

Article

Silicon Fertigation Regimes Attenuates Cadmium Toxicity and Phytoremediation Potential in Two Maize (*Zea mays* L.) Cultivars by Minimizing Its Uptake and Oxidative Stress

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Abstract: Silicon (Si) is an important plant-derived metabolite that is significantly involved in maintaining the stability of a plant's metabiological, structural and physiological characteristics under the abiotic stressed environment. We conducted the present study using maize (*Zea mays* L.) cultivars (Sadaf and EV-20) grown in sand artificially contaminated with cadmium (500 μ M) in Hoagland's nutrient solution to investigate its efficiency. Results from the present study evidenced that the toxic concentration of Cd in sand significantly reduced shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight by 88, 94, 89, 86 99 and 99%, respectively, in Sadaf while decreasing by 98, 97, 93, 99, 84 and 91%, respectively, in EV-20. Similarly, Cd toxicity decreased total chlorophyll and carotenoid content in both varieties of *Z. mays*. Moreover, the activities of various antioxidants (superoxidase dismutase, peroxidase and catalase) increased under the toxic concentration of Cd in sand which was manifested by the presence of membrane permeability, malondialdehyde (MDA), and hydrogen peroxide (H_2O_2). Results additionally showed that the toxic effect of Cd was more severe in EV-20 compared with Sadaf under the same conditions of environmental stresses. In addition, the increased concentration of Cd in sand induced a significantly increased Cd accumulation in the roots (141 and 169 $mg\ kg^{-1}$ in Sadaf and EV-20, respectively), and shoots (101 and 141 $mg\ kg^{-1}$ in Sadaf and EV-20, respectively), while; EV-20 accumulated higher amounts of Cd than Sadaf, with the values for both bioaccumulation factor (BAF) and translocation factor (TF) among all treatments being less than 1. The subsequent negative results of Cd injury can be overcome by the foliar application of Si which not only increased plant growth and biomass, but also decreased oxidative damage induced by the higher concentrations of MDA and H_2O_2 under a Cd-stressed environment. Moreover, external application of Si decreased the concentration of Cd in the roots and shoots of plants, therefore suggesting that the application of Si can ameliorate Cd toxicity in *Z. mays* cultivars and results in improved plant growth and composition under Cd stress by minimizing oxidative damage to membrane-bound organelles.

Keywords: oxidative stress; antioxidants defense system; cadmium stress; silicon; chlorophyll pigments; cereal crops

1. Introduction

Metal contamination issues are becoming increasingly common across the world, with many documented cases of metal toxicity in mining industries, foundries, smelters, coal-burning power plants and agriculture [1,2]. Among various toxic pollutants, Cadmium (Cd) is a more prominent toxic pollutant due to its severe toxicity and ability to induce damage to normal growth and development [3,4]. Cd, a known toxic heavy metal, possesses properties of water solubility, phytotoxicity, higher rates of relative mobility, and can induce toxicity to membrane-bound organelles [5,6]. Moreover, Cd is classified as a non-essential element for plant growth and development and its high accumulation in the plant organs is particularly concerning [7]. Plants absorb Cd through active transport of Fe transporters and by passive transport through ionic transcription rates by transferring through xylem and phloem transportation processes [8,9]. Hence, accumulation of Cd leads to the deficiency of iron, calcium, and magnesium by disturbing morphological and biochemical processes in plants [10]. Furthermore, Cd-toxicity damages plant cells including chloroplasts, cell nuclei, and mitochondria leading to a reduction in chlorophyll [11]. Due to increased Cd-stress, the formation of reactive oxygen species (ROS) arises that do not partake in Fenton-type reactions, which ultimately initiates destructive pathways in plants [12]. The peroxidation of lipids significantly enhances the accumulation of malondialdehyde (MDA) content [13,14]. The generation of ROS are easily handled by plants through adopting extremely effective endogenous approaches for the mitigation of ROS by implementing enzymatic and non-enzymatic antioxidants of their constituents [15,16]. The principal antioxidant enzymes are catalase (CAT), ascorbate peroxide (APX), and superoxide dismutase (SOD) which were characterized by converter hydrogen peroxide (H_2O_2), and superoxide (O^{-2}) to mitigate toxicity in plants and reduce the concentration of MDA and H_2O_2 [17–19]. Therefore, it remains a priority to decrease the concentration of Cd in nutritional crops to limit transmission into the food chain [20].

Silicon (Si) is the second most abundant metalloid found in the form of mono-silicic acid and is an essential chemical element in plant biology (required by plants, animals and microorganisms) [5,11]. It is a beneficial element for plants and improves the structural integrity of plants exposed to conditions of environmental stress, most importantly; salt, heavy metals, drought, temperature changes and freezing, pests and disease stresses [21]. From soil, uptake of Si depends on the type of growth medium, soil properties and plant species where plants are classified as high-, medium- and low-Si accumulators [22]. A number of studies revealed that Si application increased plant growth and biomass [23], mineral uptake [24], gaseous exchange attributes [25], and reduced oxidative stress by scavenging reactive oxygen species (ROS) [26], and diminished accumulation of organic acids in different plant species [11]. Moreover, maize (*Zea mays* L.) also known as corn, is the main cereal grain crop that is cultivated worldwide [27]. As (*Zea mays* L.) remains an important food crop, it is known to be a social security concern for farmers [27] and an important feed and industrial source [28]. In previous studies, *Z. mays* was used widely to cultivate under Cd-stressed environments as an efficient source of phytoextraction and plants were able to accumulate Cd contents in their roots and shoots [29]. According to previous studies, the loss in the yield of *Z. mays* mainly depended upon the accumulation of heavy metals (especially Cd) in the soil [30]. Hence, it is extremely important to find possible methods which can ameliorate Cd toxicity in *Z. mays* to able to fulfil the market demands of this cereal crop. Therefore, there remains an increasing demand for Cd minimization in *Z. mays* for the agro-environmental sustainability of world maize production and food safety.

Over the recent years, various applications such as crop residues, manure, compost, fertilizers, micronutrients, and biochar are being commonly used, and certain heavy metals have received considerable attention regarding plant morphology and physiology owing to increasing environmental exposure, which are additionally likely to bear negative impacts on cereal crops including *Z. mays*. Previously, numerous studies were conducted using *Z. mays* under the toxic environment of Cd stress, however, the foliar application of Si on various growth and physiological attributes of *Z. mays* was rarely investigated under

metal stressed conditions. Therefore, the present research was conducted to investigate (I) the effect of Si application on the growth and biomass (II) photosynthetic pigments (III) antioxidant capacity and (IV) phytoremediation potential of *Z. mays* under toxic levels of Cd in sand. Hence, results from the present study will help improve the understanding behind the mechanism of plant tolerance and mobilization of Cd concentration among the various parts of plants using foliar applications of Si.

2. Materials and Methods

The mature and healthy seeds of maize (*Z. mays*) cultivars (Sadaf and EV-20) were collected from the Ayub Agricultural Research Institute (AARI), Faisalabad 38000, Pakistan. A pot experiment was conducted in the greenhouse of the Department of Botany, Government College University, Faisalabad 38000, Punjab, Pakistan (31° 24′N, 73° 04′E). Before seed sowing, the seeds were carefully washed and sterilized in 0.1 % HgCl₂ solution for 1 min and then washed thrice with distilled water. All pots (35 cm height × 25 cm width) were covered with plastic bags. For the complete removal of cations and anions, the sand was washed with distilled water several times. After that, in each pot, about 15 seeds were sown and each pot was kept in a greenhouse where they received natural light and air. Each pot was placed in a randomized manner with three replicates per treatment being carried out. The overall average day/night temperature was 19 ± 3/10 ± 2 °C, with a relative humidity of 62.0–65.1%, and the day length averaged 10–11 hours per day, respectively. For the onset of any emergency, $\frac{1}{2}$ strength Hoagland's solution was prepared [31] and poured in each pot at an interval of every 5 days. The pH of soil was adjusted to values ranging from 6.0–6.2 using diluted H₂SO₄ solution. After one week, the propagated *Z. mays* seedlings were treated by inducing Cd stress. For the application of Cd stress, cadmium chloride (CdCl₂) salt was used. The concentration of Cd (500 µM) was added to the Hoagland's media. For the comparison group, the seedlings without Cd stress were treated as a control (Cd 0 µM). Meanwhile, the set of treatments were arranged as Si (6 mM) applied for foliar application, Cd (500 µM) for soil, and Si × Cd (6 mM and 500 µM) were applied, respectively. K₂SiO₃ salt (6 mM) with Twin-20 reagent was sprayed. After three weeks of stress treatment, the data were recorded for various biochemical and growth parameters, respectively.

2.1. Parameters

2.1.1. Growth Attributes

Experimental data were collected from the propagated shoot and root lengths, as well as the weight of fresh and dry leaves of the two cultivars, which were recorded. Root length and shoot length was measured using a measuring scale from the tips of the shoots to the bottom of the root tips. After that, fresh biomass (roots and shoots) was also measured using a weighting digital balance by selecting three randomly plants per treatment. The plant samples were oven-dehydrated at 65 °C for 72 h for Cd and ions concentration determination and the total plant dry weight was also measured. Before being oven-dried, roots were immersed in 20 mM Na₂EDTA for 15–20 min to remove Cd that had adhered to the surface of roots. Subsequently, roots were washed thrice with distilled water and finally once with deionized water then dried for further analysis.

2.1.2. Photosynthetic Pigments

Leaves were collected for the determination of chlorophyll and carotenoid contents. For chlorophyll, 0.1 g of fresh leaf sample was extracted using 8 mL of 95% acetone for 24 h at 4 °C under dark conditions. The absorbance was measured by a spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan) at 646.6, 663.6, and 450 nm. Chlorophyll content was calculated using the standard method of Arnon [32].

2.1.3. Measurement of H₂O₂ Contents

To estimate H₂O₂ content of plant tissues (root and leaf), 3 mL of sample extract was mixed with 1 mL of 0.1% titanium sulfate in 20% (v/v) H₂SO₄ and centrifuged at 6000 × g

for 15 min. The yellow color intensity was evaluated at 410 nm. The H_2O_2 level was computed by the extinction coefficient of $0.28 \text{ mmol}^{-1} \text{ cm}^{-1}$. The contents of H_2O_2 were measured by the method presented by Jana and Choudhuri [33].

2.1.4. Malondialdehyde (MDA) Contents

The degree of lipid peroxidation was evaluated as malondialdehyde (MDA) contents. Briefly, 0.1 g of frozen leaves were ground at 4°C in a mortar with 25 mL of 50 mM phosphate buffer solution (pH 7.8) containing 1% polyethene pyrrole. The homogenate was centrifuged at $10,000\times g$ at 4°C for 15 min. The mixtures were heated at 100°C for 15–30 min and then quickly cooled in an ice bath. The absorbance of the supernatant was recorded by using a spectrophotometer (xMark™ Microplate Absorbance Spectrophotometer; Bio-Rad, Hercules, CA, USA) at wavelengths of 532, 600, and 450 nm. Lipid peroxidation was expressed as 1 mol g^{-1} by using the formula: $6.45 (A_{532} - A_{600}) - 0.56 A_{450}$. Lipid peroxidation was measured using a method previously published by Heath and Packer [34].

2.1.5. Relative Membrane Permeability (RMP)

Fresh leaves from *Z. mays* seedlings (0.5 g) were cut into uniformly sized pieces and placed in a test tube with 10 ml deionized water. Test tubes were incubated at 25°C for 2 h and EC was measured. Then, the test tubes were left overnight and EC_1 was measured. Afterward, the test tubes were autoclaved at 1000°C for 1 h and EC_2 was measured. The calculation was done with the help of the formula used by researchers in [35].

2.1.6. Antioxidant Activities Determination

To evaluate enzyme activities, fresh leaves (0.5 g) were homogenized in liquid nitrogen and 5 mL of 50 mmol sodium phosphate buffer (pH 7.0), including 0.5 mmol EDTA and 0.15 mol NaCl. The homogenate was centrifuged at $12,000\times g$ for 10 min at 4°C , and the supernatant was used for measurement of superoxidase dismutase (SOD) and peroxidase (POD) activities. SOD activity was assayed in a 3 mL reaction mixture containing 50 mM sodium phosphate buffer (pH 7), 56 mM nitro blue tetrazolium, 1.17 mM riboflavin, 10 mM methionine, and 100 μL enzyme extract. Finally, the sample was measured using a spectrophotometer (xMark™ Microplate Absorbance Spectrophotometer; Bio-Rad). Enzyme activity was measured by using a method employed by Chen and Pan [36] and expressed as $\text{U g}^{-1} \text{ FW}$. POD activity in the leaves was estimated by using the method of Sakharov and Ardila [37] employed by using guaiacol as the substrate. A reaction mixture (3 mL) containing 0.05 mL of enzyme extract, 2.75 mL of 50 mM phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2 , and 0.1 mL of 4% guaiacol solution was prepared. Increases in the absorbance at 470 nm due to guaiacol oxidation was recorded for 2 min. One unit of enzyme activity was defined as the amount of the enzyme. Catalase (CAT) activity was analyzed according to Aebi [38]. The assay mixture (3.0 mL) was comprised of 100 μL enzyme extract, 100 μL H_2O_2 (300 mM), and 2.8 mL 50 mM phosphate buffer with 2 mM ETDA (pH 7.0). The CAT activity was measured by the decline in absorbance at 240 nm as a result of H_2O_2 loss ($\epsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$).

2.1.7. Determination of Cd

The ground samples were digested with pure HNO_3 at 190°C for 45 min (10 min pre-heating, 15 min heating, 20 min cooling) in a microwave oven (Mars 6, CEM Corporation, Matthews, NC, USA) with the settings described in detail by Jezek et al., [39]. Samples were diluted with 2% HNO_3 and determined by an atomic absorption spectrophotometer (AAS), model Agilent 240FS-AA.

Bioaccumulation factor (BAF) was calculated as the ratio of Cd concentration in tissues and Cd concentration in nutrient media, by using the following formula:

$$\text{BAF} = \text{Cd concentration (plant tissues)} / \text{Cd concentration (nutrient media)}$$

while the translocation factor (TF) was determined by estimating the concentration of Cd in one part of the plant with respect to the other parts as follows:

$$TF = \text{Cd concentration (shoots)} / \text{Cd concentration (roots)}$$

2.1.8. Statistical Analysis

Statistical analysis of data were performed with analysis of variance (ANOVA) by using a statistical program Co-Stat version 6.2, Cohorts Software, 2003, Monterey, CA, USA. All the data obtained were tested by two-way analysis of variance (two-way ANOVA). Thus, the differences between treatments were determined by using ANOVA, and the least significant difference test ($p < 0.05$) was used for multiple comparisons between treatment means. Logarithmic or inverse transformations were performed for data normalization, where necessary, prior to analysis. Pearson's correlation analysis was performed to quantify relationships between various analyzed variables. The graphical presentation was carried out by using Origin-Pro 2017. The Pearson correlation coefficients and the principal component analysis between the measured variables of *Z. mays* were also calculated using RStudio software.

3. Results

3.1. Effect of Foliar Application of Si on Plant Growth and Biomass in Both Varieties of *Z. mays* Grown under the Toxic Concentration of Cd in the Sand

In the present study, various growth and biomass parameters were also measured in both varieties of *Z. mays* (Sadaf and EV-20) grown under the application of Si (3 mM) with or without the toxic concentration of Cd (500 μM) in the sand. The data regarding root and shoot length, fresh and dry biomass in both varieties of *Z. mays* is presented in Figure 1. According to the results, it was noticed that the toxic amount of Cd (500 μM) in the sand significantly ($p < 0.05$) decreased root length, shoot length, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight in both cultivars of *Z. mays*, compared with the plants grown in the control treatment (0 μM Cd concentration) (Figure 1; Table 1). Compared with the control treatment, maximum decreases of 88, 94, 89, 86, 99 and 99% in root length, shoot length, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight, respectively, were observed in Sadaf, while decreases of 98, 97, 93, 99, 84 and 91%, respectively, in EV-20, were observed in the plants grown under the toxic concentration of Cd (500 μM) in the sand. Results also showed that the toxic effect of Cd was more severe in EV-20 compared with Sadaf under the same environmental stressed condition in terms of plant growth and biomass attributes. Although, plant growth and biomass of *Z. mays* cultivars under the toxic concentration of the Cd in the sand could be improved by the foliar application of Si (Figure 1; Table 1). The application of Si increased the root length, shoot length, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight by 0.6, 0.7, 0.2, 0.6, 0.7 and 0.6%, respectively, in the Sadaf, and also increased by 1.2, 0.5, 0.1, 0.7, 0.4 and 0.3%, respectively, in the EV-20 plants grown under the application of Si (3 mM), compared with plants grown in sand without the application of Si (0 mM).

3.2. Effect of Foliar Application of Si on Chlorophyll Pigments in Both Varieties of *Z. mays* Grown under the Toxic Concentration of Cd in the Sand

We also demonstrated various photosynthetic pigments in both cultivars (Sadaf and EV-20) of *Z. mays* grown under the toxic concentration of Cd (500 μM) in sand with or without the foliar application of Si (Figure 2; Table 2). Results showed that plants which were grown in the toxic amount of Cd in the sand significantly ($p < 0.05$) decreased chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content in both cultivars of *Z. mays* compared with the plants grown in the control treatment. However, the results also advocated that Sadaf showed a greater concentration of photosynthetic pigments compared with EV-20 under the same level of stress in the sand. The concentrations of chlorophyll and carotenoid could be enhanced in both cultivars of *Z. mays* by the foliar

application of Si which increased these pigments significantly even in the Cd-contaminated sand. Our results showed that the application of Si increased chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents by 0.026, 0.034, 0.31 and 0.06%, respectively, in Sadaf while also increasing by 0.03, 0.046, 0.42 and 0.08%, respectively, in EV-20 in the plants which were grown in sand containing the toxic concentration of Cd upon foliar application of Si (3 mM) compared with plants which were grown without the foliar application of Si.

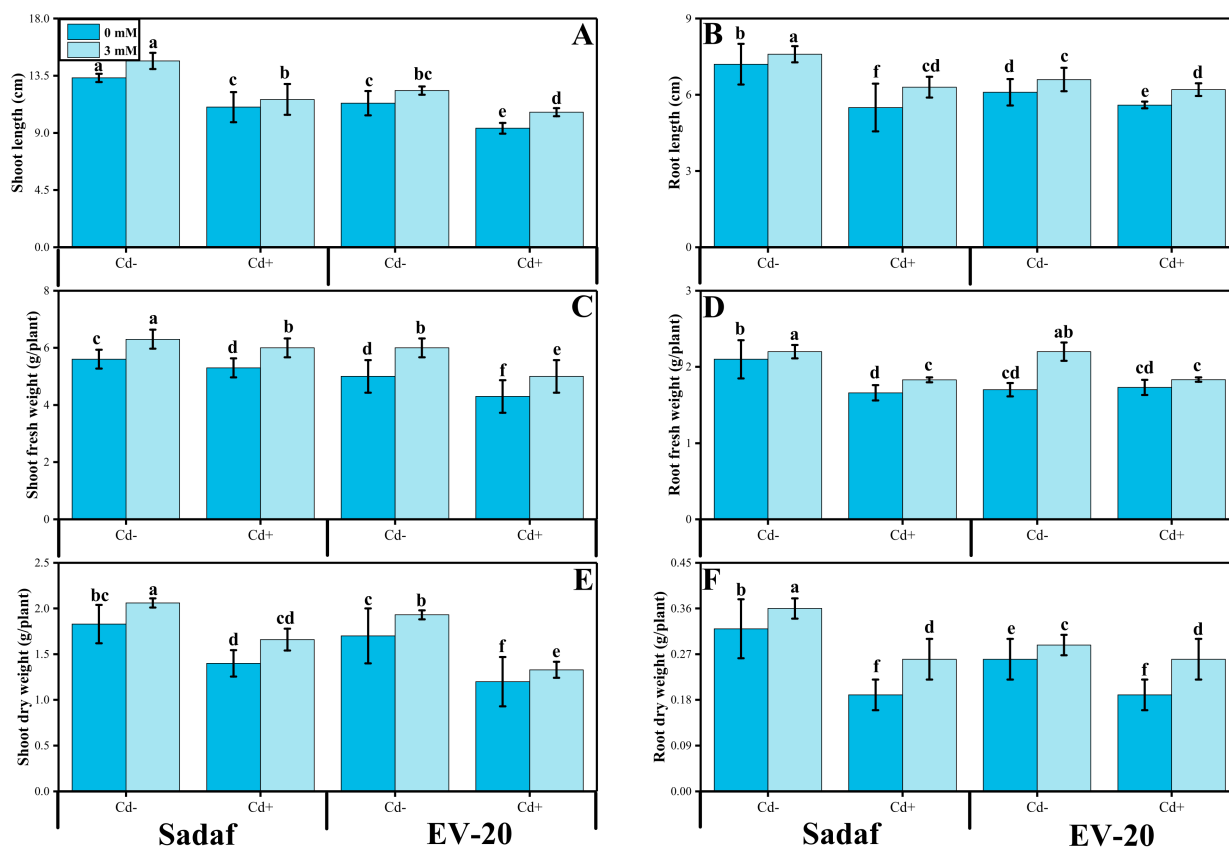


Figure 1. Effect of different levels of Si (0 and 6 mM) on shoot length (A), root length (B), shoot fresh weight (C), root fresh weight (D), shoot dry weight (E) and root dry weight (F) under the toxic concentration of Cd (500 μ M) in the sand in both cultivars of *Z. mays* (Sadaf and EV-20). All the data represented were the average of three replications (n = 3). Error bars represent the standard deviation (SD) of three replicates. Different lowercase letters on the error bars indicate a significant difference between the treatments.

Table 1. Mean square values from ANOVA of data for foliar applied silicon modulate growth in *Z. mays* cultivars grown under cadmium stress conditions.

Source of Variance	df	Shoot Length	Root Length	Shoot Fresh Weight	Shoot Dry Weight	Root Fresh Weight	Root Dry Weight
Varieties (V)	1	18.47 ***	420.74 ***	661.54 ***	92.0 **	0.02ns	0.082ns
Cd stress (S)	1	166.05 ***	1858.70 ***	18.64 **	80.05 ***	31.15 ***	2.38 ***
Treatments (T)	1	67.04 ***	355.80 ***	160.17 ***	35.04 ***	0.95 ***	0.449 ***
V \times S	1	4.04 **	47.35 ***	25.16 ***	27.04 ***	0.05ns	0.16 *
V \times T	1	6.04 ***	21.05 ***	27.16 ***	23.37 ***	0.01ns	0.01ns
S \times T	1	1.04ns	14.88 ***	28.16ns	16.04 **	0.042ns	0.071ns
V \times S \times T	1	2.03ns	0.04 ***	1.15	3.03 *	0.060ns	0.02ns
Error	16	3.45	0.70	1.25	3.83	0.032	0.026

*, **, *** = significant at 0.05, 0.01 and 0.001 levels, respectively. ns = non-significant. Abbreviations: SL = shoot length; RL = Root length; SFW = Shoot fresh weight; RFW = Root fresh weight; SDW = shoot dry weight; RDW = root dry weight.

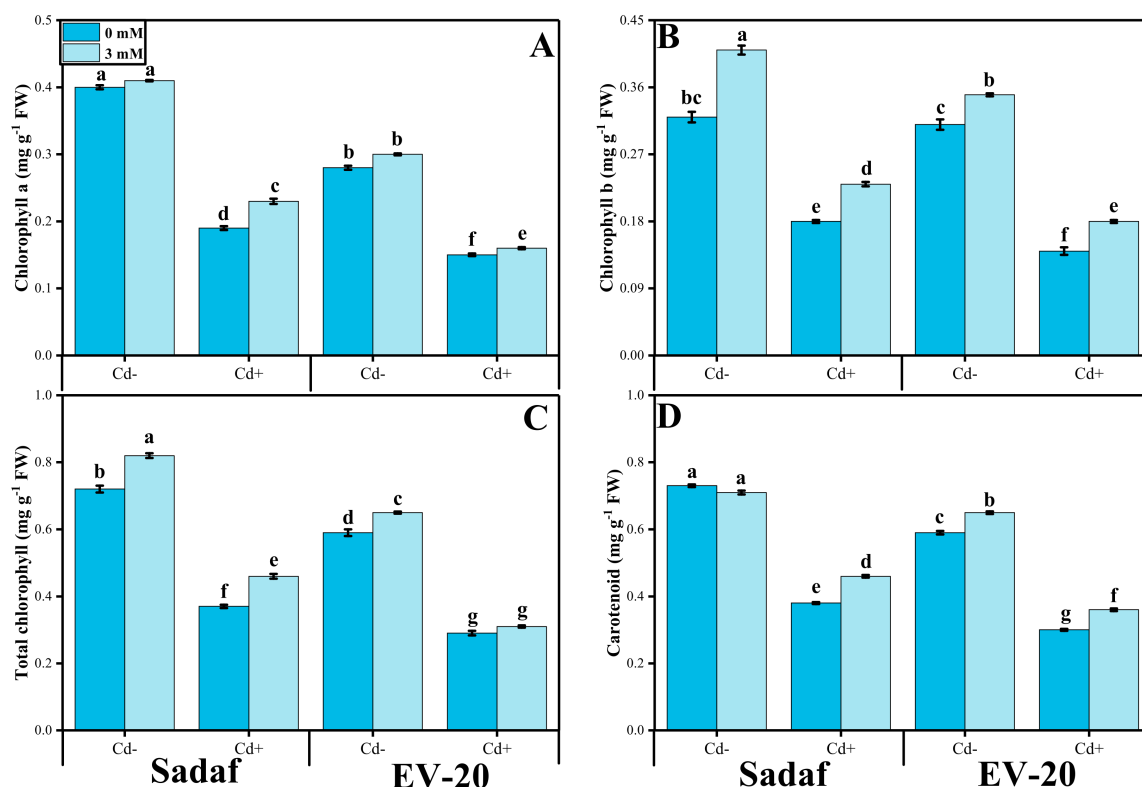


Figure 2. Effect of different levels of Si (0 and 6 mM) on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and carotenoid (D) contents under the toxic concentration of Cd (500 μ M) in the sand in both cultivars of *Z. mays* (Sadaf and EV-20). All the data represented are the average of three replications (n = 3). Error bars represent the standard deviation (SD) of three replicates. Different lowercase letters on the error bars indicate a significant difference between the treatments.

Table 2. Mean square values from ANOVA of data for foliar applied silicon modulate photosynthetic pigments in *Z. mays* cultivars grown under cadmium stress conditions.

Source of Variance	df	Chl. a	Chl. b	Carotenoids	Total Chl.
Varieties (V)	1	4.77 **	31.04 **	0.145 ***	0.03ns
Cd stress (S)	1	4.60 ***	42.56 ***	0.314 ***	0.34ns
Treatments (T)	1	3.456 **	91.49 **	0.404 **	15.37 **
V \times S	1	5.16 ns	9.25ns	0.081 *	0.03ns
V \times T	1	6.08 **	8.85 **	0.07ns	0.04ns
S \times T	1	4.132 **	9.54 **	0.02ns	0.04 **
V \times S \times T	1	0.14	6.023	0.17ns	1.0 ns
Error	16	0.354	0.13	0.09	3.46

*, **, *** = significant at 0.05, 0.01 and 0.001 levels, respectively. ns = non-significant. Abbreviations: Chl. a = chlorophyll a, Chl. b = chlorophyll b; Car. Carotenoids; Total. Chl. = Total chlorophyll.

3.3. Effect of Foliar Application of Si on Oxidative Stress Biomarkers, Relative Membrane Permeability and Antioxidant Capacity in Both Varieties of *Z. mays* Grown under the Toxic Concentration of Cd in the Sand

Moreover, we measured various oxidative stress biomarkers such as malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) contents and relative membrane permeability (RMP) in *Z. mays* cultivars under the toxic concentration of the Cd in sand with or without the foliar application of Si (Figure 3; Table 1). Results showed that the toxic concentration of Cd in the sand significantly ($p < 0.05$) increased the contents of MDA, H₂O₂ and RMP in both cultivars of *Z. mays*, compared with the plants which were grown in normal sand (Figure 3; Table 1). Similarly, result showed that the activities of various antioxidants such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were also

increased in the plants which were grown in sand with the toxic concentration of Cd (500 μM), compared with the plants which were grown in the Cd free sand (Figure 3; Table 1). However, the concentrations of MDA, H_2O_2 and RMP were higher in the Sadaf while the activities of antioxidants were higher in the EV-20. We also provided evidence that the application of Si significantly ($p < 0.05$) decreased the contents of MDA, H_2O_2 and RMP in both cultivars of *Z. mays*, compared with the plants which were not treated with Si. In contrast, results additionally illustrated that the application of Si further increased the activities of various antioxidants (SOD, POD and CAT) in both cultivars of *Z. mays*, compared with the plants which were not treated with Si.

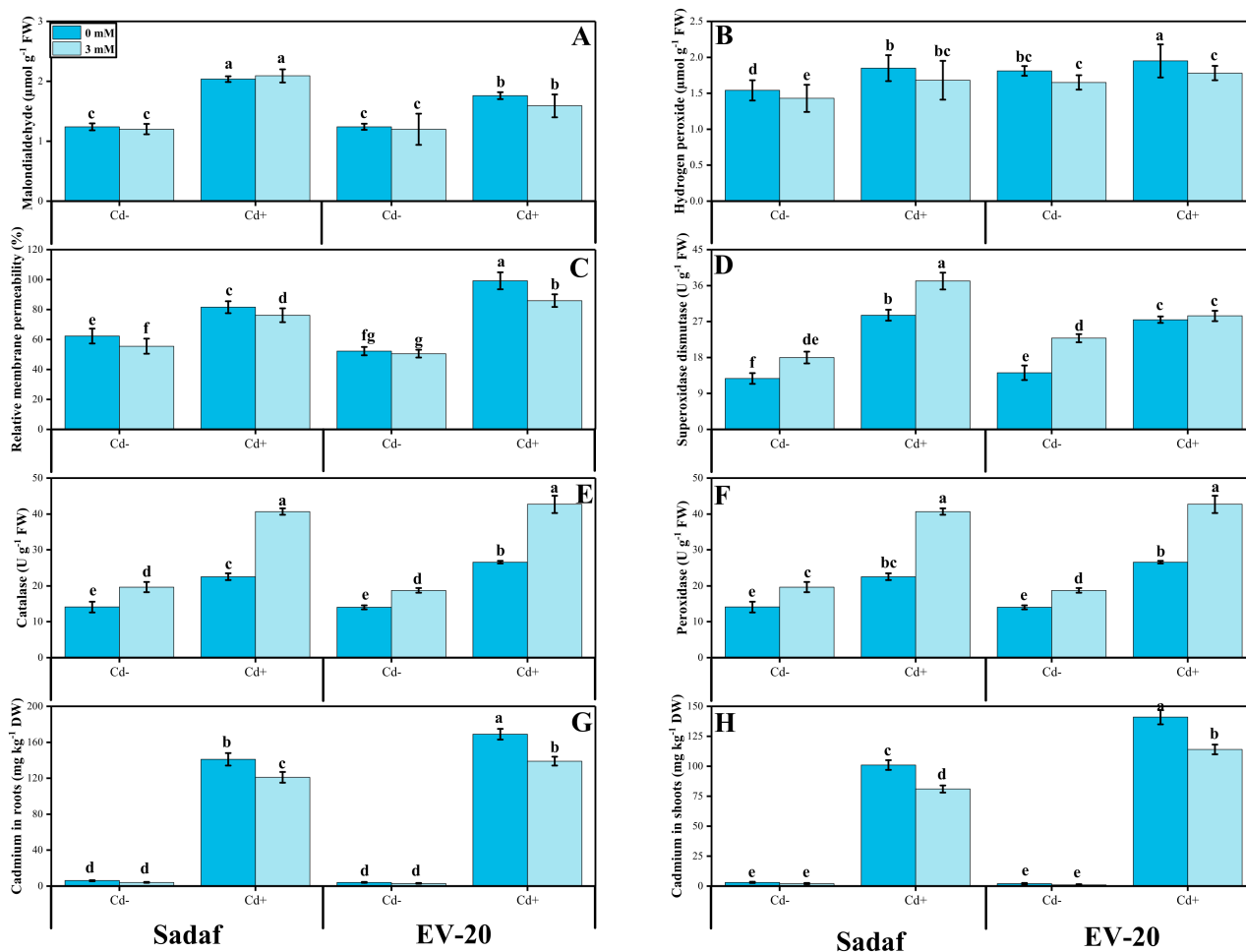


Figure 3. Effect of different levels of Si (0 and 6 mM) on malondialdehyde (A), hydrogen peroxide (B), relative membrane permeability (C), superoxide dismutase SOD (D), catalase CAT (E), peroxidase POD (F), cadmium concentration in the roots (G) and cadmium concentration in the shoots (H) under the toxic concentration of Cd (500 μM) in the sand in both cultivars of *Z. mays* (Sadaf and EV-20). All the data represented are the average of three replications ($n = 3$). Error bars represent the standard deviation (SD) of three replicates. Different lowercase letters on the error bars indicate a significant difference between the treatments.

3.4. Cd Accumulation

The concentration of Cd was additionally detected from the roots and shoots of Sadaf and EV-20 with or without the foliar application of Si when grown in sand containing the toxic concentration of Cd (Figure 3; Table 3). Results from the present study showed that the toxic concentration of Cd in the sand significantly ($p < 0.05$) increased Cd uptake in the roots and shoots of both cultivars of *Z. mays* compared with the plants which were grown without the addition of Cd in the sand. In addition, increased concentration of Cd in the

sand induced a significant increase of Cd accumulation in the roots (141 and 169 mg kg⁻¹ in Sadaf and EV-20, respectively), and shoots (101 and 141 mg kg⁻¹ in Sadaf and EV-20, respectively), while; EV-20 accumulated in a higher amount of Cd than Sadaf, and the values for both bioaccumulation factor (BAF) and translocation factor (TF) at all treatments were less than 1. (Figure 3; Tables 3 and 4). Moreover, results showed that Si application decreased the levels of Cd in the roots and shoots of the plants compared with plants which were not treated with Si. The decrease in Cd accumulation was observed in both varieties of *Z. mays* (Sadaf and EV-20) under the controlled treatment or Cd-stressed environment (Figure 3; Table 3). Similarly, application of Si also decreased the values of BAF (roots and shoots) and TF in both cultivars of *Z. mays* when grown in the Cd-contaminated sand mixture (Table 4).

Table 3. Mean square values from ANOVA of data for foliar applied silicon oxidative defense system and Cd uptake in *Z. mays* cultivars under the cadmium-contaminated soil.

Source of Variance	df	MDA	RMP	H ₂ O ₂	SOD	POD	CAT	Cd-R	Cd-S
Varieties (V)	1	0.16 *	0.06 ns	0.083 ns	5.76 ns	734.78 ***	8.57 ns	0.14 **	0.13 **
Cd stress (S)	1	6.27 ***	6.70 ***	1.03ns	1085.52 ***	588.89 ***	1730.47 ***	0.436 ***	0.219 ***
Treatments (T)	1	5.13 ***	6.35 ***	1.30 **	204.71 **	54.84 **	839.16 **	0.566 ***	0.467 **
V × S	1	0.02 ns	0.08 ns	0.03ns	87.30ns	14.07 ns	19.67ns	0.624 **	0.246 **
V × T	1	0.13 ns	0.03 ns	2.34 **	5.66 ***	9.66 ns	2.067ns	0.008 ns	0.04 ns
S × T	1	0.07 **	0.12**	0.05 ns	8.13 ***	36.58 **	314.07***	0.476 ***	0.268 ***
V × S × T	1	0.09 ns	0.02	0.04 ns	47.32 *	83.92 ***	1.55 ns	0.746 **	0.67 ***
Error	16	0.06	0.17	0.14	7.27	19.13	3.45	16.4	5.6

*, **, *** = significant at 0.05, 0.01 and 0.001 levels, respectively. ns = non-significant. Abbreviations: MDA = malondialdehyde; RMP = Relative membrane permeability; H₂O₂ = hydrogen peroxide; SOD = superoxide dismutase; POD = peroxidase; CAT = catalase; Cd-R = Cadmium concentration in the roots and; Cd-S = Cadmium concentration in the shoots.

Table 4. Effect of Si application on bioaccumulation factor (BAF) and translocation factor (TF) of different varieties of *Z. mays* grown under the cadmium-contaminated soil.

Treatments	Varieties	BAF (Roots)	BAF (Shoots)	TF
	Sadaf			
Cd+ Si-		0.28	0.20	0.71
Cd+ Si+		0.24	0.16	0.66
	EV-20			
Cd+ Si-		0.33	0.28	0.83
Cd+ Si+		0.27	0.22	0.82

Different treatments used in the table are as follows: Cd+ Si- (Cd = 500 µM + Si = 0 mM) and Cd+ Si+ (Cd = 500 µM + Si = 3 mM).

3.5. Relationship between Various Morpho-Physiological Parameters with Cd Uptake and Accumulation in the Various Parts of the Plants

A Pearson's correlation was illustrated to depict a relationship between various growth and physiological parameters of *Z. mays* cultivars grown under Cd stress with or without the foliar application of Si (Figure 4). As both varieties showed the same trend under a Cd-stressed environment, thus we have studied the tolerant variety i.e., Sadaf in this relationship. Cd concentration in the root was positively correlated with CAT, POD, RMP, MDA, SOD, H₂O₂ and Cd concentration in the shoots while negatively correlated with Chl-a, TC, Carot, SDW, SFW, RFW, Chl-b, RDW, SL and RL. Similarly, Cd concentration in the shoots were positively correlated with CAT, POD, RMP, MDA, SOD, H₂O₂ and Cd concentration in the roots while negatively correlated with Chl-a, TC, Carot, SDW, SFW, RFW, Chl-b, RDW, SL and RL. This relationship showed a close connection between various growth parameters studied in this experiment.

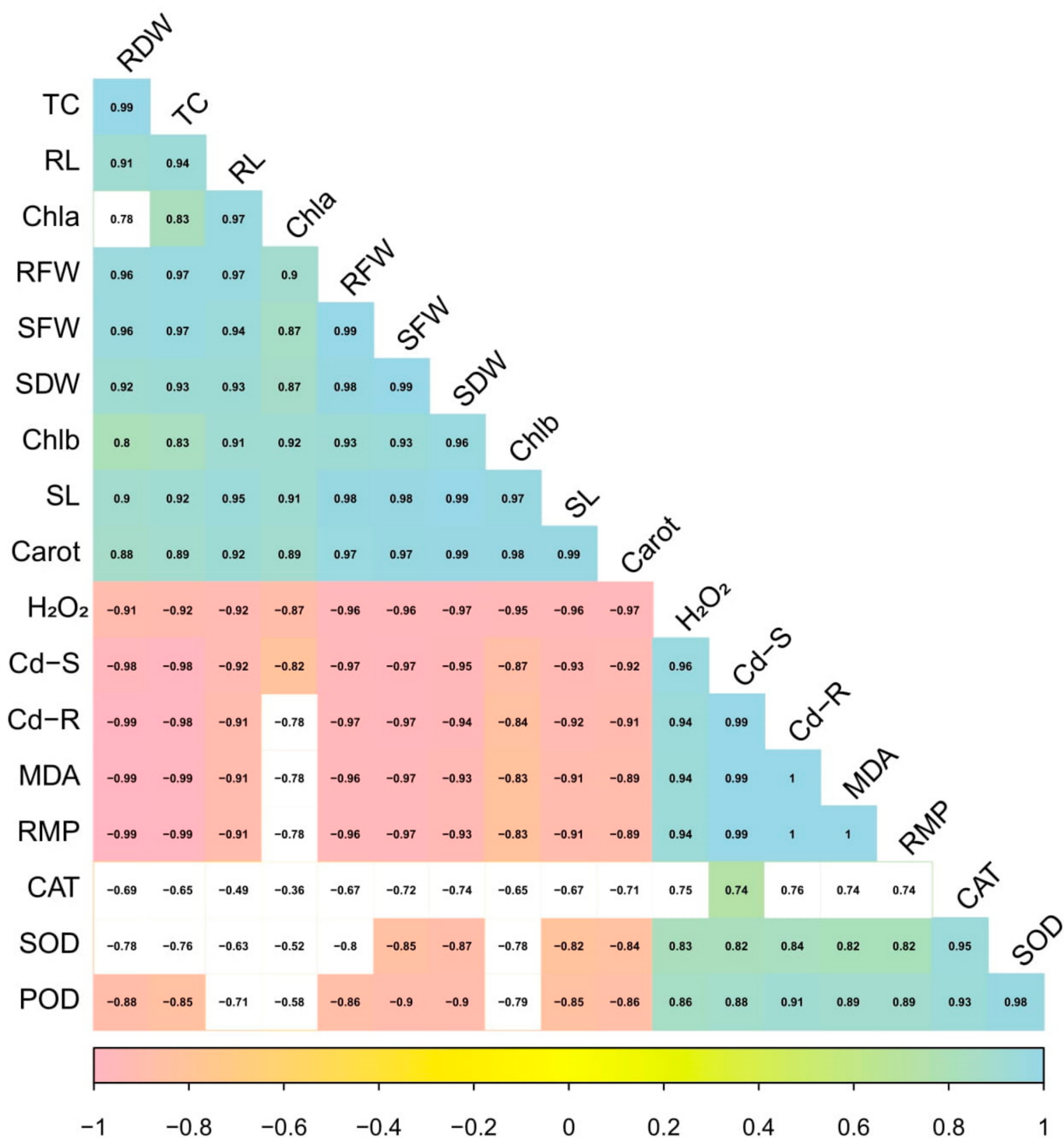


Figure 4. Pearson’s correlation between various morpho-physio-biochemical attributes of *Z. mays* cultivars grown under the application of Si in Cd-contaminated soil. Different abbreviations used in the figure are as follows: TC (total chlorophyll content), RL (root length), Chla (chlorophyll a content), RFW (root fresh weight), SFW (shoot fresh weight), SDW (shoot dry weight), Chlb (chlorophyll b content), SL (shoot length), Carot (carotenoid content), H₂O₂ (hydrogen peroxide content), Cd-S (cadmium concentration in the shoots), Cd-R (cadmium concentration in the roots), MDA (malondialdehyde content), RMP (relative membrane permeability), CAT (catalase activity), SOD (superoxidase activity) and POD (peroxidase activity).

3.6. Principal Component Analysis

The loading plots of principal component analysis (PCA) depicted a close connection between various growth and physiological parameters in *Z. mays* under the application of Si in a Cd-stressed environment as presented in Figure 5. Among the entire database, Dim1 and Dim2 exhibited the maximum contribution and occupied more than 96% of the database. Among which Dim1 exhibited (89.4%) and Dim2 exhibited (6.7%) of contributions from the total database. All studied parameters were distributed successfully in the database which gave a clear indication that Cd stress caused a significant impact to the growth and eco-physiology of *Z. mays*. From the results, it can be indicated that Cd concentration in the roots, CAT, POD, RMP, MDA, SOD, H₂O₂ and Cd concentration in the shoots were positively correlated in the database. While; Chl-a, TC, Carot, SDW, SFW, RFW, Chl-b, RDW, SL and RL were negatively correlated, when compared with all other variables in the database.

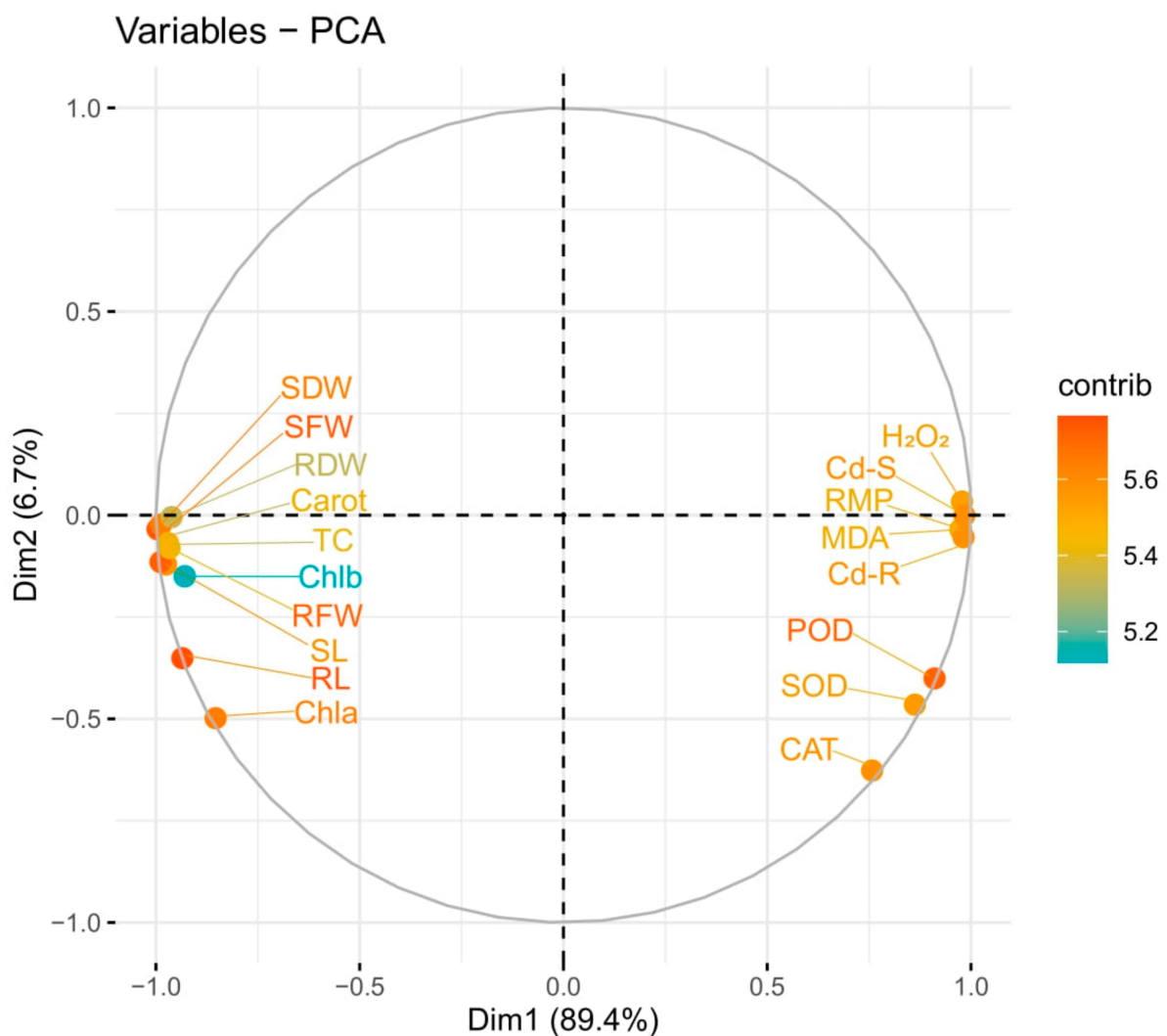


Figure 5. The loading plots of principal component analysis of *Z. mays* under the application of Si with or without the toxic concentration of Cd in the sand. Different abbreviations used in the figure are as follows: TC (total chlorophyll content), RL (root length), Chla (chlorophyll a content), RFW (root fresh weight), SFW (shoot fresh weight), SDW (shoot dry weight), Chlb (chlorophyll b content), SL (shoot length), Carot (carotenoid content), H₂O₂ (hydrogen peroxide content), Cd-S (cadmium concentration in the shoots), Cd-R (cadmium concentration in the roots), MDA (malondialdehyde content), RMP (relative membrane permeability), CAT (catalase activity), SOD (superoxidase activity) and POD (peroxidase activity).

4. Discussion

Heavy metal contamination of the environment through anthropogenic activities and/or natural processes presents a widespread and serious problem [40,41]. Heavy metals occur in various forms in soil, which differ greatly with respect to their solubility and bioavailability. The geochemical behavior of heavy metals in soil, their uptake by plants, and effects on crop productivity are affected by various physicochemical properties of soil [42,43]. Subsequently, the plants grown in soil containing high levels of Cd showed visible symptoms of injury reflected in terms of chlorosis, growth inhibition, browning of root tips and eventually death [44]. The inhibiting effect of Cd on fresh and dry mass accumulation, height, root length, leaf area, and other biometric parameters of plants were reported in almost all investigations. Differences in the degree of expressed phytotoxicity due to various Cd concentrations applied to the root medium, the duration of treatment, as well as the characteristics of species and cultivars were established [12,45]. Cd inhibited the growth of most plant species [10,44,46]. Moreover, in the present study we observed the same pattern, i.e., root length, shoot length, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight (Figure 1) and also decreased chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content (Figure 2) in both varieties of *Z. mays*. Decreased plant growth and biomass under excess Cd exposure in plants has already been reported as a phenomenon in many studies dependent upon the number of environmental factors including plant species, treatments, growth medium and soil–Cd contents [10,12,45]. Plant growth and biomass variation under the same environmental conditions in different *Z. mays* cultivars might be due to the low availability of water, poor stomatal regulation, and perturbed root architecture as described by Saleem et al., [47] in Cu-contaminated soil. These results coincide with the findings of Bashir et al., [48] in *Oryza sativa*, Rizwan et al., [49] in *Triticum turgidum* and Javed et al., [50] in *Z. mays*. Excessive Cd concentrations affected the level of net photosynthesis due to two important factors; (i) stomatal factors and (ii) non-stomatal factors. Ascorbic acid mediated the closure of the stomata under excess concentration of Cd and caused a reduction in stomatal numbers under the influence of stomatal factors [48,51].

Stress conditions can disturb the dynamic equilibrium of reactive oxygen species (ROS) production and elimination under normal growth in plants [52–54], which promotes ROS accumulation and membrane lipid peroxidation, and disrupts the structure and function of the cell membrane system [55,56]. It was reported that an excess of Cd can increase lipid peroxidation and MDA, an oxidized product of membrane lipids, indicating the prevalence of oxidative stress and membrane damage [12,44,46]. This ROS accumulation in plants is removed by a variety of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) which were also increased in the plants grown in the toxic concentration of Cd (500 μ M), compared with the plants grown in Cd-free sand (Figure 3). However, the expression of antioxidative enzymes, such as SOD, POD and CAT under a Cd-stressed environment plays a significant role in reducing Cd toxicity, which was reported in a number of studies among various plant species [45,46,48]. Our results were similar to those of the study undertaken by Howlader et al., [20] which showed that Cd-treated plants boosted the endogenous levels of antioxidants SOD, POD, CAT, which detoxified ROS at the cellular level. In the present study, we additionally noticed that the Cd concentration in the plant parts were increased by an increasing concentration of Cd in the sand (Figure 3). It is well known that Cd toxicity in crops depend on the bioavailability of Cd in soils and the concentration of elements, which can compete with Cd during plant uptake [57]. Cd uptake in *Z. mays* plants varies with soil pH and organic matter content present in the soil [58]. After absorption by the roots, Cd is transported to the stele by passing through the endodermis, and Casparian strips, where the Cd is then translocated to the shoot via xylem and finally accumulates in the grains [5,11]. The translocation factor (TF) and bioaccumulation factor (BCF) are important in screening hyperaccumulators for phytoremediation of heavy metals [59]. Screening of hyperaccumulators depend on BCF and TF values (both of them are greater than 1) for evaluation and selection of plants for

phytoremediation [60,61]. The TF is the capacity of plants to transfer metals from roots to shoots and BCF expresses the ability of plants to accumulate metals from the soil to tissues [62,63]. Another requirement for the criteria of plants that determine whether it qualifies as a Cd hyperaccumulator species or not, is Cd accumulation in shoots. Although, in the present study we noted that the BAF and TF values were less than 1 (Table 4), which shows that both *Z. mays* cultivars are not hyperaccumulator species for Cd. Similar results for *Z. mays* were also displayed by Anwer et al., [64], and Tanwir et al., [65] where they found Cd accumulated highly in the roots then transferred to the shoots which depicts *Z. mays* as not being a hyperaccumulator species for Cd-contaminated soil.

Silicon (Si) is found abundantly in the earth's crust and is believed to be an important constituent in soil where it efficiently neutralizes the hazardous impacts of different stresses such as salinity, temperature and various metal stresses on plants [49]. Although, Si is not deliberated as an indispensable plant nutrient but rather plays a major role in plant growth, especially under stressful conditions [11]. Silicon bears an ability to be promptly transported through specified transporters located in the cellular membranes of plant roots, and translocation from root cells to the aerial parts of plants is carried out through influx transporters identified in the xylem parenchyma cells [20,21]. Numerous investigations have reported the ameliorating effects of Si against heavy metals in *Triticum aestivum* [5], *Trachyspermum ammi* [11] and *Triticum turgidum* [66]. Under conditions of metal stress, the application of Si reduced the metal contents of plant organs and increased plant growth and composition, improved photosynthetic machinery, decreased in planta oxidative stress via increased antioxidative compounds, increased uptake of minerals and influenced the exudation of organic acids from plant roots which were discussed in detail in reviews by Adrees et al., [67] and Jia-Wen et al., [25]. Research findings depicted that the application of Si increased plant growth and biomass (Figure 1), and also increased photosynthetic pigments (Figure 2) in both *Z. mays* cultivars grown under the Cd-contaminated soil. The application of Si bears a protective role and increases plant morphology and physiology under Cd [68], Cu [69] and Zn [23] stress. This might result from the fact that Si application leads to a secretion of secondary metabolites which assist in ameliorating metal stressed conditions and additionally due to the dilution effects of Si application which increase morpho-physiological traits by decreasing the contents of Cd in plant roots and shoots [24,25,67]. The oxidative stress in plant cells and tissues can be reduced by the application of Si which increases the activities of antioxidants and capturing of stress induced ROS [5,20,21]. Our results illustrated that Si application decreased oxidative stress indicators (Figure 3) and increased the activities of various antioxidant compounds such as SOD, POD and CAT in both varieties of *Z. mays* (Figure 3). The application of Si induces the activities of antioxidative enzymes and, therefore, can be considered as an indicator of enhanced ROS production and extenuation (Figure 3). Moreover, our results showed that the application of Si decreased the uptake of Cd concentration in the roots and shoots of both *Z. mays* cultivars under Cd-contaminated sand (Figure 3). This might be due to the Si restricting apoplasmic transport of heavy metals and thus decreasing the concentration of free Cd ions in apoplasm [58,70]. The schematic presentation of mechanistic role of Si alleviating the Cd toxicity in *Z. mays* is presented in Figure 6.

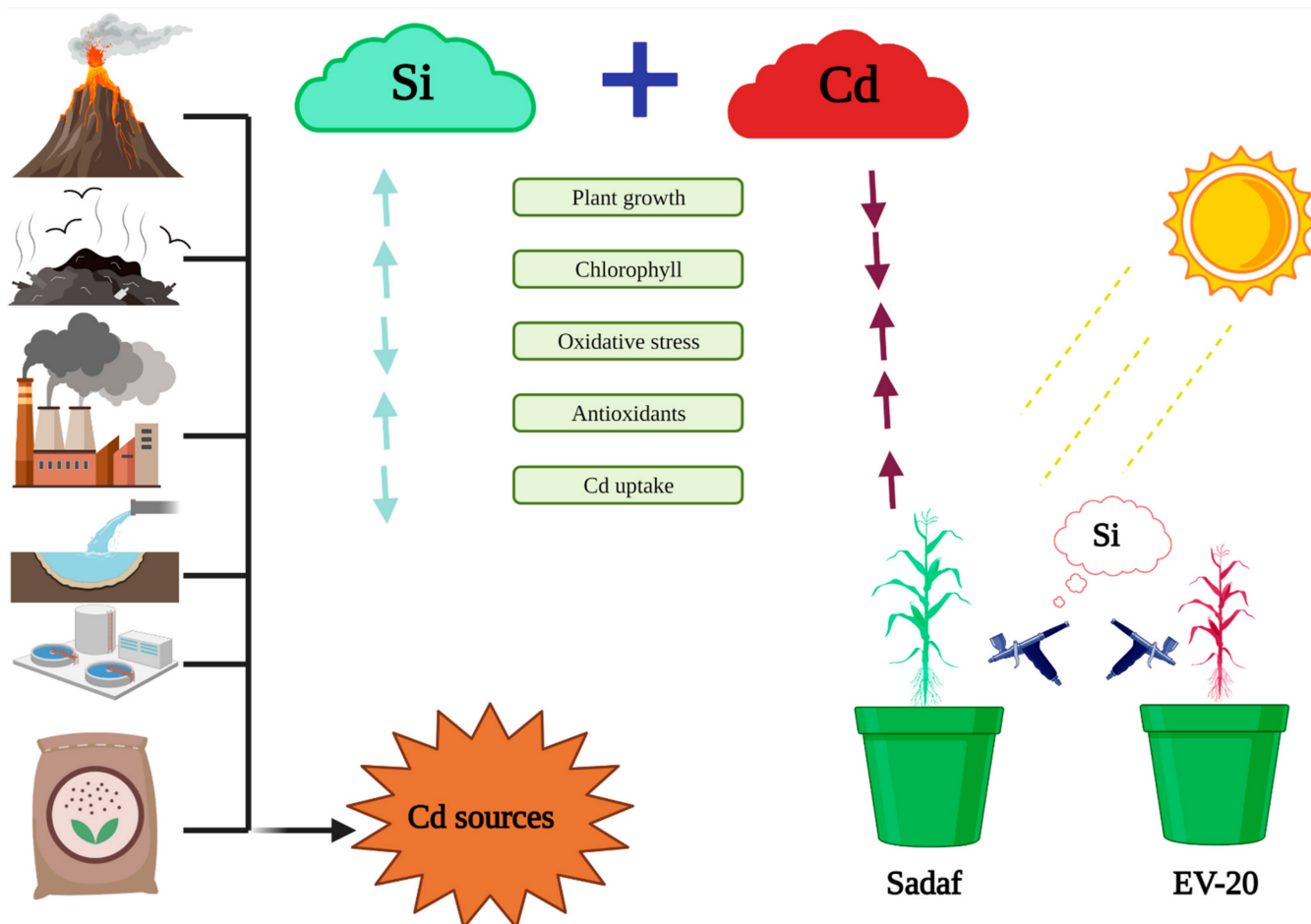


Figure 6. Schematic presentation interpreting the mechanistic role of Si in alleviating the Cd toxicity in *Z. mays*. The Cd toxicity inhibited plant growth characteristics and higher Cd concentration was accumulated in the roots and shoots of *Z. mays*. In contrast, the application of Si significantly alleviated Cd toxicity and improved root and shoot growth and alleviated Cd induced inhibition of photosynthetic efficiency. Si addition regulated the antioxidant defense system while reduced the oxidative stress and relative membrane permeability. The current study demonstrated that Si could relieve Cd toxicity in *Z. mays* by reducing Cd uptake at the root surface and its translocation (root to shoot), and regulating proficient antioxidant coordination in the leaves of *Z. mays*.

5. Conclusions

On the basis of these findings, it can be concluded that the negative impact of Cd toxicity can be overcome by the application of Si. Moreover, our results depicted that Cd toxicity induced severe metal toxicity in *Z. mays* cultivars by increased generation of ROS in the form of oxidative stress and increased the concentration of Cd in the roots and shoots of the plants, which ultimately decreased plant growth and yield and photosynthetic efficiency. In addition, we noticed that the values of BAF and TF were less than 1 which revealed that *Z. mays* cultivars were not a hyperaccumulator species of the Cd-contaminated environment. Hence, Cd toxicity was eliminated by the external application of Si, which also decreased the Cd concentration in the plant tissues, degenerated ROS, and increased the activities of antioxidants. Therefore, long-term field studies should be executed to draw parallels among plants and crops root exudations, metal stress, nutrient mobility patterns, and plant growth in order to gain further insights into underlying mechanisms.

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S.A.; Visualization, M.H.S.; Writing—original draft, M.H.S., S.U.K., X.W., H.A., M.A.E.-S. and S.A.; Writing—review & editing, M.H.S., M.A.E.-S. and S.A. All authors have read and agreed to the published version of the manuscript.

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