phytoremediation, especially in Cd-contaminated soils (Huang et al., 2020; Raza et al., 2020). Plant tolerance to heavy metals largely depends on the efficiency of uptake, translocation, and sequestration in specialized tissues and cell organelles (Boros-Lajszner et al., 2020). Maize is commonly used for glucose preparation, edible oil production and several other products (Shah et al., 2016). The maize is also one of the highest growing crops and has the survival ability in the metalpolluted soils and metal accumulation capacity concerning other crops (Wuana and Okieimen, 2010). Maize is considered among 400 plants that accumulate heavy metals and survive in Cd-polluted soils but maybe with low biomass production (Waseem et al., 2014). The maize production is decreasing due to the heavy metal accumulation in soils. Therefore, to find the resistant maize varieties are necessary to fulfill the food requirement of the worldwide increasing population.

Pakistan is one among the agricultural countries in the world and its large population (> 70%) living in the villages directly or indirectly depends on agriculture (Bashir et al., 2012). Pakistan is the  $20^{th}$  largest producer of maize in the world. However, rapid urbanization and an increase in population demand healthier and higher crop yields of rice, sugarcane, wheat and maize (Ali et al., 2017). In Pakistan, the vegetables and crops are commonly irrigated with canal water that gets polluted by industrial wastes when passing by industrial areas, which is commonly contaminated with heavy metals (Naveed et al., 2020). Among these heavy metals, Cd is of major concern. Maize fabrication is significantly reduced in Pakistan owing to the use of sewage water (Waseem et al., 2014). Industrial wastewater is extensively used for irrigation in the agricultural areas of Pakistan. The wide distribution of Cd in wastewater has been reported in the different areas of Pakistan (Waseem et al., 2014; Najam et al., 2015). The maximum amount of Cd (5.35 mg/L) in wastewater was recorded in the Korangi region of Karachi (Amin et al., 2014), which exceeded the 0.10 mg/L permissible threshold set by NEQS-Pak (Waseem et al., 2014). In addition, the Cd concentration in

wastewater was also found above the said threshold limit in the Punjab province (0.18 to 0.37 mg/L) (Mahmood and Malik, 2014) and the Khyber Pakhtunkhwa (KPK) province (0.19 to 0.62 mg/L) (Rehman et al., 2008).

Thus, in this context, it is urgent to investigate the strategies to reduce the bioavailability of these heavy metals for plants, particularly crops and vegetables. Moreover, it is also required to find out the Cd-tolerant varieties. There are few reports on the varietal differences for accumulation, translocation and partitioning of Cd in different plant tissues (DPT) under induced Cd toxicity. So, the present study was conducted: 1) To screen out the Cd tolerant variety and to investigate the varietal performance of maize to induced Cd toxicity; 2) To determine the extent of the accumulation, partitioning and translocation of Cd in different parts (crown tissue, leaves and roots) of maize varieties, and 3) To estimate the consequence of Cd appliance on the growth and biochemical traits of various maize varieties.

## **Materials and Methods**

#### Study location

The experiment was conducted in the rain protected warehouse of the Old Botanical Garden, University of Agriculture, Faisalabad-Pakistan under natural conditions. The geographical location of the experimental site is 31°45′ N, 73°14′ E at 180 m above the sea level with a semi-arid climate. The mean annual precipitation and evaporation are 375 mm and 1600 mm, respectively. The mean annual temperature is 24.8°C and the temperature normally ranges from 50°C to 4°C in the summer and winter season, respectively (Akram, 2020).

## **Experimental details**

The experiment was designed to assess the Cd-accumulation in different maize parts: crown tissue (CT), leaves (L) and roots (R) and to check the effect of Cd-toxicity on growth and biochemical traits as well as the growth response of maize cultivars. The seeds of six maize cultivars (EV-1098, Sahiwal-2002/SA-2002/Sahiwal, EV-5098, Agati-2002/AG-2002/Agaiti, EV-6098, and Sadaf) were obtained from the Maize and Millets Research Institute (MMRI), Yusafwala, Sahiwal District, Pakistan. The 20 seeds per pot were sown in the plastic pots (20 cm diameter  $\times$  28 cm height) that filled with 10 kg of river sand. The sand was thoroughly washed with tap water and then thrice with distilled water before filling in the pots. The pots were placed in the warehouse under natural conditions of light and temperature. The experiment was arranged in a Completely Randomized Design (CRD) with a factorial arrangement.

## Treatment application

Three different Cd-concentrations (0, 500 and 1000  $\mu$ M) were made by dissolving cadmium chloride (CdCl<sub>2</sub>.2.5H<sub>2</sub>O) of Merck Company in distilled water. After seeds germination (three days), the seedlings were thinned to keep 5 uniform and healthy seeds per pot. Then, the plants were irrigated with Hoagland's nutrient containing solution (Hoagland and Arnon, 1950; Zhang et al., 2020) for a regular period of three days, until the completion of the experiment. After that, the 28-days old plants of all the varieties were treated with enhanced Cd-concentrations (0, 500 and 1000  $\mu$ M solution) after every three-day interval seven times. Two factors (there different Cd-

concentrations and six maize cultivars) were combined to make 18-treatment and each treatment was replicated thrice (54-pots). Only the water was supplied to the control plants. The Cd-treated and control plants were grown for another 21-day.

# Growth traits

After 21 days of Cd-treatment, the morphological parameters such as the number of leaves, shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW) and stem diameter (SD) was recorded. Before uprooting, the shoot length (SL), the number of green leaves per plant (NOL) and stem diameter (SD) of each plant was measured. The SL was measured from the stem base to the top of the plant. The NOL for each treatment was counted and means values were calculated from three replicates. The SD was measured with Vernier Calliper. The RL was measured from the bottom of the plant to the stem base. After that, all the plants were harvested. The SFW and RFW of three plants per replicate were recorded immediately after uprooting the plants and the mean value per plant was calculated. Then, washed with distilled water twice, blotted dry and transferred to paper bags and stored for 24-48 hours in an oven set at 65°C and used for subsequent analysis.

# Digestion of plant material for chemical analysis and determination of $K^+$ , $Ca^{2+}$ , P and $Cd^{2+}$

The plant parts including crown tissue (CT), leaves (L) and roots (R) were separated and shade dried to determine the K<sup>+</sup>, Ca<sup>2+</sup>, phosphorus (P) and cadmium (Cd<sup>2+</sup>) contents. 0.5 g dried well ground plant material and 5 mL of Conc. HNO<sub>3</sub> was taken in digestion flasks and incubated overnight at room temperature. Then, 0.5 mL of HNO<sub>3</sub> was added into each digestion flask to boost the reaction and the flasks were placed on the hot plate. The temperature was gradually increased up to 200°C for 30-45 minutes until the complete transparent digestion. After cooling the flasks, the extract was filtered by filter paper and the volume was raised to 50 mL by adding distilled H<sub>2</sub>O in the volumetric flask and used for the determination of K<sup>+</sup>, Ca<sup>2+</sup>, P and Cd<sup>2+</sup>.

The potassium (K<sup>+</sup>) and calcium (Ca<sup>2+</sup>) contents were recorded using Flame Photometer (Jenway PFP7). Phosphorus (P) was determined by the ammonium molybdate method (Sparks et al., 1996; Akram et al., 2020). The Cd<sup>2+</sup> amount in different plant parts was determined by the atomic absorption spectrophotometer (AAS) (Perkin Elmer Analyst-100).

## Data analysis

The following indices were determined.

## Bioconcentration factor (BCF) and transportation index (Ti)

Cadmium uptake was assessed by the bioconcentration factor (BCF) and bioaccumulation factor (BAF). The concentrations of plant and soil/substrate Cd was determined according to dry weight. BCF and BAF indicate the plant's ability to accumulate a specific metal regarding its concentration in the growing medium/substrate. They were calculated by the following formulas (Retamal-Salgado et al., 2017):

$$BAF_{CT} = \frac{C_{CdCT}}{C_{Substrate}}$$
(Eq.1)

$$BAF_{L} = \frac{C_{CdL}}{C_{Substrate}}$$
(Eq.2)

$$BCF_{R} = \frac{C_{CdR}}{C_{Substrate}}$$
(Eq.3)

where  $C_{CdCT}$ ,  $C_{CdL}$ , and  $C_{CdR}$  are the concentrations of Cd in the crown tissue, leaves, and roots, respectively; and  $C_{Substrate}$  in the growing medium/substrate.

#### Translocation factor (TF)

The translocation factor (TF) gives the aerial parts/root concentration of metal and shows the plant's ability to transfer the metal from plant roots to the aerial parts (crown tissue and leaves). TF was calculated by the following formulas (Retamal-Salgado et al., 2017):

$$TF_{CT} = \frac{C_{CdCT}}{C_{CdR}}$$
(Eq.4)

$$TF_{L} = \frac{C_{CdL}}{C_{CdR}}$$
(Eq.5)

where  $C_{CdCT}$ ,  $C_{CdL}$ , and  $C_{CdR}$  are the concentrations of Cd in the crown tissue, leaves, and roots, respectively.

#### Tolerance index (TI)

The tolerance index (TI) shows the plant's ability to tolerate Cd toxicity. TI was calculated using the following equation (Retamal-Salgado et al., 2017):

$$TI = \frac{DBM_{Cd-treatments}}{DBM_{Control}}$$
(Eq.6)

where DBM<sub>Cd-treatments</sub> and DBM<sub>Control</sub> are the dry biomass (DBM) of each Cd treatment and DBM of the control treatment (no added Cd), respectively.

#### Statistical analysis

To confirm the data variability and result's validity, all the data were analyzed at two levels. Firstly, all the data were used to treat all observations at the same time by principal component analysis (PCA) to assess the correlation among all the plant traits, Cd-treatments and growth parameters. Secondly, the data were analyzed at the variety level for each plant trait, Cd-treatments and plant parts. Tukey's test was conducted to observe the significant differences within-and-between Cd-treatment means. The Pearson correlations were conducted using the "lm" function in the R package (Akram et al., 2020). Mean values of the Ca<sup>2+</sup>, K<sup>+1</sup>, P and Cd<sup>2+</sup> in different plant parts (crown tissue, leaves and roots) were mapped against each cultivar to observe the whole data patterns. The linear regressions were constructed to assess the bivariate relationship between different plant traits and parts of all the cultivars. All the values in figures and tables expressed as mean (triplicate)  $\pm$  standard error (SE) and the values were considered significant at p < 0.05. All the statistical data were arranged in Excel sheets and analyzed using SPSS 21.0 and R software (version 3.6.0, R Development Core Team 2018).

## Results

# Effect of Cd on the plant biomass and related growth traits

Varieties and Cd treatments were found to be significant for all the growth traits, such as shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), shoot length (SL), root length (RL), stem diameter (SD) and the number of leaves plant<sup>-1</sup> (NOL) (*Table 1*). The SFW, RFW, SDW, RDW, SL, RL, SD and NOL interacted and significantly changed with the Cd treatments (*Figs. S1, S2*). Under control conditions, the varietal effect of all the growth traits was noted significantly different for all the six varieties, which exhibited EV-1098 as the highest biomass yielding and SA-2002 lowest biomass yielding variety among all other varieties (Table 1; Figs. S1, S2). The EV-1098 performed better for all traits than other varieties. However, the biomass and all the other growth-related traits showed a significant decreasing trend for all the six varieties with an increased level of Cd. The reduction in the SFW, RFW, SDW, RDW, SL, RL, SD and NOL was recorded 56%, 45%, 34%, 30%, 61%, 45%, 35% and 63% (from control level), respectively. Maximum reduction was found in the NOL and minimum in the RDW, 63% and 30% reduction from the control level, respectively. All the varieties and treatments were found significant, while variety  $(V) \times$  treatment (T) interactions were noted non-significant for all the growth traits, except stem diameter (SD) (Table 1). Significant positive correlations were observed between the different growth traits (Fig. S3).

| Traits                    |                                | SFW      | RFW     | SDW     | RDW    | SL       | RL       | SD      | NOL    |
|---------------------------|--------------------------------|----------|---------|---------|--------|----------|----------|---------|--------|
| Maize<br>varieties<br>(V) | EV-1098                        | 32.95 a  | 24.04 a | 9.19 a  | 5.63 a | 67.87 a  | 45.67 a  | 0.54 a  | 4.56 a |
|                           | SA-2002                        | 23.45 d  | 20.00 b | 6.38 c  | 3.70 c | 54.25 d  | 30.83 e  | 0.36 d  | 4.22ab |
|                           | EV-5098                        | 25.89bcd | 16.53 c | 6.93 c  | 3.90 c | 57.93 cd | 33.69 de | 0.39 cd | 3.78ab |
|                           | AG-2002                        | 29.89ab  | 22.43ab | 8.41ab  | 5.12ab | 63.77ab  | 42.70ab  | 0.49 b  | 3.44 b |
|                           | EV-6098                        | 24.52 cd | 19.84 b | 7.58bc  | 4.49bc | 59.76bc  | 39.91bc  | 0.42 c  | 3.89ab |
|                           | Sadaf                          | 28.51abc | 21.95ab | 7.30bc  | 4.12 c | 61.19bc  | 37.14 cd | 0.43 c  | 4.11ab |
| Treatments<br>(T)         | To<br>(Control)                | 34.71 a  | 26.95 a | 10.98 a | 6.41 a | 73.33 a  | 52.49 a  | 0.63 a  | 5.00 a |
|                           | T1 (500<br>μM)                 | 28.34 b  | 20.24 b | 8.20 b  | 5.11 b | 64.12 b  | 39.00 b  | 0.46 b  | 3.83 b |
|                           | T2 (1000<br>μM)                | 19.56 c  | 15.20 c | 3.71 c  | 1.95 c | 44.95 c  | 23.48 c  | 0.22 c  | 3.17 c |
| ANOVA                     | V                              | ***      | ***     | ***     | ***    | ***      | ***      | ***     | *      |
|                           | Т                              | ***      | ***     | ***     | ***    | ***      | ***      | ***     | ***    |
|                           | $\mathbf{V} \times \mathbf{T}$ | ns       | ns      | ns      | ns     | ns       | ns       | *       | ns     |

**Table 1.** Shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), shoot length (SL), root length (RL), stem diameter (SD) and the number of leaves per plant (NOL) for the different Cd treatments

Different letters in the same column indicate the significant differences compared with Cd treatments and maize varieties according to Tukey's test (p < 0.05)

## Cd uptake, translocation, partitioning and its effect on the different biochemical traits

The uptake of cadmium in the crown tissue (CT) was significantly affected by the varieties (V), Cd treatments (T) and interaction between them (V  $\times$  T) (*Table 2; Fig. S4*). The contrast to different Cd levels (*Table 2*), the maximum Cd accumulation was

noted with 1000  $\mu$ M CdCl<sub>2</sub>. While the contrast between different varieties (*Table 2*), the highest uptake of Cd in the crown tissue was recorded in two varieties, such as SA-2002 and EV-6098 (0.10 mg g<sup>-1</sup>) the same amount of Cd in both varieties; and the lowest Cd uptake was found in the EV-1098 (0.03 mg g<sup>-1</sup>) variety with significant differences (*Table 2, Fig. 1*).



Figure 1. The concentration of Cd (mean  $\pm$  SE) in the different plant parts of the six maize varieties

The Cd uptake in the leaves (L) was substantially influenced by the varieties (V), Cd treatments (T) and interaction between them (V × T) (*Table 2*). In comparison to CdCl<sub>2</sub> levels (*Table 2; Fig. S4*), the significant higher uptake of Cd was observed with 1000  $\mu$ M CdCl<sub>2</sub> (0.17 mg g<sup>-1</sup>) followed by 500  $\mu$ M CdCl<sub>2</sub> (0.11 mg g<sup>-1</sup>). The contrasting between different varieties (*Table 2*), it was noted that the significantly higher amount of Cd has accumulated in the leaves of the SA-2002 (0.12 mg g<sup>-1</sup>) variety and the lowest in the EV-1098 variety (*Table 2, Fig. 1*).

As for the roots (R), the Cd uptake was significantly affected by the varieties (V), Cd treatments (T) and interaction between variety (V) × treatments (T) (*Table 2*). In the comparison between the Cd levels (*Table 2*), the highest uptake of Cd was recorded with 1000  $\mu$ M CdCl<sub>2</sub> (0.22 mg g<sup>-1</sup>) followed by 500  $\mu$ M CdCl<sub>2</sub> (0.15 mg g<sup>-1</sup>) with significant differences. In the case of varieties (*Table 2*), the higher Cd absorption in the roots of EV-6098 and Sadaf varieties with the values 0.16 mg g<sup>-1</sup> and 0.14 mg g<sup>-1</sup>, respectively. The significant lower Cd uptake was found in the EV-1098 (0.10 mg g<sup>-1</sup>) variety (*Table 2, Fig. 1*). The mean values (mean ± standard error) for Cd uptake in the different plant parts and whole plant by all the studied varieties were shown in *Fig. 1*. The highest amount of Cd for the whole plant was uptake by two varieties: SA-2002 and EV-6098. The lowest Cd was uptake by the EV-1098 variety (*Fig. 1*).

| Traits                    |                                | Cd-CT  | Cd-L   | Cd-R   | Ca-CT  | Ca-L   | Ca-R   | К-СТ   | K-L    | K-R     | P-CT   | P-L     | P-R    |
|---------------------------|--------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|---------|--------|---------|--------|
|                           | EV-1098                        | 0.03 d | 0.05 e | 0.10 d | 4.12 a | 4.40 b | 4.53 b | 8.05 a | 9.17 a | 10.85 a | 0.25 a | 0.27 a  | 0.23 a |
| Maize<br>varieties<br>(V) | SA-2002                        | 0.10 a | 0.12 a | 0.13 c | 3.55 b | 3.70 c | 3.77 c | 3.73 d | 4.27 e | 5.37 e  | 0.17 e | 0.18 d  | 0.16 d |
|                           | EV-5098                        | 0.07 b | 0.09 c | 0.12 c | 3.75 b | 4.48ab | 4.77 a | 5.22 c | 6.17 c | 7.48 c  | 0.21bc | 0.22 b  | 0.21 b |
|                           | AG-2002                        | 0.04 c | 0.08 d | 0.09 e | 3.57 b | 4.61 a | 4.67ab | 5.86 b | 7.21 b | 8.83 b  | 0.22 b | 0.20 c  | 0.22 a |
|                           | EV-6098                        | 0.10 a | 0.12 b | 0.16 a | 3.74 b | 4.33 b | 4.48 b | 5.00 c | 5.38 d | 6.66 d  | 0.19 d | 0.20 c  | 0.21 b |
|                           | Sadaf                          | 0.04 c | 0.09 c | 0.14 b | 3.69 b | 3.76 c | 3.89 c | 3.27 e | 5.02 d | 6.61 d  | 0.21 c | 0.19 cd | 0.19 c |
| Treatments<br>(T)         | To (Control)                   | 0.00 c | 0.00 c | 0.00 c | 4.10 a | 4.52 a | 4.68 a | 6.58 a | 7.87 a | 9.52 a  | 0.22 a | 0.23 a  | 0.22 a |
|                           | T1 (500 μM)                    | 0.07 b | 0.11 b | 0.15 b | 3.74 b | 4.24 b | 4.34 b | 5.25 b | 6.01 b | 7.47 b  | 0.21 b | 0.21 b  | 0.21 b |
|                           | Τ2 (1000 μΜ)                   | 0.12 a | 0.17 a | 0.22 a | 3.37 c | 3.88 c | 4.03 c | 3.74 b | 4.73 c | 5.92 c  | 0.19 c | 0.19 c  | 0.19 c |
| ANOVA                     | V                              | ***    | ***    | ***    | ***    | ***    | ***    | ***    | ***    | ***     | ***    | ***     | ***    |
|                           | Т                              | ***    | ***    | ***    | ***    | ***    | ***    | ***    | ***    | ***     | ***    | ***     | ***    |
|                           | $\mathbf{V} \times \mathbf{T}$ | ***    | ***    | ***    | **     | **     | **     | ***    | ***    | ***     | ns     | ns      | ns     |

**Table 2.** The concentration of Cd in crown tissue (Cd-CT), leaves (Cd-L) and roots (Cd-R); Ca concentration in crown tissue (Ca-CT), leaves (Ca-L), roots (Ca-R); K concentration in crown tissue (K-CT), leaves (K-L), roots (K-R); and P concentration in crown tissue (P-CT), leaves (P-R)

Means sharing different letters in the same column indicate the significant differences compared with Cd treatments and maize varieties according to Tukey's test (p < 0.05)

The Cd, Ca, K and P contents in the different plant tissues (such as crown tissues, leaves and roots) were significantly affected by varieties, Cd levels and interaction between them (*Table 2; Figs. 1,2*). The maximum Cd was accumulated in the roots and minimum in the crown tissues of all varieties (*Fig. S4*). Additionally, the roots retained maximum Cd rather than crown tissues and leaves. In general, the EV-1098 variety showed a minimum uptake of Cd compared with other varieties (*Fig. 1*). However, the same variety performed better in terms of Ca, K and P contents in different plant tissues as compared with other varieties (*Fig. 2*). In short, Cd contents in all plant tissues tended to increase with increased Cd level.



*Figure 2.* The concentration of Ca, K, P and Cd (mean  $\pm$  SE) in the six maize varieties

The Ca, K and P contents interacted and significantly changed with the increasing Cd treatments (*Table 2*). In control conditions, the varietal effect of Ca, K and P contents were observed different significantly. However, an opposite trend was followed by Ca, K and P contents in all plant parts under Cd treatments i.e. an increase in the Cd level resulted in a decrease in maize plant biochemical traits (Ca, K and P contents) in the crown tissues, leaves and roots (*Table 2*).

The reduction in the plant total Ca, K and P contents from the control level were recorded 85%, 60% and 85%, respectively. All the varieties and treatments were found highly significant for the Cd, Ca, K and P contents in all plant parts (CT, L and R), while variety (V) × treatment (T) interactions were recorded non-significant only for the P contents in all plant parts (*Table 2*). The Ca, K and P contents exhibited significant positive correlations in three different plant parts (*Fig. S5*).

## Correlations between Cd, plant morphological and biochemical traits

The regression lines were drawn to determine the correlation among different studied traits, such as plant total dry weight; and total Ca, K and P (*Fig. 3*). Results revealed that there existed a significantly positive correlation among plant total dry weight (PTDW); and total Ca (PT-Ca), K (PT-K) and P (PT-P). The linear regression

coefficient reached to significant level for total Ca, K and P, respectively (*Fig. 3*). However, a significant negative correlation was observed among plant total Cd (PT-Cd), PT-Ca, PT-K and PT-P, total plant dry (PTDW) and fresh weight (PTFW) and plant height as shown in *Fig. 3*. The negative significant linear regression coefficient was recorded for total Ca, K, P, total dry weight, total fresh weight and plant height (*Fig. 3*).



**Figure 3.** The relationship between plant total dry weight (PTDW) and total Ca, K and P contents (PT-Ca, PT-K and PT-P, respectively) (in panel a, b, c); plant total Cd (PT-Cd) and total Ca, K and P contents (PT-Ca, PT-K and PT-P, respectively) (in panel d, e, f); and plant total Cd (PT-Cd) and total plant dry weight (PTDW), fresh weight (PTFW) and plant height (in panel g, h, i)

## Pearson correlation and principal component analysis (PCA)

Pearson correlation for plant different traits was shown in *Table 3*. The different significant correlations (both positive and negative) were found among maize plant growth traits (shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), shoot length (SL), root length (RL), stem diameter

(SD) and the number of leaves per plant (NOL); biochemical traits (Ca concentration in crown tissue (Ca-CT), leaves (Ca-L), roots (Ca-R), K concentration in crown tissue (K-CT), leaves (K-L), roots (K-R), P concentration in crown tissue (P-CT), leaves (P-L), roots (P-R); and Cd related traits, such as Cd concentration in crown tissue (Cd-CT), leaves (Cd-L) and roots (Cd-R) (*Table 3*). Particularly, the Cd in all the plant parts (CT, L and R) showed significant negative correlations with all the growth and biochemical traits of plants. Conversely, all growth traits exhibited significant positive correlations with all the biochemical traits (*Table 3*).

The principal component analysis (PCA) for this study is shown in *Fig. 4*. The first two PCA components exhibited maximum variation (84.1%) for all the parameters tested within the dataset. The PC1 and PC2 explained 74.6% and 9.5% variations, respectively. Moreover, the PC1 was positively loaded by the variables SDW, RFW, SDW, RDW, SL, RL, SD, NOL, CaCT, CaL, CaR, KCT, KL, KR, PCT, PL and PR; and PC2 was positively loaded by the variables CdCT, CdL and CdR. In comparison, a strong negative correlation was observed between the PC1 and PC2 parameters (*Fig. 4*).

# Effect of Cd on the plant biomass and related growth traits, different factors/indices for Cd translocation, bioaccumulation, bioconcentration and tolerance

## Translocation factors (TF)

The translocation factors (TF) was used to determine the plant's ability to translocate Cd from the lower part (roots) to the aerial parts (crown tissue and leaves) of the plant, which are the mean values of different Cd treatments and maize varieties shown in *Table 4*. The variations in TF<sub>CT</sub> and TF<sub>L</sub> showed accordingly with the different treatments and varieties. TF<sub>CT</sub> and TF<sub>L</sub> values observed at 1000  $\mu$ M (0.54 and 0.76) were similar and not statistically different from those measured at 500  $\mu$ M (0.51 and 0.74) (*Table 4*).

## Bioconcentration factors (BCF)

For BCF, neither significant differences were noted among Cd treatments (p > 0.05) and nor for the different maize varieties (*Table 4*). The BCF showed an inverse relationship with Cd treatments, therefore with the increasing Cd supply, the BCF decreased (*Table 4*).

## Bioaccumulation factors (BAF)

Conversely, BAF indicates the plant's capacity to accumulate Cd in the aerial part (crown tissue and leaves) of the plant, concerning the concentration of Cd in the substrate/soil. The variations in BAF<sub>CT</sub> and BAF<sub>L</sub> showed accordingly in *Table 4*. Maximum BAF<sub>CT</sub> and BAF<sub>L</sub> measured at 500  $\mu$ M (1.29 and 1.87) and 1000  $\mu$ M (1.05 and 1.49) were not statistically different (*Table 4*).

## *Tolerance index (TI)*

The significant differences (p< 0.05) for TI were found at different treatments (Control, 500  $\mu$ M and 1000  $\mu$ M) with TI values 1.01, 0.77, and 0.33, respectively (*Table 4*). While, no significant differences (p> 0.05) were observed between the varieties (EV-1098, SA-2002, EV-5098, AG-2002, EV-6098 and Sadaf) with TI values 0.75, 0.64, 0.65, 0.74, 0.72, and 0.66, respectively. TI was decreased with the increasing Cd supply (*Table 4*).

| Traits | SFW          | RFW          | SDW          | RDW          | SL           | RL           | SD           | NOL          | CdCT         | CdL      | CdR      | CaCT         | CaL          | CaR          | KCT          | KL           | KR           | РСТ          | PL           | PR |
|--------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|----------|----------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|----|
| SFW    | 1            |              |              |              |              |              |              |              |              |          |          |              |              |              |              |              |              |              |              |    |
| RFW    | $0.818^{**}$ | 1            |              |              |              |              |              |              |              |          |          |              |              |              |              |              |              |              |              |    |
| SDW    | 0.865**      | 0.861**      | 1            |              |              |              |              |              |              |          |          |              |              |              |              |              |              |              |              |    |
| RDW    | 0.831**      | 0.823**      | 0.932**      | 1            |              |              |              |              |              |          |          |              |              |              |              |              |              |              |              |    |
| SL     | 0.863**      | 0.821**      | 0.933**      | 0.937**      | 1            |              |              |              |              |          |          |              |              |              |              |              |              |              |              |    |
| RL     | $0.867^{**}$ | $0.841^{**}$ | 0.930**      | 0.925**      | 0.937**      | 1            |              |              |              |          |          |              |              |              |              |              |              |              |              |    |
| SD     | $0.877^{**}$ | $0.884^{**}$ | $0.960^{**}$ | 0.936**      | 0.949**      | 0.937**      | 1            |              |              |          |          |              |              |              |              |              |              |              |              |    |
| NOL    | $0.584^{**}$ | $0.714^{**}$ | $0.682^{**}$ | 0.636**      | 0.625**      | 0.631**      | $0.676^{**}$ | 1            |              |          |          |              |              |              |              |              |              |              |              |    |
| CdCT   | -0.804**     | -0.777**     | -0.822**     | -0.797**     | -0.819**     | -0.834**     | -0.837**     | -0.629**     | 1            |          |          |              |              |              |              |              |              |              |              |    |
| CdL    | -0.832**     | -0.828**     | -0.889**     | -0.852**     | -0.871**     | -0.887**     | -0.906**     | -0.712**     | 0.959**      | 1        |          |              |              |              |              |              |              |              |              |    |
| CdR    | -0.811**     | -0.840**     | -0.873**     | -0.839**     | -0.840**     | -0.863**     | -0.892**     | -0.689**     | $0.879^{**}$ | 0.939**  | 1        |              |              |              |              |              |              |              |              |    |
| CaCT   | $0.748^{**}$ | $0.740^{**}$ | $0.789^{**}$ | 0.771**      | 0.765**      | $0.774^{**}$ | $0.789^{**}$ | $0.658^{**}$ | -0.670**     | -0.760** | -0.704** | 1            |              |              |              |              |              |              |              |    |
| CaL    | $0.592^{**}$ | $0.462^{**}$ | 0.651**      | 0.649**      | 0.630**      | 0.669**      | $0.666^{**}$ | 0.257        | -0.510**     | -0.574** | -0.590** | 0.551**      | 1            |              |              |              |              |              |              |    |
| CaR    | $0.572^{**}$ | 0.397**      | $0.600^{**}$ | $0.602^{**}$ | $0.570^{**}$ | $0.625^{**}$ | 0.619**      | $0.285^{*}$  | -0.486**     | -0.559** | -0.565** | $0.523^{**}$ | $0.917^{**}$ | 1            |              |              |              |              |              |    |
| КСТ    | 0.709**      | 0.629**      | 0.725**      | 0.749**      | 0.709**      | 0.734**      | 0.741**      | $0.472^{**}$ | -0.622**     | -0.670** | -0.668** | $0.718^{**}$ | $0.781^{**}$ | 0.756**      | 1            |              |              |              |              |    |
| KL     | $0.780^{**}$ | 0.683**      | $0.750^{**}$ | 0.749**      | 0.749**      | 0.783**      | $0.776^{**}$ | $0.518^{**}$ | -0.751**     | -0.755** | -0.719** | 0.731**      | $0.769^{**}$ | 0.737**      | 0.945**      | 1            |              |              |              |    |
| KR     | $0.787^{**}$ | $0.712^{**}$ | $0.752^{**}$ | 0.761**      | 0.756**      | $0.781^{**}$ | $0.792^{**}$ | $0.520^{**}$ | -0.750**     | -0.759** | -0.714** | 0.735**      | $0.771^{**}$ | 0.735**      | 0.923**      | 0.983**      | 1            |              |              |    |
| РСТ    | 0.719**      | 0.613**      | 0.686**      | $0.680^{**}$ | 0.692**      | $0.708^{**}$ | 0.713**      | $0.404^{**}$ | -0.745**     | -0.710** | -0.587** | 0.661**      | $0.679^{**}$ | $0.642^{**}$ | $0.784^{**}$ | $0.871^{**}$ | $0.884^{**}$ | 1            |              |    |
| PL     | 0.610**      | 0.499**      | 0.589**      | 0.611**      | 0.613**      | 0.611**      | 0.616**      | $0.440^{**}$ | -0.615**     | -0.643** | -0.534** | $0.744^{**}$ | 0.583**      | $0.572^{**}$ | 0.832**      | 0.852**      | 0.841**      | 0.829**      | 1            |    |
| PR     | $0.676^{**}$ | $0.579^{**}$ | $0.700^{**}$ | $0.711^{**}$ | $0.701^{**}$ | 0.729**      | $0.724^{**}$ | 0.319*       | -0.671**     | -0.683** | -0.602** | $0.660^{**}$ | $0.838^{**}$ | $0.801^{**}$ | $0.812^{**}$ | $0.838^{**}$ | $0.848^{**}$ | $0.892^{**}$ | $0.742^{**}$ | 1  |

Table 3. Pearson correlation for plant growth traits, biochemical traits and Cd related traits (Cd concentration in different plant tissues)

SFW: shoot fresh weight; RFW: root fresh weight; SDW: shoot dry weight; RDW: root dry weight; SL: shoot length; RL: root length; SD: stem diameter; NOL: number of leaves; CdCT: Cd concentration in crown tissue; CdL: Cd concentration in leaves; CdR: Cd concentration in roots; CaCT: Ca concentration in crown tissue; CdL: Ca concentration in roots; KCT: K concentration in crown tissue; KL: K concentration in leaves; KR: K concentration in leaves; PCT: P concentration in crown tissue; PL: P concentration in leaves; PR: P concentration in roots. Correlation is significant at \*\*  $p \le 0.01$  and \*  $p \le 0.05$ 



*Figure 4.* The expression of growth traits, biochemical traits and Cd (in the different plant parts) on the two principal components analysis (PCA) axes. Loading values for PC axis 1 and 2; and color's difference show the contribution of each trait/variable

**Table 4.** Bioaccumulation factor (BAF), bioconcentration factor (BCF), translocation factor (TF) and tolerance index (TI) values for cadmium in the crown tissue (CT), leaves (L) and roots (R) according to different Cd treatments and maize varieties

| To store                  |              | BA                                 | AF   | DCE  | Т    | тт      |       |
|---------------------------|--------------|------------------------------------|------|------|------|---------|-------|
| ractors                   |              | BAF <sub>CT</sub> BAF <sub>L</sub> |      | всг  | ТЕст | FCT TFL |       |
| Treatments<br>(T)         | To (Control) | 0.00 0.00                          |      | 0.00 | 0.00 | 0.00    | 1.01a |
|                           | T1 (500µM)   | 1.29                               | 1.87 | 2.60 | 0.51 | 0.74    | 0.77b |
|                           | Τ2 (1000μΜ)  | 1.05                               | 1.49 | 1.97 | 0.54 | 0.76    | 0.33c |
| Maize<br>varieties<br>(V) | EV-1098      | 0.35                               | 0.58 | 1.19 | 0.20 | 0.33    | 0.75  |
|                           | SA-2002      | 1.26                               | 1.54 | 1.65 | 0.52 | 0.64    | 0.64  |
|                           | EV-5098      | 0.83                               | 1.13 | 1.48 | 0.37 | 0.50    | 0.65  |
|                           | AG-2002      | 0.50                               | 0.95 | 1.02 | 0.33 | 0.62    | 0.74  |
|                           | EV-6098      | 1.26                               | 1.47 | 2.00 | 0.42 | 0.49    | 0.72  |
|                           | Sadaf        | 0.50                               | 1.04 | 1.81 | 0.19 | 0.39    | 0.66  |

Different letters in the same column indicate the significant differences compared with Cd treatments and maize varieties according to Tukey's test (p < 0.05)

## Discussion

#### Effect of Cd on the plant biomass and related growth traits

The growth traits such as SFW, RFW, SDW, RDW, SL, RL, SD and NOL interacted and significantly changed with the Cd treatments (*Table 1*). The growth traits of all varieties were reduced at applied Cd treatments and the highest reduction in these traits was pragmatic at 1000 µM CdCl<sub>2</sub> treatment level (*Table 1*). The growth traits decreased with the increasing Cd supply in the substrate/soil (Rascio et al., 1993; Klaus et al., 2013; Alia et al., 2015; Anjum et al., 2015). The fresh and dry weights of shoot and root; and shoot and root length reduced with increasing Cd level (Table 1). This reduction in the fresh and dry biomass as well as the length of maize plants may be due to Cd toxicity as well as nutrient disparity, lower water rate and nutrient uptake in plants (Ghani, 2010; Faizan et al., 2011; Alia et al., 2015; Rizwan et al., 2017; Abedi and Mojiri, 2020). Moreover, this could be due to a reduction in xylem transport caused by the interruption of the transpiration process in metal hassle, especially Cd (Hayat et al., 2020). The Cd toxicity also decreased the SD and NOL of maize plants i.e. the decreased trend with the exceeding Cd supply was observed (Table 1) in previous studies (Aslam et al., 2015; Figlioli et al., 2019). Generally speaking, the EV-1098 and AG-2002 variety performed better for all the growth traits than other varieties. However, the growth traits showed a significant decreasing trend for all the six varieties with an increased level of Cd (Table 1).

#### Cd uptake, translocation, partitioning and its effect on the different biochemical traits

The Cd uptake, translocation, partitioning and its effect on the different biochemical traits were observed in the different plant parts (leaves, crown tissue, and roots) of all varieties of maize at different Cd levels (*Table 2*). The Cd partitioning in the crown tissue, leaves and roots was substantially affected by the varieties, Cd levels and their interactions; particularly at higher Cd levels. The Cd accumulation gradually increased with increasing applied Cd levels in all the studied plant parts (Table 2), as observed in the former study (Nguyen et al., 2016). The Cd accumulation trend was recorded as: crown tissue < leaves < roots, 58%, 65% and 68%, respectively. The maize extraction capacity exhibit that the maize has the potential to accumulate heavy metals especially Cd and can be used for phytoextraction purposes (Retamal-Salgado et al., 2017). The maximum Cd was accumulated in the roots of all the six maize varieties rather than aerial plant parts (*Table 2*), similar results were found in previos studies (Zhao, 2011; Stritsis and Claassen, 2013; Singh and Srivastava, 2016; Ling et al., 2017; Boros-Lajszner et al., 2020), these results are in contrast to that the maximum Cd was accumulated in the plant aerial parts of maize rather than roots (Retamal-Salgado et al., 2017). It may be due to that the root first comes into contact with Cd and due to its immobility, more Cd retained in the roots (Anjum et al., 2015). Moreover, the Cd translocation from roots to aerial plant parts also limited by the suberization of endoderm and lignification of root cortex cells (Kaznina and Titov, 2014). For varieties, the minimum Cd concentration was noted for the EV-1098 variety (Table 2, Fig. 1), this is because the higher dry mass production was recorded in the same variety (Table 1) than other varieties (Trejo et al., 2016; Retamal-Salgado et al., 2017). The limitations of Cd accumulation in the roots and translocation from roots to other plant parts and several other factors could be involved to deal with Cd toxicity in maize (Yang et al., 2014). It may suggest that maize has effective and strong defense mechanisms to reduce Cd toxic effect than all other crops, including the Cd accumulation in the roots (Retamal-Salgado et al., 2017). Secondly, the Zn availability in the soils plays an important antagonistic role for Cd accumulation in the maize plants (Rizwan et al., 2019). Finally, the lower Cd levels possibly attributed to the higher efficiency of growth traits (*Table 1*) and biochemical traits/nutrients (*Table 2*), which result in the dilution of Cd toxic effect on maize plant (Retamal-Salgado et al., 2017; Rizwan et al., 2019). Overall, the total Cd extraction is also found significantly higher in the roots than the crown tissue and leaves (*Table 2*) (Singh and Srivastava, 2016; Boros-Lajszner et al., 2020). The same pattern was found in the other plants like wheat (Abedi and Mojiri, 2020) and mustard and oats (Boros-Lajszner et al., 2020).

The micro- and macro, both types of nutrients are required to perform the normal functioning, growth, and development in the plants. The deficiency of these nutrients, especially of macronutrients such as potassium, phosphorus, or calcium significantly affects the plant's metabolic processes (Sitko et al., 2019). The process of photosynthesis can't occur without the nutrients supply and directly dependent on mineral nutrition in plants (Engels et al., 2012). The uptake of heavy metals occurs in competition with other metals/elements such as Zn, Cu, Mn, Cd and Fe and may reduce the Fe, K, P, Zn and Ca uptake in the plants (Engels et al., 2012; Alia et al., 2015). The Ca, K and P are among essential nutrients that are required for plant growth (Engels et al., 2012). The Cd uptake, accumulation and partitioning adversely affect the nutrients uptake and distribution in plants (Alia et al., 2015). The Ca, K and P contents in the different plant tissues were significantly affected by varieties, Cd levels and interaction between them (Fig. 3, Table 2). In short, the Ca, K and P contents interacted and significantly decreased with the increasing applied Cd levels in all the studied plant parts of all the six maize varieties (Table 2) (Alia et al., 2015; Nguyen et al., 2016). The higher Cd concentration interrupts the ATPase and other enzymes functioning which uptake the K and as a consequence reduced K availability for plants (Erel et al., 2015). The K deficiency indirectly influences the photosynthetic activity and stomatal conductance of plants (Akram et al., 2020). Thus, a sufficient amount of K contents is necessary for the higher rate of plant water contents, transpiration, and stomatal conductance (Jin et al., 2011; Sitko et al., 2019). Ca plays an active role in stress signals transduction and works as an intracellular messenger (Engels et al., 2012; Hochmal et al., 2015). The distribution and uptake of Ca also considerably reduced because of the Cd treatment (Table 2), which may be due to the disruption of essential nutrient uptake supply like K, Ca and Zn by Cd (Alia et al., 2015). Moreover, the increase in Cd concentration also decreased the phosphorus contents in all parts of the maize plant (Table 2) (Shareef et al., 2018). Phosphorus is one of the main macronutrients that are necessary for nucleic acids, membrane lipids and the synthesis of ATP in addition to other metabolites (Akram et al., 2020). Moreover, P deficiency can disturb the regulation of stomata and transpiration (Singh et al., 2017; Sitko et al., 2019). In varieties, the EV-1098 variety performed better in terms of Ca, K and P contents in different plant parts and uptake maximum Ca, K and P as compared with other varieties (Table 2, Fig. 2) because minimum Cd uptake was noted for the same variety (Table 2). It also supports that increasing Cd levels decrease the minerals uptake and vice versa (Table 2) (Alia et al., 2015; Nguyen et al., 2016).

#### Pearson correlation and principal component analysis (PCA)

The positive correlations between growth traits (SFW, RFW, SDW, RDW, SL, RL, SD and NOL) with biochemical traits (CaCT, CaL, CaR, KCT, KL, KR, PCT, PL and PR) (*Table 3*) exhibit the contribution of biochemical traits in the growth and biomass production of maize varieties. The adverse effects of Cd on the plant growth and biochemical traits in all plant parts (*Tables 1-3*) may be attributed to the disruption of essential plant nutrients absorption pattern by decreasing the efficacy of root proliferation. Moreover, the distribution and uptake of Cd associated with metal transporters (divalent cations), as the nutrients uptake higher, may result in the form of direct conflict with Cd for transportation in the plants (Naveed et al., 2020). Further, all the Cd treatments and studied plant traits were effectively displaced along with the first two PC axes (*Fig. 4*), which suggests the applied Cd treatments had a deteriorative effect on the studied traits of maize plants to the control (Kaznina and Titov, 2014; Nguyen et al., 2016).

# Different factors/indices for Cd translocation, bioaccumulation, bioconcentration and tolerance

The plant's phytoextraction capacity is defined as TF, which is the measure of Cd concentration in the aerial parts and the roots of the plant (Retamal-Salgado et al., 2017). For all the treatments,  $TF_{CT}$  and  $TF_L$  values were found <1 (*Table 4*), which meant that the Cd concentration in the roots was higher than other plant tissues (crown tissue and leaves). As for 0 and 1000  $\mu$ M, the big difference between the concentrations of roots and other plant parts suggests that there is an internal restriction for the Cd transport from roots to other plant tissues, resulting in higher Cd concentrations in roots rather than crown tissue and leaves (Table 2). The apoplastic barriers in the roots of maize played a significant role under Cd stress. Higher Cd concentrations may result in harm to root apoplastic barriers, which would be helpful in retarding Cd ion's transport from roots to other plant tissues (Ling et al., 2017). The TF values in this study (Table 4) don't coincide with the findings of the earlier researchers and TF values found lower than the reported values (Liu et al., 2013; Azzi et al., 2017; Retamal-Salgado et al., 2017), which may be affected by soil pH and differences of Cd treatment that result in the reduction of Cd supply from roots to the plant aerial parts (Liu et al., 2013). This result indicates the low Cd translocation efficiency of maize varieties used in this study.

The Cd absorption capacity of plants from substrate/soil is defined as BCF, which is the relationship of Cd concentration in the plant roots and the substrate/soil. The BCF values vary from 1.02 to 2.60 (*Table 4*) and found higher than the results of other authors (Retamal-Salgado et al., 2017), which may be due to difference in the Cd treatments and maize cultivars/varieties response to Cd stress (Ghani, 2010; Nguyen et al., 2016; Shah et al., 2016; Rizwan et al., 2017). The BCF values >1 (*Table 4*) exhibit the high Cd bioaccumulation capacity of maize at the root level (*Table 2*) (Singh and Srivastava, 2016). However, most of the BAF values found comparatively lower than BCF values (*Table 4*). It is an indication of an intrinsic limitation on Cd transport from roots to the aerial parts (crown tissue and leaves), resulting in higher Cd concentrations retains in the roots rather than other plant parts (*Table 2*) (Ling et al., 2017). Consequently, high Cd concentration interrupts the apoplastic pathway and retard the Cd transport from roots to aerial parts (Ling et al., 2017; Retamal-Salgado et al., 2017). The dry mass production in the different parts (crown tissue, leaves and roots) of maize plants were impaired by the different Cd levels (*Tables 1, 4*); whereas no significant differences were noted between the varieties for TI values (*Table 4*). It may be due to the higher Cd concentration that damaged the plant roots, affect nutrients uptake in the roots and subsequently reduce/inhibit the plant growth (Klaus et al., 2013; Kaznina and Titov, 2014; Yang et al., 2014). The Cd levels (Yang et al., 2014) were lower than those used in this study, it suggests that Cd levels applied in substrate/soil are higher than the threat threshold (3.5 mg kg<sup>-1</sup>) (Yang et al., 2014). The decreased TI value with the increasing Cd supply (*Table 4*) further strengthens that the high Cd concentrations reduce the plant growth (Ghani, 2010).

# Conclusions

The growth (plant biomass, height, stem diameter and the number of leaves) and biochemical traits (Ca, K and P) are found sensitive to Cd stress and negatively influenced by different Cd levels. The Cd toxic effect directly depended on the applied Cd stress, an increase in the Cd level resulted in a decrease in maize plant growth and biochemical traits. A considerable positive correlation was noted between the growth and biochemical traits. The uptake, translocation and partitioning of Cd were varied between the different plant parts and varieties, while, the Cd contents in all plant parts tended to increase with increased Cd level. The translocation factor (TF < 1) values emphasize that maize roots have some internal restriction, so the low Cd is translocated from the substrate to roots. Moreover, the maximum Cd accumulated and retained in the roots rather than crown tissues and leaves of studied maize varieties. The EV-1098 variety performed better for all growth and biochemical traits than other varieties. Further, the EV-1098 variety was found tolerant to Cd stress even at higher Cd levels (1000 µM); while SA-2002 and EV-6098 sensitive. The results revealed the diverse varietal performance/response of studied maize varieties to induced Cd toxicity. Consequently, the EV-1098 variety could be good in terms of the growth in Cd-polluted soils. In future, molecular studies are suggested at gene level to better understand the Cd interaction with the plant's physiological and metabolic processes.

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#### APPENDIX

#### **Supplementary information**



Figure S1. Effects of Cadmium (Cd) treatments on shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW) and root dry weight (RDW) in the six maize cultivars. Bars (mean values  $\pm$  SE) having different letters show mean values that are significantly different (p<0.05)

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*Figure S2.* Effects of Cadmium (Cd) treatments on shoot length (SL), root length (RL), stem diameter (SD) and number of leaves per plant (NOL) in the six maize cultivars. Bars (mean values  $\pm$  SE) having different letters show mean values that are significantly different (p<0.05)



*Figure S3. Relationship between shoot fresh weight (SFW), root fresh weight (RFW), plant total fresh weight (PTFW), shoot dry weight (SDW), root dry weight (RDW), plant total dry weight (PTDW), shoot length (SL) and root length (RL)* 



Figure S4. Cadmium (Cd) partitioning in the crown tissues, leaves and roots of six maize cultivars at three different Cd treatments (Mean  $\pm$  SE). Bars (mean values  $\pm$  SE) having different letters show mean values that are significantly different (p<0.05)

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Figure S5. The relationships between Calcium (Ca), Potassium (K), Phosphorus (P) and Cadmium (Cd) in the three different plant parts