

Zinc toxicity and phytoremediation potential of Oleander: How silicon can help?

Elkadri LEFI

Center of Biotechnology of Borj Cédria

Hasna ELLOUZI

Center of Biotechnology of Borj Cédria

Khawla ATIA

Center of Biotechnology of Borj Cédria

Mokded RABHI

Qassim University

Chedly ABDELLY

Center of Biotechnology of Borj Cédria

Walid ZORRIG (✉ zorrigwalid@gmail.com)

Center of Biotechnology of Borj Cédria

Research Article

Keywords: Antioxidant enzymes, oxidative stress markers, gas exchange, PSI activity, PSII activity

Posted Date: September 22nd, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3348417/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Soil contamination by heavy metals, such as zinc, has significant environmental consequences. Phytoremediation, among various remediation techniques, has been developed and applied to restore contaminated soils. However, phytoremediation has limitations related to slow plant growth, influenced by contaminant toxicity. This study aims to investigate the effect of the interaction between zinc and silicon on growth, hydration, photosynthetic and biochemical behaviours, as well as the phytoremediation capacity in oleander (*Nerium oleander*), a promising species for phytoremediation.

Oleander plants were exposed to four treatments, including two adequate zinc treatments (0.76 μM Zn) with two different silicon concentrations (0 mM and 0.5 mM Si) and two zinc toxic treatments (1800 μM Zn) with the same silicon concentrations (0 mM and 0.5 mM Si). The results revealed that zinc toxicity negatively affected most of the measured parameters. However, the depressive effects of zinc toxicity were significantly mitigated under silicon treatments. Adding silicon alleviated leaf chlorosis and improved biomass production, water status, photosynthetic pigment contents, photosynthetic gas exchange, oxidation state of photosystem I (PSI), membrane integrity, soluble protein content, and antioxidant enzyme activity (especially guaiacol peroxidase). Overall, silicon had beneficial effects on the phytoremediation capacity of *N. oleander*. Therefore, fertilization rich in silicon could represent an effective solution for enhancing the phytoremediation capacity of this species, minimizing one of the major disadvantages of phytoremediation, namely low biomass production influenced by toxicity.

1. Introduction

Zinc (Zn) is an essential micronutrient for plant growth and development, but excessive levels of this metal in soil can have harmful effects on plants, including reduced growth, impaired photosynthesis and respiration, mineral nutrient imbalances, and the generation of Reactive Oxygen Species (ROS). The optimal range of zinc concentration required for suitable plant growth varies between 30 and 200 μg per gram dry weight. Zinc is involved in several structural and catalytic functions such as cell division, cell expansion, and protein synthesis. However, its toxicity can lead to decreased germination capacity and biomass production, ultimately resulting in reduced yield and quality [1].

The reduction in plant growth is one of the most prominent physiological responses to zinc toxicity [1,2]. Excess zinc affects both leaf area and number, which can result from inhibition of cell division, elongation, or both [3]. Moreover, high levels of zinc damage mitochondrial organization, leading to decreased levels of nicotinamide (a group B vitamin) and accelerating the degradation of NAD^+ (Nicotinamide Adenine Dinucleotide), thereby lowering energy metabolism. This may explain the overall decrease in plant growth [4]. Zinc is typically more accumulated in roots, which could be a resistance strategy adopted by plants to maintain lower zinc concentrations in aerial organs [5,6]. Thus, excess zinc at the root level inhibits primary root elongation and stimulates lateral roots to grow vertically, which is likely a compensatory response to explore deeper areas of potentially less contaminated soil [7,8].

Zinc toxicity decreases Relative Water Content (RWC) in plant tissues and disrupts water relations [9], inducing severe dehydration [10]. These effects could be due to shoot zinc accumulation, but the reduction in the RWC could also be induced by a decrease in root surface area or in the vascular system (xylem vessels), thus disrupting xylem flow [11]. The decrease in water content results in a loss of turgor, which in turn reduces the cell expansion process, ultimately leading to growth inhibition [2]. At excessive concentrations, zinc interferes with plant physiological balance by competing with other ions at their root uptake sites (Mg, Mn, Cu, Fe, etc.) [12]. Additionally, excess zinc inhibits root growth, damages the elongation zones in the roots, or even causes cell death, resulting in a reduction in nutrient absorption [13,14]. Changes in nutrient contents in under excessive zinc conditions could be due to impaired functioning of membrane transporters and ion channels, as well as membrane depolarization in root cells, which affects membrane permeability [15]. High levels of zinc not only impede the absorption of nutrients but also their transport to different parts of the plant [9,16].

Zinc toxicity decreases net photosynthesis, but it is not clear how it affects mesophyll conductance, stomatal conductance, respiration, photochemistry, leaf biochemistry or their combination [17,18]. Generally, excess zinc induces increased resistance of mesophyll to CO₂ diffusion, possibly due to the physiological drought caused by zinc toxicity. High zinc concentrations were associated with reduced stomatal conductance, root hydraulic reductions, and decreased photosynthesis rate [19]. Excess zinc appears also to cause a decline in water uptake, induce stomatal closure, and reduce photosynthesis rate, resulting in a reduction in plant biomass. Furthermore, excess zinc decreases the efficiency of photosystem II (PSII) and non-cyclic photophosphorylation by disrupting the structure of the central complex of PSII [16]. Finally, it is widely reported that plants exposed to excess zinc exhibit various photosynthetic responses depending on the species, zinc concentration, or genetic variability. There is no specific target for the photosynthetic toxicity of zinc, but rather a cascade of events that could be triggered by the stress.

Zinc is a non-redox heavy metal that cannot directly produce ROS such as superoxide radicals (O₂^{•-}), singlet oxygen (¹O₂), hydroxyl radical (OH[•]), and hydrogen peroxide (H₂O₂) *via* Haber-Weiss reactions. However, the overproduction of ROS and the induction of oxidative stress in plants due to zinc toxicity may be due to the disruption of various metabolic pathways and/or electron transfer mechanisms. Phytotoxic concentrations of zinc have been reported to increase lipoxygenase (LOX) activity, stimulate lipid peroxidation (malondialdehyde (MDA)), and influence membrane integrity and permeability [20]. Despite its harmful effects at high levels, zinc cannot be completely eliminated because of its dual functions. On the one hand, it is an essential micronutrient, and on the other hand, it is a toxic environmental contaminant. To cope with the destruction triggered by ROS, plant cells have an antioxidant defence machinery comprising enzymatic and non-enzymatic components [21]. In plants subjected to excess zinc, an improvement is often observed in the activity of ROS-scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), and glutathione reductase (GR). This behaviour suggests an adaptive and positive defence response that helps maintain the ROS balance and prevents oxidative damage [22,23]. However, a

decrease in enzymatic antioxidant activities was also noticed in plants exposed to zinc toxicity, indicating an excessive oxidative load [24].

Due to the serious implications of soil contamination caused by heavy metals, numerous soil remediation approaches such as phytoremediation have been developed and applied to recover contaminated soils [25–27]. Although phytoremediation is a fascinating technique, it has certain limitations, mainly related to the slow plant growth caused by the toxicity of the contaminant. Therefore, it would be wise to find a solution that can improve plant growth and their phytoremediation capacity under phytotoxicity conditions caused by contaminants.

In this context, numerous research studies suggest the use of silicon (Si), a highly promising element, to enhance plant tolerance to metallic toxicity. Silicon is the second most abundant mineral element in the Earth's crust, after oxygen. Although silicon is not considered an essential element for plants, various studies have demonstrated that its application to plants increases their tolerance to numerous abiotic stresses, including salinity [28–32], drought [33], nutritional deficiencies [34–36], and metal toxicity [37].

Multiple studies have demonstrated that the addition of silicon to the plant medium is a sustainable and straightforward approach to enhance plant performance in the presence of zinc toxicity. Silicon advantageous effects might be attributed to the reduction in zinc bioavailability in the growth medium, which is the consequence of an increase in pH and the creation of biologically inactive metallic silicates [38–40]. Silicon presence lowers zinc toxicity and leads to an increase in biomass production [41]. Furthermore, silicon addition induces the formation of 'polysilicates' in the cell wall, altering metal-binding capacity (particularly that of zinc) and strengthening cell walls [42]. Incorporating silicate compounds into root cell walls also enhances the number of specific zinc ion binding sites, boosting the amount of zinc in the cell wall matrix [43]. This plays a crucial role in the detoxification process by sequestering metals, including zinc, in the cell walls, preventing toxic cations from reaching more sensitive cells like mesophyll ones [44,45].

Adding silicon affects zinc distribution in plants. This is because zinc and silicon have similar distributions, with a preference for the root endodermis. Silicates increase zinc sequestration in the cell wall due to complexation, leading to a decrease in its levels in the xylem sap by forming insoluble complexes between silicon and zinc [46]. Moreover, the deposition of these precipitates might partially restrict zinc loading in the xylem, resulting in a further reduction in zinc transport [47].

One of the goals of phytoremediation researches is to improve plant yield, enhance plant tolerance to heavy metals, and increase their phytoremediation capacity by enriching the culture media with silicon.

Therefore, the aim of this study was to investigate the effect of zinc-silicon interaction on plant growth, tissue hydration, photosynthetic and antioxidant responses, and phytoremediation capacity in *Nerium oleander*. The selection of this plant species was based on its known tolerance to heavy metals, as well as its rapid growth and high biomass production, making it easy to be cultivated and harvested. These factors collectively make *N. oleander* a compelling candidate for phytoremediation.

2. Material and methods

2.1. Plant material and growth conditions

Oleander (*Nerium oleander*), locally known as 'Defla', is a shrub species belonging to the Apocynaceae family. The cultivation of *N. oleander* plants took place in a glass greenhouse (natural light) at the 'Centre of Biotechnology of Borj-Cedria'. At five months old, plants obtained by cuttings were transplanted into 5.75-liter buckets (with three plants per bucket) and hydroponically-grown. The buckets were filled with Hewitt's nutrient solution (T/2 for macronutrients, T for micronutrients, and T for iron). The nutrient solutions were continuously aerated using aquarium pumps. Two months later,

plants were subjected to four treatments for 45 days. The first treatment, considered as the control, received a nutrient solution containing a basic concentration of zinc ($0.76 \mu\text{M Zn}$ added as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) (C treatment); the second received a basic concentration of zinc supplemented with 0.5 mM sodium silicate (Na_2SiO_3 , Sigma-Aldrich, extra pure) (C + Si treatment); the third received a toxic concentration of zinc ($1800 \mu\text{M Zn}$; added as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) (Zn treatment); and the fourth received a toxic concentration of zinc supplemented with 0.5 mM sodium silicate (Zn + Si treatment).

To ensure maximum homogenization, the growing conditions, arrangement, and orientation of the buckets were randomly altered every two days. Throughout the experiment, the nutrient solution was replaced every seven days. After 45 days of treatment, the harvest took place.

2.2. Plant harvest

At the harvest, plant roots, stems, and leaves were separated. Roots were washed three times, initially with a 0.01 M HCl solution (maintained at 4°C) and then twice with distilled water (maintained at 4°C). Next, roots were thoroughly dried with filter paper. Additional samples taken for biochemical assays and were stored at -80°C .

2.3. Biomass production and water content

Fresh and dry weights were determined using a RADWAG AS 160/C/2 analytical balance at $1/10 \text{ mg}$, before and after oven-drying at 60°C for 15 days, respectively.

Relative growth was determined using the following formula:

$$\text{Relative growth} = (\text{biomass of treated plants} / \text{biomass of control plants}) \times 100$$

Tissue hydration was expressed in $\text{ml H}_2\text{O g}^{-1} \text{ DW}$ using the following formula:

Water content ($\text{ml H}_2\text{O g}^{-1} \text{ DW}$) = $(\text{FW} - \text{DW}) / \text{DW}$, where FW and DW stand respectively for fresh and dry weights.

2.4. Photosynthetic pigment contents

The extraction of photosynthetic pigments was performed according to the method of Lichtenthaler [48]. Fresh leaf discs (50 mg) were placed in vials containing 3 ml of 80% acetone. The vials were then stored in the dark at 4°C for 7 days. The absorbance of the extract was determined at 470, 646, and 663 nm using a UV-visible spectrophotometer: Specord 210 Plus, Analytik Jena.

Chlorophyll (*a* and *b*) and carotenoid contents were calculated based on the following formulas:

$$\text{Chlorophyll } a (\mu\text{g ml}^{-1}) = (12.21 \times A_{663}) - (2.81 \times A_{646}).$$

$$\text{Chlorophyll } b (\mu\text{g ml}^{-1}) = (20.13 \times A_{646}) - (5.03 \times A_{663}).$$

$$\text{Carotenoids } (\mu\text{g ml}^{-1}) = ((1000 \times A_{470}) - (3.27 \times [\text{Chl. } a]) - (104 \times [\text{Chl. } b]))/229.$$

Where A470, A646 and A663 stand for extract absorbance values at 470 nm, 646 nm and 663 nm, respectively.

2.5. Photosynthetic gas exchange

Various parameters were measured using an infrared gas analyser (LCpro + portable photosynthesis system, ADC BioScientific Ltd). The measurements were carried out on the same leaf stage using saturating light with an ambient CO₂ concentration of 403 ± 1 μmol mol⁻¹ at a leaf temperature of 29 ± 1°C and a measuring chamber temperature of 27 ± 2°C. The measurements were taken between 11 a.m. and 12 a.m. The parameters measured were net CO₂ assimilation rate (*A*), internal CO₂ concentration (*C*_i), and transpiration rate (*E*).

Water Use Efficiency was also calculated as *A/E*.

2.6. Photosystem (PSI and PSII) activities

The measurement of PSII and PSI activities was performed using Dual-PAM-100 (PAM: Pulse Amplitude Modulation; Heinz Walz, Effeltrich, Germany), a measurement system for the simultaneous evaluation of chlorophyll fluorescence and P700 measurement, as described by Klughammer and Schreiber [49] and Klughammer and Schreiber [50], respectively. Measurements were carried out on *N. oleander* leaves pre-adapted to the dark (stored before measurements for 30 minutes in the dark).

A light curve was initiated by applying an increasing range of light intensities (0, 6, 12, 21, 56, 107, 146, 257, 412, 652, and 1017 μmol photons m⁻² s⁻¹). Each period of illumination lasted 30 seconds.

Regarding PSII activity, the measurement and analysis of fluorescence allow the estimation of several parameters, which in turn provide information on the efficiency of the photochemical and biochemical processes of photosynthesis that can be used to assess the physiological state of plants. This combination of lights makes it possible to evaluate different parameters related to the functional aspect of PSII, which will be detailed below [49]:

F0: minimal fluorescence yield of the dark-adapted state; Fm: maximal fluorescence yield of the dark-adapted state; Fv: variable fluorescence (Fm-F0); Fv/Fm: maximal quantum yield of PSII photochemistry; Y(II): quantum yield of photochemical energy conversion in PSII; ETR(II): electron transfer rate (II).

Regarding PSI activity, the Dual-PAM-100 is also equipped with a dual-wavelength transmitter (at 830 nm and 875 nm), which makes it possible to study PSI activity according to the method proposed by Klughammer and Schreiber [50].

This combination of lights allows the evaluation of several parameters related to the functional aspect of PSI, which will be detailed below [50]: Y(I): quantum yield of photochemical energy conversion in PSI; ETR(I): electron transfer rate (I); P700ox: oxidized PSI; P700m: maximal fluorescence yield of dark-adapted samples with all PSI centres closed.

2.7. Zinc content

After determining the dry biomass, all the individual samples were reduced to a fine powder using agate mortar and pestle. To adequate aliquots of dry root, stem and leaf powder were added 15 ml sulphuric acid (H_2SO_4 ; 1 N) in plastic pill boxes that were then incubated in a water bath at 80°C for 1 hour to allow complete extraction of the sample elements and then left for 24 hours at room temperature. Subsequently, sample filtration was performed using filter paper [51,52]. Zinc ions were assayed using the concentrated extract using an atomic absorption spectrophotometer (VARIAN, SpectrAA 220 FS).

2.8. Malondialdehyde content

The determination of malondialdehyde (MDA) content was carried out by hot condensation in an acidic medium with thiobarbituric acid (TBA) [53]. Fresh leaf samples of *N. oleander* were ground at 4°C in a 5% (w/v) trichloroacetic acid (TCA) solution. The ground material was centrifuged for 15 min at 10,000 *g* at 4°C. The procedure involved incubating a reaction medium solution (one volume of the supernatant plus one volume of TBA (0.5%)/TCA (20%)) for 30 min at 95°C. The reaction was stopped by immediate cooling in an ice bath. After centrifugation at 10,000 *g* for 15 min, the supernatant was recovered for colorimetric assay at 532 nm using a UV-visible spectrophotometer (Specord 210 Plus, Analytik Jena, Germany).

2.9. Hydrogen peroxide content

To assay hydrogen peroxide (H_2O_2), samples of *N. oleander* leaves were ground in a solution of 0.1% trichloroacetic acid (TCA). The ground material from each sample was then centrifuged at 6000 *g* for 15 min at 4°C. A volume of supernatant was incubated in the presence of potassium iodide (1 M) added to 10 mM of a phosphate buffer ($\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$; pH 7). Optical density was determined at 390 nm [54] using a UV-visible spectrophotometer (Specord 210 Plus, Analytik Jena, Germany). The hydrogen peroxide contents were deduced from calibration curves carried out using known concentrations of H_2O_2 .

2.10. Soluble protein content

Soluble proteins were assayed according to the method of Bradford [55] which is based on the reaction between Bradford reagent and proteins, causing a shift in the maximum absorption peak of the dye from 465 to 595 nm. To perform the assay, 200 µl of Bradford reagent and 795 µl of distilled water or extraction buffer were added to a 5 µl aliquot of the supernatant. The colour developed within a minimum of 5 minutes. After 15 minutes of incubation, the absorbance was read at 595 nm against a blank that did not contain protein extract. The soluble protein content was determined by referencing a standard range prepared from Bovine Serum Albumin (BSA).

2.11. Antioxidant enzyme activities

2.11.1. Plant extract preparation

Leaf samples previously stored at -80°C were ground in a mortar containing liquid nitrogen and kept cold (in a crushed ice bin). The grinding was carried out in a phosphate buffer ($\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$; pH 7.8) containing ethylenediamine-tetraacetic acid (EDTA; 10 mM), dithiothreitol (DTT; 1 mM), and polyvinylpyrrolidone (PVP). The PVP binds phenols, thus preventing their oxidation into toxic quinones (Bradford, 1976).

2.11.2. Superoxide dismutase

The superoxide dismutase (SOD) substrate (EC 1.15.1.1) is very unstable and has a very short lifespan (9–10 seconds). Thus, the methods of determining SOD are indirect. The method proposed by Scebba et al. [56] evaluates SOD through its ability to inhibit superoxide anion flux generated by illuminated riboflavin. The superoxide radicals produced by this system reduce nitro blue tetrazolium (NBT) to blue Formosan.

Two sets of tubes were prepared. The first group was considered as control plants and kept in the dark. It contained a mixture in a 50 mM phosphate buffer (pH 7.8) composed of EDTA (0.1 mM), methionine (13 mM), NBT (75 µM), riboflavin (2 µM), and an enzymatic extract. The second set of tubes were intended for the determination of SOD activity. These tubes contained the same reaction mixture except that they were kept for 15 min under an illumination of 15 W.

The absorbance measurement was carried out at 560 nm using a UV-visible spectrophotometer (Specord 210 Plus, Analytik Jena). One enzymatic unit of SOD corresponds to the amount of the plant extract capable of inducing a 50% inhibition of the NBT reduction reaction.

2.11.3. Catalase

Catalase (CAT) activity (EC 1.11.1.6) was assessed by monitoring the decomposition of H_2O_2 , which directly leads to a decrease in absorbance at 240 nm. The change in extinction (ΔE_{240}) per unit time corresponds to CAT activity [57]. The reaction mixture contained 10 mM H_2O_2 solution (prepared in 50 mM phosphate buffer, pH 7) and the enzyme extract. The measurement involved the determination of the

time (in seconds) required for a reduction in OD₂₄₀ from 0.45 to 0.4. One CAT unit is defined as the amount of enzyme that degrades half of the H₂O₂ in 100 seconds at 25°C.

2.11.4. Guaiacol peroxidase

The activity of guaiacol peroxidase (GPX, EC 1.11.1.7) was estimated using the method of Fielding and Hall [58]. This activity was measured by the increase in absorbance at 470 nm due to the polymerization of guaiacol to tetraguaiacol in the presence of hydrogen peroxide. The molar extinction coefficient of tetraguaiacol is 26.6 mM⁻¹ cm⁻¹. The reaction was initiated by the addition of the protein extract. The reaction medium consisted of KH₂PO₄/K₂HPO₄ (25 mM, pH 7), H₂O₂ (10 mM), and guaiacol (9 mM). GPX activity was expressed in units per mg of soluble protein.

2.12. Graphic presentation and statistical analysis

Microsoft Office Excel software (version 2021) was used for calculations and graphical representations. All statistical analyses were carried out with XLSTAT software (2014 version) (www.xlstat.com).

In this study, three types of statistical analysis were performed. First, multiple mean comparisons using Duncan's test at $p \leq 0.05$ were conducted to determine statistically significant differences between the means of different groups. Second, correlation analysis (Pearson (n)) was used to identify the most interesting correlations between different variables, facilitating the visualization and interpretation of relationships between variables in the dataset. Finally, Principal Component Analysis (PCA), a multivariate statistical method that centres variables on their means and normalizes them with a standard deviation of 1, was also made.

3. Results

3.1. Morphological appearance of plants

After 45 days of treatment, plants exposed to an excessive concentration of zinc showed severe impairment of growth compared to control plants treated with adequate Zn as well as leaf chlorosis, as sign of zinc toxicity (Fig. 1). Nevertheless, plants treated with 0.5 mM Si together with excess Zn showed significantly improved growth compared to plants subjected to only zinc toxicity. Leaf chlorosis was also considerably reduced upon Si application (Zn + Si treatment).

3.2. Biomass production

In the presence of an adequate concentration of zinc, root DW of the control plants was approximately 0.6 g. With an excess of zinc, it decreased to almost 0.08 g (Fig. 2). As regards stems, their DW decreased from almost 5 g to 2 g under excess zinc conditions.

Similarly, leaf and whole plant DWs were reduced by excess zinc from almost 4 g to 0.5 g and from almost 10 g to 3 g, respectively. Interestingly, silicon application improved biomass production by more than 400% for leaves and more than 150% for the whole plant compared to plants subjected to excess

zinc. It is important to note that silicon treatment did not appear to improve biomass production in the absence of excess zinc.

3.3. Leaf number

With an excess of zinc, the number of leaves, which was around 35 in the control, decreased by 67% to reach a value of about 12 (Fig. 2). However, the addition of silicon improved the number of leaves by about 60%, even in the presence of excess zinc. Under normal Zn level conditions, Si application did not appear to stimulate the emergence of new leaves in oleander.

3.4. Length growth

With an excess of zinc, root length, which was approximately 23 cm in the control plants, was reduced by nearly 27% to almost 17 cm (Fig. 3). However, the application of silicon did not significantly improve root length in Zn-stressed plants. By contrast, it greatly alleviated the detrimental effect of Zn toxicity on stem and whole plant lengths. Conversely, both stem and whole plant lengths were reduced by Si application at normal Zn level.

3.5. Tissue hydration

Zinc toxicity resulted in a significant reduction in root, stem, and leaf water contents (Fig. 4). But, the beneficial effect of Si on tissue hydration under Zn excess conditions was observed only in leaves.

3.6. Photosynthetic pigment contents

Zinc toxicity led to substantial reductions in the levels of chlorophyll *a*, chlorophyll *b*, and carotenoids, by approximately 25%, 42%, and 14%, respectively (Fig. 5). In the presence of excess zinc, silicon supply to the medium elicited a statistically significant effect on the concentrations of chlorophyll *a* and carotenoids, with enhancements of nearly 12% and 10%, respectively. The increase in the ratio of carotenoid content to chlorophyll content (*a* + *b*) indicated that zinc toxicity increased carotenoid content relative to chlorophyll content.

3.7. Photosynthetic gas exchange

Figure 6 displays gas exchange parameters in the four treatments. *A* showed a marked difference between plants subjected and non-subjected to zinc toxicity. It decreased from 9.8 to 0.8 ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), indicating a reduction of nearly 92% in Zn-stressed plants compared to the control. *E* was diminished by 79% in comparison with control plants. Consequently, *WUE* was reduced from 5.8 to 1.9 (Fig. 6). The internal CO_2 concentration showed no significant variation. Interestingly, Si supply alleviated the substantial effect of excess zinc on *A*, *E*, and in particular on *WUE*.

3.8. Photosystem (PSI and PSII) activities

In an initial step, without any limitation at the level of the electron transfer chain, chlorophyll fluorescence was measured for dark-adapted leaves (dark test) to assess the maximum potential of PSII (Fig. 7).

Figure 7 depicts the results obtained for minimum fluorescence (F0), maximum fluorescence (Fm), and variable fluorescence (Fv). Our findings indicated that excess zinc caused a pronounced reduction in variable fluorescence compared to control plants. However, silicon exhibited no enhancing effect in the presence of excess zinc.

Additionally, plants exposed to zinc toxicity displayed a substantial decrease in the maximal photosystem II (PSII) quantum yield of dark-adapted samples, as Fv/Fm significantly decreased compared to the control. In contrast, plants with excess zinc supplied with silicon maintained Fv/Fm values at levels approaching those of control plants (Fig. 7).

Regarding PSI, in the absence of any limitation in the electron transfer chain, the activity of PSI was measured for dark-adapted leaves to evaluate the maximum potential of PSI (Fig. 8). In the presence of Zn toxicity, certain processes occur upstream and downstream of PSI to protect it from ROS by increasing its oxidation (P700ox). Consequently, an increase in P700ox is considered a beneficial response for plants. The results displayed in Fig. 8 demonstrate the detrimental effect of excess zinc, causing a significant decrease in P700ox and P700m signals of approximately 43% and 44%, respectively. In the presence of excess zinc, the addition of silicon enhanced PSI oxidation with a significant increase of nearly 22% for both parameters. However, silicon exhibited no effect in the absence of toxicity.

Figure 9 illustrates the impact of the interaction between zinc and silicon on photosystem activity in *N. oleander* during leaf adaptation to light.

In summary, the analysis of our findings suggests that zinc toxicity results in a more pronounced reduction in quantum yield of photochemical energy conversion (Y) and Electron Transfer Rate (ETR) in PSI compared to PSII. However, silicon exhibited no ameliorative effect on these two parameters.

Concerning PSI oxidation, it is essential to note that certain processes safeguard this photosystem from ROS under toxicity by increasing its oxidation (P700ox). Based on the results depicted in Fig. 10, zinc toxicity induced a significant reduction in both P700ox and P700m' parameters when compared to control plants. Nevertheless, an attenuation of this detrimental effect was observed in the presence of silicon. Consequently, silicon helps in mitigating the depressive impact of excess zinc, thus protecting and preserving the function of PSI.

3.9. MDA and H₂O₂ contents

The fluctuations in MDA contents, which serve as an indicator of lipid peroxidation, are depicted in Fig. 11. Our results showed that excess zinc significantly increased MDA by nearly 40% in the absence of silicon.

The addition of silicon to the nutrient medium alleviated the effect of excess zinc by decreasing MDA values by approximately 32% in a statistically significant manner. Thus, silicon exhibited a positive impact by reducing lipid peroxidation induced by zinc toxicity.

The effects of zinc excess and silicon supply on H_2O_2 contents are displayed in Fig. 11. A statistically significant increase in H_2O_2 contents was observed due to excessive zinc. Conversely, the advantageous effect of silicon in mitigating this excess was evident.

3.10. Soluble protein content

The fluctuations in soluble protein content are shown in Fig. 12. Our findings revealed that excess zinc induced a considerable reduction in soluble protein contents in the absence of silicon. However, the addition of silicon in the presence of excess zinc resulted in an increase in protein contents of over 36%.

3.11. Antioxidant enzyme activity

Figure 13 shows the impact of various concentrations of zinc and silicon on the activities of antioxidant. Under the influence of zinc toxicity, SOD activity exhibited a marked increase of 69%; however, this increase disappeared in the presence of silicon. Regarding GPX activity, a weak activity of this enzyme was observed in almost all studied treatments, except for the 'Zn + Si' treatment, which triggered a significant increase in this activity. Nonetheless, no significant effect of the two studied elements (Zn and Si) was detected on CAT activity (Fig. 13).

3.12. Zinc accumulation

3.12.1. Zinc contents

'Zn' treatment induced a statistically significant increase in zinc content in the roots, approximately 98 times higher than in control plants. With the addition of silicon, zinc content was reduced by 37% compared to 'Zn' treatment. For stems, the presence of excess zinc increased zinc contents by around 96% when compared to control plants. In leaves, zinc content showed an increase of nearly 4 times under zinc toxicity. However, adding silicon to the medium did not significantly alter zinc contents in the stems and leaves. Therefore, the effect of silicon was only apparent in root content in the presence of excess zinc (Fig. 14).

3.12.2. Zinc quantities

For all three studied organs, 'Zn' treatment led to a statistically significant increase in zinc quantities compared to the control. The application of silicon resulted in a substantial increase in zinc quantities in roots and leaves, with increases of 108% and 227%, respectively. However, no difference was observed between 'Zn' and 'Zn + Si' treatments in stems. Consequently, silicon contributed to a rise in zinc quantities only in roots and leaves, likely due to the increase in their biomass in the presence of silicon (Fig. 15).

3.13. Correlation Analysis and Principal Component Analysis

To identify any correlations between the various studied parameters and the applied treatments, both correlation analysis (Table 1) and Principal Component Analysis (PCA) were performed (Fig. 16). These

analyses were exclusively conducted on the results obtained in the presence of excess zinc (1800 μM Zn) to concentrate on the primary objective of our study, which pertains to the effects of silicon in the presence of Zn excess.

Indeed, the analysis revealed several statistically significant correlations (positive or negative). '1800 μM Zn + 0.5 mM Si' treatment appeared to exhibit contrasting behaviour compared to '1800 μM Zn + 0 mM Si'. In fact, numerous significant positive correlations were observed, including root dry weight ($r=0.77$), stem dry weight ($r=0.81$), leaf dry weight ($r=0.93$), whole plant dry weight ($r=0.94$), leaf number ($r=0.86$), stem length ($r=0.83$), whole plant length ($r=0.77$), leaf water content ($r=0.79$), whole plant water content ($r=0.76$), chlorophyll *a* content ($r=0.82$), chlorophyll *b* content ($r=0.85$), chlorophyll *a* content + chlorophyll *b* content ($r=0.85$), carotenoid content ($r=0.78$), net CO_2 assimilation rate ($r=0.72$), transpiration rate ($r=0.52$), intercellular CO_2 concentration ($r=0.57$), water use efficiency ($r=0.64$), soluble protein content ($r=0.80$), guaiacol peroxidase activity ($r=0.96$), catalase activity ($r=0.71$), root zinc quantity ($r=0.92$), and leaf zinc quantity ($r=0.80$).

These correlations demonstrate that the presence of silicon, along with an excess of zinc, favoured an increase in all these parameters. This treatment also supported a decrease in chlorophyll *a* content/chlorophyll *b* content ($r=-0.84$), malondialdehyde content ($r=-0.82$), hydrogen peroxide content ($r=-0.8$), superoxide dismutase activity ($r=-0.75$), and root zinc content ($r=-0.97$). PCA confirmed the beneficial effects of applying 0.5 mM silicon in the presence of excess zinc. Furthermore, the obtained correlations corroborate the results reported for individual traits throughout this section (Table 1; Fig. 16).

4. Discussion

Zinc is a crucial building block for plant growth, as it performs essential functions in numerous metabolic pathways. However, zinc concentrations in soils dramatically increase through various anthropogenic sources (primarily industrial and agricultural activities). Zinc contamination of soils disrupts the ecosystem balance, degrades soil properties, affects agricultural productivity, and poses risks to human health [27,59,60].

Considering the severe consequences of soil contamination, various remediation techniques, such as phytoremediation, have been developed and implemented for the restoration of contaminated soils [25–27].

In this context, the current study aims to investigate the effects of zinc and silicon interaction on growth, tissue hydration, photosynthetic performance, biochemical responses, and phytoremediation capacity of bay laurel rose (*N. oleander*), a promising species for phytoremediation.

The present study demonstrated that zinc toxicity reduced dry biomass production in all plant organs (roots, stems, leaves, and whole plant) (Fig. 1; Fig. 2). Our findings align with numerous studies that reported growth reduction as one of the most evident physiological responses of plants exposed to zinc toxicity [1,2]. This response may arise from the inhibition of cell division, elongation, or both [3].

Furthermore, zinc disrupts mitochondrial organization and results in decreased nicotinamide (group B vitamin) levels when present in high concentrations, consequently accelerating NAD⁺ (Nicotinamide Adenine Dinucleotide) degradation. This reduction may account for the decline in overall plant growth by impairing energy metabolism [4].

Similarly, *N. oleander* plants exposed to 1800 µM Zn exhibited severe symptoms of chlorosis (Fig. 1). Indeed, leaf chlorosis followed by necrosis is often associated with zinc toxicity, which can be attributed to decreased V-ATPase levels and activity [61]. Our findings indicated that silicon treatment had a beneficial effect, resulting in enhanced dry biomass production. This outcome revealed strong positive correlations between silicon application and dry biomass production in roots (correlation coefficient (r) = 0.77; $p \leq 0.05$), stems ($r = 0.81$; $p \leq 0.05$), leaves ($r = 0.93$; $p \leq 0.001$), and whole plant ($r = 0.94$; $p \leq 0.001$). In fact, numerous studies reported that silicon mitigates zinc toxicity on plant growth, subsequently increasing biomass production [41]. Similarly, silicon addition prompts the formation of 'polysilicates' in the cell wall, modifying metal-binding capacity, particularly for zinc, and thus strengthening cell walls [42].

It has also been reported that the application of zinc at 1800 µM leads to a reduction in water content. Indeed, zinc toxicity disrupts water relations [9] by interfering with water transport and transpiration, resulting in severe dehydration [10]. These effects could be attributed to zinc accumulation in shoot tissues. This disturbance might also be due to a reduction in root surface area or in the vascular system (xylem vessels), consequently disrupting the ascent of raw sap [11]. Similarly, this disruption of hydration results in the loss of turgor, which in turn diminishes the cell expansion process, ultimately inhibiting growth [2].

Interestingly, this behaviour was restored by the addition of silicon (Fig. 4). Strong positive correlations were revealed between silicon application and the water content of leaves ($r = 0.79$; $p \leq 0.05$) as well as of the whole plant ($r = 0.76$; $p \leq 0.05$). This enhancing effect of silicon under abiotic stresses was extensively discussed by Romero-Aranda et al. [62] and Zhu et al. [63], who demonstrated beneficial effects in several plant species. In fact, Zhu et al. [63] indicated that silicon induces the overexpression of genes from the PIP2 (Plasma membrane Intrinsic Proteins 2) subfamily in roots. According to Zhu et al. [63], this overexpression is accompanied by a modification of the osmotic potential, promoting better water absorption. PIPs (Plasma membrane Intrinsic Proteins) are highly conserved plant aquaporins that represent the primary route of water exchange between cell membranes [64]. Consequently, the reduction in water uptake and transport caused by abiotic stresses appears to be mitigated by silicon through the overexpression of genes encoding these aquaporins. In addition to the well-known aquaporin family, NIP (Nodulin-like Intrinsic Protein) is directly involved in silicon transport [65]. However, another mechanism was suggested by Savant et al. [66]. According to these authors, silicon could actually prevent water loss by inhibiting transpiration. This effect could be due to the formation of silica deposits around the leaf epidermal cells. In line with this notion, Vandegheer et al. [67] suggested that silicon improves plant water status by forming a deposit in the shape of a layer at the cuticle level. This layer could act as a physical barrier preventing water loss by reducing cuticular conductance.

We also found that excess zinc resulted in a reduction of photosynthetic pigment content (Fig. 5) and photosynthetic gas exchange (Fig. 6). Indeed, zinc toxicity decreases the content of photosynthetic pigments as well as net photosynthesis. However, it remains unclear whether this decrease is limited by mesophyll conductance, stomatal conductance, enhanced respiration, reduced photochemistry, weakened biochemistry, or a combination of all these factors [17,18].

Interestingly, the application of silicon exhibited an enhancing effect on chlorophyll and carotenoid contents (with correlation coefficients ranging from 0.78 to 0.85) as well as photosynthetic gas exchange (with correlation coefficients ranging from 0.52 to 0.72).

Regarding photochemical yields (Y (I) and Y (II)) and Electron Transfer Rates in both photosystems (ETR (I) and ETR (II)), excess zinc caused a decrease in these parameters (Fig. 9). According to the scientific literature, an excess of zinc reduces the efficiency of PSII and non-cyclic photophosphorylation by disrupting the structure of PSII central complex [16].

It has also been reported that plants grown in the presence of silicon did not show significant improvement in these parameters. This suggests that the positive effects of silicon with excess zinc are not modulated by an enhancement in photochemical yields and Electron Transfer Rates in photosystems. In addition, treating plants with silicon showed a notable beneficial effect on PSI. Indeed, silicon alleviated the depressive effect of zinc toxicity on P700m and P700ox signals (Fig. 10). Thus, silicon appears to improve the level of light absorption when PSI is subjected to a saturating flash in the presence of actinic light (P700m') as well as the oxidation state of PSI reaction centres (P700ox). In plants, certain processes are positioned upstream and downstream of PSI to protect it against ROS by increasing the oxidation of P700 [68,69]. It seems that silicon contributes to the enhancement of these protective mechanisms in the presence of zinc toxicity. Consequently, our results demonstrated that zinc toxicity significantly increased membrane lipid peroxidation and H₂O₂ content (Fig. 11). In fact, zinc is a non-redox heavy metal incapable of directly producing ROS, including H₂O₂, *via* Haber-Weiss reactions. However, the generation of ROS and the manifestation of oxidative stress in plants due to zinc toxicity might be possible due to the disruption of various metabolic pathways and/or electron transport mechanisms. Phytotoxic zinc concentrations have been reported to increase lipoxygenase (LOX) activity, stimulate lipid peroxidation (malondialdehyde (MDA)), and affect membrane integrity and permeability [20]. Interestingly, our study showed that silicon application reduced MDA contents. Hence, our results are consistent with several studies reporting that silicon helps maintain better cell membrane integrity, thus reducing their permeability under various abiotic stresses [28,70].

Our results clearly confirm the stimulating effect of zinc on SOD activity (Fig. 13). Despite its harmful effects in high doses, zinc cannot be completely eliminated due to its dual function. It is indeed an essential micronutrient on one hand and a toxic environmental contaminant on the other. To cope with the damage triggered by ROS, plant cells have an antioxidant defence machinery comprising enzymatic and non-enzymatic components [21]. In plants subjected to excess zinc, an improvement in the activity of ROS-scavenging enzymes such as SOD was observed in our study (Fig. 13). This behaviour suggests an

adaptive and positive defence response that helps maintain ROS balance and prevents oxidative damage [22,23]. This behaviour was completely restored in the presence of silicon, suggesting a reduction in toxicity (Fig. 13). Similarly, a dramatic response from increased GPX activity was demonstrated in the presence of silicon. This behaviour suggests that the positive effects of silicon against the deleterious effects of zinc toxicity could be modulated by the overexpression of GPX (Fig. 13).

As regards the accumulation of zinc (Fig. 14; Fig. 15), our results showed that Zn^{2+} contents increased considerably in plants subjected to excess zinc, especially in roots. Similarly, zinc contents were significantly higher in roots, which may be a resistance strategy adopted by plants to maintain a lower zinc concentration in leaves [5,6].

Under excess zinc, it is believed that silicon induces a tendency to decrease Zn^{2+} contents only in roots. However, the addition of silicon did not considerably reduce these levels in the aerial parts.

For the three studied organs, it was noticed that 'Zn' treatment resulted in a very marked increase in the quantities of zinc compared to the control. The application of silicon led to a substantial rise in Zn quantities in roots (+ 108%) and leaves (+ 227%) (Fig. 15). This was undoubtedly due to the increase in their biomass in the presence of silicon. Hence, silicon improved the phytoremediation capacity of *N. oleander* by more than 200% under our culture conditions.

5. Conclusion

In conclusion, the present work investigated the interaction between zinc and silicon on Oleander (*N. oleander*), a promising species for phytoremediation, by examining its impact on growth, photosynthesis, some metabolic responses, and phytoremediation capacity. Our results showed that the application of silicon in the presence of severe zinc toxicity can lead to improvements in plant biomass production, plant hydration, photosynthetic pigment contents, photosynthetic gas exchange, light absorption and oxidation state of photosystem I (PSI), membrane integrity, soluble protein content, antioxidant enzyme activity (especially GPX), and phytoremediation capacity.

Overall, our study confirms the beneficial effects of silicon against the deleterious effects of zinc toxicity on *N. oleander* and its phytoremediation capacity. The use of silicon-rich fertilization could be an effective solution for enhancing the phytoremediation capacity of this species, helping to address one of the major limitations of phytoremediation: the low production of biomass under the influence of toxicity. Therefore, the use of fertilization rich in silicon could represent a very effective solution for improving the phytoremediation capacity of this species.

Declarations

Author contribution statement

Study conception and design were performed by Elkadri LEFI and Walid ZORRIG. Material preparation, data collection, and analysis were performed by Elkadri LEFI, Hasna ELLOUZI, Khawla ATIA, Chedly ABDELLY, and Walid ZORRIG. The first draught of the manuscript was written by Elkadri LEFI and Walid ZORRIG. Mokded RABHI commented on previous versions of the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgment

This study was conducted in the Laboratory of Extremophile Plants (LPE) of the Centre of Biotechnology of Borj-Cedria (CBBC), Tunisia. The authors express their gratitude to the technical and administrative staff at CBBC for their support during the study.

Funding

This work was funded by the Tunisian Ministry of Higher Education and Scientific Research (LR15CBBC02). Furthermore, some of this research was supported by the PHC Maghreb 2019 network (Partenariat Hubert CURIEN Maghreb 2019 no. 19MAG41) and the Young Researchers Encouragement Program (Programme d'Encouragement des Jeunes Chercheurs - 21PEJC D4P1).

The authors declare that they have no competing financial interests or personal relationships that could have affected the work presented in this paper.

Ethics approval

We confirm that all authors have consented to adhere to ethical standards.

Consent to participate

We confirm that all authors consent to participation.

Consent for publication

We confirm that all authors have given their consent to participate.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Not applicable

References

1. H. Mahmoudi, I. Ben Salah, W. Zaouali, W. Zorrig, A. Smaoui, T. Ali, M. Gruber, Z. Ouerghi, K. Hosni, Impact of Zinc Excess on Germination, Growth Parameters and Oxidative Stress of Sweet Basil (*Ocimum basilicum* L.), Bull Environ Contam Toxicol. 106 (2021). <https://doi.org/10.1007/s00128-021-03188-6>.
2. H. Kaur, N. Garg, Zinc toxicity in plants: a review, Planta. 253 (2021). <https://doi.org/10.1007/s00425-021-03642-z>.
3. S. Glińska, M. Gapińska, S. Michlewska, E. Skiba, J. Kubicki, Analysis of *Triticum aestivum* seedling response to the excess of zinc, Protoplasma. 253 (2016). <https://doi.org/10.1007/s00709-015-0816-3>.
4. Y. Zhang, Y. Wang, Z. Ding, H. Wang, L. Song, S. Jia, D. Ma, Zinc stress affects ionome and metabolome in tea plants, Plant Physiology and Biochemistry. 111 (2017). <https://doi.org/10.1016/j.plaphy.2016.12.014>.
5. N. Garg, H. Kaur, Response of Antioxidant Enzymes, Phytochelatins and Glutathione Production Towards Cd and Zn Stresses in *Cajanus cajan* (L.) Millsp. Genotypes Colonized by Arbuscular Mycorrhizal Fungi, J Agron Crop Sci. 199 (2013) 118–133.
6. T.L. Tiecher, T. Tiecher, C.A. Ceretta, P.A.A. Ferreira, F.T. Nicoloso, H.H. Soriani, L. De Conti, M.S.S. Kulmann, R.O. Schneider, G. Brunetto, Tolerance and translocation of heavy metals in young grapevine (*Vitis vinifera*) grown in sandy acidic soil with interaction of high doses of copper and zinc, Sci Hortic. 222 (2017). <https://doi.org/10.1016/j.scienta.2017.05.026>.
7. P. Zhang, L. Sun, J. Qin, J. Wan, R. Wang, S. Li, J. Xu, cGMP is involved in Zn tolerance through the modulation of auxin redistribution in root tips, Environ Exp Bot. 147 (2018). <https://doi.org/10.1016/j.envexpbot.2017.10.025>.
8. G. Feigl, Á. Molnár, R. Szöllősi, A. Ördög, K. Törőcsik, D. Oláh, A. Bodor, K. Perei, Z. Kolbert, Zinc-induced root architectural changes of rhizotron-grown *B. napus* correlate with a differential nitro-oxidative response, Nitric Oxide. 90 (2019). <https://doi.org/10.1016/j.niox.2019.06.003>.
9. N. Garg, S. Singh, Arbuscular Mycorrhiza *Rhizophagus irregularis* and Silicon Modulate Growth, Proline Biosynthesis and Yield in *Cajanus cajan* L. Millsp. (pigeonpea) Genotypes Under Cadmium and Zinc Stress, J Plant Growth Regul. 37 (2018). <https://doi.org/10.1007/s00344-017-9708-4>.
10. A. Ben Ghnaya, A. Hourmant, S. Cerantola, N. Kervarec, J.Y. Cabon, M. Branchard, G. Charles, Influence of zinc on soluble carbohydrate and free amino acid levels in rapeseed plants regenerated in vitro in the presence of zinc, Plant Cell Tissue Organ Cult. 102 (2010). <https://doi.org/10.1007/s11240-010-9721-9>.
11. L. Koleva, I. Semerdjieva, A. Nikolova, A. Vassilev, others, Comparative morphological and histological study on zinc-and cadmium-treated durum wheat plants with similar growth inhibition., General and Applied Plant Physiology. 36 (2010) 8–11.
12. R.K. Tewari, P. Kumar, P.N. Sharma, Morphology and physiology of zinc-stressed mulberry plants, Journal of Plant Nutrition and Soil Science. 171 (2008). <https://doi.org/10.1002/jpln.200700222>.

13. A. Vassilev, A. Perez-Sanz, A. Cuypers, J. Vangronsveld, Tolerance of two hydroponically grown *Salix* genotypes to excess zinc, *J Plant Nutr.* 30 (2007). <https://doi.org/10.1080/01904160701555671>.
14. M. Šimon, Z.J. Shen, K. Ghoto, J. Chen, X. Liu, G.F. Gao, A. Jemec Kokalj, S. Novak, B. Drašler, J.Y. Zhang, Y.P. You, D. Drobne, H.L. Zheng, Proteomic investigation of Zn-challenged rice roots reveals adverse effects and root physiological adaptation, *Plant Soil.* 460 (2021). <https://doi.org/10.1007/s11104-020-04772-1>.
15. N. Bazihizina, C. Taiti, L. Marti, A. Rodrigo-Moreno, F. Spinelli, C. Giordano, S. Caparrotta, M. Gori, E. Azzarello, S. Mancuso, Zn²⁺-induced changes at the root level account for the increased tolerance of acclimated tobacco plants, *J Exp Bot.* 65 (2014). <https://doi.org/10.1093/jxb/eru251>.
16. R. Sagardoy, F. Morales, A.F. López-Millán, A. Abadía, J. Abadía, Effects of zinc toxicity on sugar beet (*Beta vulgaris* L.) plants grown in hydroponics, *Plant Biol.* 11 (2009). <https://doi.org/10.1111/j.1438-8677.2008.00153.x>.
17. D. Di Baccio, R. Tognetti, A. Minnocci, L. Sebastiani, Responses of the *Populus × euramericana* clone I-214 to excess zinc: Carbon assimilation, structural modifications, metal distribution and cellular localization, *Environ Exp Bot.* 67 (2009). <https://doi.org/10.1016/j.envexpbot.2009.05.014>.
18. A. Vassilev, A. Nikolova, L. Koleva, F. Lidon, Effects of Excess Zn on Growth and Photosynthetic Performance of Young Bean Plants, *Journal of Phytology.* 3 (2011).
19. H. Fatemi, C. Zaghdoud, P.A. Nortes, M. Carvajal, M. del Carmen Martínez-Ballesta, Differential Aquaporin Response to Distinct Effects of Two Zn Concentrations after Foliar Application in Pak Choi (*Brassica rapa* L.) Plants, *Agronomy.* 10 (2020). <https://doi.org/10.3390/agronomy10030450>.
20. A. Goodarzi, S. Namdjoyan, A.A. Soorki, Effects of exogenous melatonin and glutathione on zinc toxicity in safflower (*Carthamus tinctorius* L.) seedlings, *Ecotoxicol Environ Saf.* 201 (2020). <https://doi.org/10.1016/j.ecoenv.2020.110853>.
21. G. Miller, V. Shulaev, R. Mittler, Reactive oxygen signaling and abiotic stress, *Physiol Plant.* 133 (2008). <https://doi.org/10.1111/j.1399-3054.2008.01090.x>.
22. R. Jain, S. Srivastava, S. Solomon, A.K. Shrivastava, A. Chandra, Impact of excess zinc on growth parameters, cell division, nutrient accumulation, photosynthetic pigments and oxidative stress of sugarcane (*saccharum* spp.), *Acta Physiol Plant.* 32 (2010). <https://doi.org/10.1007/s11738-010-0487-9>.
23. P.I. Michael, M. Krishnaswamy, The effect of zinc stress combined with high irradiance stress on membrane damage and antioxidative response in bean seedlings, *Environ Exp Bot.* 74 (2011). <https://doi.org/10.1016/j.envexpbot.2011.05.016>.
24. A. Song, P. Li, Z. Li, F. Fan, M. Nikolic, Y. Liang, The alleviation of zinc toxicity by silicon is related to zinc transport and antioxidative reactions in rice, *Plant Soil.* 344 (2011). <https://doi.org/10.1007/s11104-011-0749-3>.
25. H.I. Gomes, Phytoremediation for bioenergy: challenges and opportunities, *Environmental Technology Reviews.* 1 (2012). <https://doi.org/10.1080/09593330.2012.696715>.

26. L. Liu, W. Li, W. Song, M. Guo, Remediation techniques for heavy metal-contaminated soils: Principles and applicability, *Science of the Total Environment*. 633 (2018).
<https://doi.org/10.1016/j.scitotenv.2018.03.161>.
27. V. Shah, A. Daverey, Phytoremediation: A multidisciplinary approach to clean up heavy metal contaminated soil, *Environ Technol Innov*. 18 (2020). <https://doi.org/10.1016/j.eti.2020.100774>.
28. I. Laifa, M. Hajji, N. Farhat, A. Elkhouni, A. Smaoui, A. M'nif, A.H. Hamzaoui, A. Savouré, C. Abdelly, W. Zorrig, Beneficial Effects of Silicon (Si) on Sea Barley (*Hordeum marinum* Huds.) under Salt Stress, *Silicon*. (2020). <https://doi.org/10.1007/s12633-020-00770-1>.
29. I. Laifa, H. Ellouzi, M. Idoudi, N. Farhat, M. Rabhi, H. Mahmoudi, A. Smaoui, A. Debez, C. Cabassa-Hourton, A. Savouré, others, Silicon (Si) Treatment has Preferential Beneficial Effects on Photosystem I Photochemistry in Salt-Treated *Hordeum marinum* (Huds.) Plants, *J Soil Sci Plant Nutr*. (2023) 1–17.
30. M. Falouti, H. Ellouzi, F. Bounaouara, N. Farhat, A.M. Aggag, A. Debez, M. Rabhi, C. Abdelly, I. Slama, W. Zorrig, Higher activity of PSI compared to PSII accounts for the beneficial effect of silicon on barley (*Hordeum vulgare* L.) plants challenged with salinity, *Photosynthetica*. 60 (2022).
<https://doi.org/10.32615/ps.2022.031>.
31. H. Ellouzi, M. Rabhi, S. Khedher, A. Debez, C. Abdelly, W. Zorrig, Silicon Seed Priming Enhances Salt Tolerance of Barley Seedlings through Early ROS Detoxification and Stimulation of Antioxidant Defence, *Silicon*. 15 (2023). <https://doi.org/10.1007/s12633-022-02001-1>.
32. N. Lamsaadi, R. Hidri, W. Zorrig, A. El Moukhtari, A. Debez, A. Savouré, C. Abdelly, M. Farissi, Exogenous silicon alleviates salinity stress in fenugreek (*Trigonella foenum graecum* L.) by enhancing photosystem activities, biological nitrogen fixation and antioxidant defence system, *South African Journal of Botany*. 159 (2023) 344–355.
33. M. Ahsan, M. Valipour, F. Nawaz, M. Raheel, H.T. Abbas, M. Sajid, A. Manan, S. Kanwal, E.A. Mahmoud, R. Casini, H.O. Elansary, E. Radicetti, H. Zulfikar, Evaluation of Silicon Supplementation for Drought Stress under Water-Deficit Conditions: An Application of Sustainable Agriculture, *Agronomy*. 13 (2023). <https://doi.org/10.3390/agronomy13020599>.
34. W. Benslimma, W. Zorrig, M. Bagues, C. Abdelly, C. Hafsi, Silicon mitigates potassium deficiency in *Hordeum vulgare* by improving growth and photosynthetic activity but not through polyphenol accumulation and the related antioxidant potential, *Plant Soil*. 477 (2022).
<https://doi.org/10.1007/s11104-021-05188-1>.
35. W. Benslimma, H. Ellouzi, W. Zorrig, C. Abdelly, C. Hafsi, Beneficial effects of silicon on growth, nutrient dynamics, and antioxidative response in barley (*Hordeum vulgare* L.) plants under potassium deficiency, *J Soil Sci Plant Nutr*. 22 (2022). <https://doi.org/10.1007/s42729-022-00832-w>.
36. M. Ksaa, N. Farhat, M. Rabhi, A. Elkhouni, A. Smaoui, A. Debez, C. Cabassa-Hourton, A. Savouré, C. Abdelly, W. Zorrig, Silicon (Si) Alleviates Iron Deficiency Effects in Sea Barley (*Hordeum marinum*) by Enhancing Iron Accumulation and Photosystem Activities, *Silicon*. 14 (2022).
<https://doi.org/10.1007/s12633-021-01376-x>.

37. S.M. Mousavi, Silicon and nano-silicon mediated heavy metal stress tolerance in plants, in: *Silicon and Nano-Silicon in Environmental Stress Management and Crop Quality Improvement: Progress and Prospects*, 2022. <https://doi.org/10.1016/B978-0-323-91225-9.00012-1>.
38. K.M. Cocker, D.E. Evans, M.J. Hodson, The amelioration of aluminium toxicity by silicon in higher plants: Solution chemistry or an in planta mechanism?, *Physiol Plant*. 104 (1998). <https://doi.org/10.1034/j.1399-3054.1998.1040413.x>.
39. J.F. Ma, M. Sasaki, H. Matsumoto, Al-induced inhibition of root elongation in corn, *Zea mays* L. is overcome by Si addition, *Plant Soil*. 188 (1997). <https://doi.org/10.1023/A:1004274223516>.
40. V. Paradisone, S. Landi, S. Esposito, Roles of Silicon in Alleviating Zinc Stress in Plants, in: *Metalloids in Plants*, 2020. <https://doi.org/10.1002/9781119487210.ch17>.
41. H.H. Gu, H. Qiu, T. Tian, S.S. Zhan, T.H.B. Deng, R.L. Chaney, S.Z. Wang, Y.T. Tang, J.L. Morel, R.L. Qiu, Mitigation effects of silicon rich amendments on heavy metal accumulation in rice (*Oryza sativa* L.) planted on multi-metal contaminated acidic soil, *Chemosphere*. 83 (2011). <https://doi.org/10.1016/j.chemosphere.2011.03.014>.
42. H.J. Brasser, G.C. Krijger, T.G. Van Meerten, H.T. Wolterbeek, Influence of silicon on cobalt, zinc, and magnesium in baker's yeast, *Saccharomyces cerevisiae*, *Biol Trace Elem Res*. 112 (2006). <https://doi.org/10.1385/BTER:112:2:175>.
43. H.A. Currie, C.C. Perry, Silica in plants: Biological, biochemical and chemical studies, *Ann Bot*. 100 (2007) 1383–1389. <https://doi.org/10.1093/aob/mcm247>.
44. D. Neumann, U.Z. Nieden, W. Schwieger, I. Leopold, O. Lichtenberger, Heavy metal tolerance of *Minuartia verna*, *J Plant Physiol*. 151 (1997). [https://doi.org/10.1016/S0176-1617\(97\)80044-2](https://doi.org/10.1016/S0176-1617(97)80044-2).
45. M.J. Hodson, A.G. Sangster, Aluminium/silicon interactions in conifers, in: *J Inorg Biochem*, 1999. [https://doi.org/10.1016/S0162-0134\(99\)00119-1](https://doi.org/10.1016/S0162-0134(99)00119-1).
46. L. Wang, Y. Wang, Q. Chen, W. Cao, M. Li, F. Zhang, Silicon induced cadmium tolerance of rice seedlings, *J Plant Nutr*. 23 (2000). <https://doi.org/10.1080/01904160009382110>.
47. K.P.V. Da Cunha, C.W.A. Do Nascimento, Silicon effects on metal tolerance and structural changes in Maize (*Zea mays* L.) grown on a cadmium and zinc enriched soil, *Water Air Soil Pollut*. 197 (2009). <https://doi.org/10.1007/s11270-008-9814-9>.
48. H.K. Lichtenthaler, Chlorophyll and Carotenoid Determination: pigments of photosynthetic biomembranes., *Methods in Enzymology*. 8 (1987).
49. C. Klughammer, U. Schreiber, Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method, *PAM Application Notes*. 1 (2008) 201–247.
50. C. Klughammer, U. Schreiber, Saturation Pulse method for assessment of energy conversion in PS I, *PAM Application Notes*. (2008).
51. W. Zorrig, A. Rouached, Z. Shahzad, C. Abdelly, J.C. Davidian, P. Berthomieu, Identification of three relationships linking cadmium accumulation to cadmium tolerance and zinc and citrate

- accumulation in lettuce, *J Plant Physiol.* 167 (2010) 1239–1247.
<https://doi.org/10.1016/j.jplph.2010.04.012>.
52. W. Zorrig, J.-Y. Cornu, B. Maisonneuve, A. Rouached, C. Sarrobert, Z. Shahzad, C. Abdelly, J.-C. Davidian, P. Berthomieu, Genetic analysis of cadmium accumulation in lettuce (*Lactuca sativa*), *Plant Physiology and Biochemistry.* 136 (2019) 67–75.
<https://doi.org/10.1016/j.plaphy.2019.01.011>.
 53. D.M. Hodges, J.M. DeLong, C.F. Forney, R.K. Prange, Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds, *Planta.* 207 (1999). <https://doi.org/10.1007/s004250050524>.
 54. I. Sergiev, V. Alexieva, E. Karanov, Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants, *Proceedings of the Bulgarian Academy of Sciences.* 51 (1997).
 55. M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal Biochem.* 72 (1976). [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3).
 56. F. Scebba, L. Sebastiani, C. Vitagliano, Protective enzymes against activated oxygen species in wheat (*Triticum aestivum* L.) seedlings: Responses to cold acclimation, *J Plant Physiol.* 155 (1999).
[https://doi.org/10.1016/S0176-1617\(99\)80094-7](https://doi.org/10.1016/S0176-1617(99)80094-7).
 57. H. Luck, Catalase in Methods of Enzymatic Analyses, *Methods of Enzymatic Analyses.* (1965).
 58. J.L. Fielding, J.L. Hall, A biochemical and cytochemical study of peroxidase activity in roots of *Pisum sativum*: I. A comparison of dab-peroxidase and guaiacol-peroxidase with particular emphasis on the properties of cell wall activity, *J Exp Bot.* 29 (1978). <https://doi.org/10.1093/jxb/29.4.969>.
 59. X. Mao, R. Jiang, W. Xiao, J. Yu, Use of surfactants for the remediation of contaminated soils: a review, *J Hazard Mater.* 285 (2015) 419–435.
 60. R. Ahmad, Z. Tehsin, S.T. Malik, S.A. Asad, M. Shahzad, M. Bilal, M.M. Shah, S.A. Khan, Phytoremediation Potential of Hemp (*Cannabis sativa* L.): Identification and Characterization of Heavy Metals Responsive Genes, *Clean (Weinh).* 44 (2016).
<https://doi.org/10.1002/clen.201500117>.
 61. Y. Fukao, A. Ferjani, R. Tomioka, N. Nagasaki, R. Kurata, Y. Nishimori, M. Fujiwara, M. Maeshima, iTRAQ analysis reveals mechanisms of growth defects due to excess zinc in Arabidopsis, *Plant Physiol.* 155 (2011). <https://doi.org/10.1104/pp.110.169730>.
 62. M.R. Romero-Aranda, O. Jurado, J. Cuartero, Silicon alleviates the deleterious salt effect on tomato plant growth by improving plant water status, *J Plant Physiol.* 163 (2006).
<https://doi.org/10.1016/j.jplph.2005.05.010>.
 63. Y.X. Zhu, X. Bin Xu, Y.H. Hu, W.H. Han, J.L. Yin, H.L. Li, H.J. Gong, Silicon improves salt tolerance by increasing root water uptake in *Cucumis sativus* L., *Plant Cell Rep.* 34 (2015).
<https://doi.org/10.1007/s00299-015-1814-9>.

64. A. Yaneff, V. Vitali, G. Amodeo, PIP1 aquaporins: Intrinsic water channels or PIP2 aquaporin modulators?, *FEBS Lett.* 589 (2015). <https://doi.org/10.1016/j.febslet.2015.10.018>.
65. R. Deshmukh, H. Sonah, R.R. Belanger, New evidence defining the evolutionary path of aquaporins regulating silicon uptake in land plants, *J Exp Bot.* 71 (2020). <https://doi.org/10.1093/jxb/eraa342>.
66. N.K. Savant, G.H. Korndörfer, L.E. Datnoff, G.H. Snyder, Silicon nutrition and sugarcane production: A review, *J Plant Nutr.* 22 (1999). <https://doi.org/10.1080/01904169909365761>.
67. R.K. Vandegeer, C. Zhao, X. Cibils-Stewart, R. Wuhler, C.R. Hall, S.E. Hartley, D.T. Tissue, S.N. Johnson, Silicon deposition on guard cells increases stomatal sensitivity as mediated by K⁺ efflux and consequently reduces stomatal conductance, *Physiol Plant.* 171 (2021). <https://doi.org/10.1111/ppl.13202>.
68. D. Takagi, K. Ishizaki, H. Hanawa, T. Mabuchi, G. Shimakawa, H. Yamamoto, C. Miyake, Diversity of strategies for escaping reactive oxygen species production within photosystem I among land plants: P700 oxidation system is prerequisite for alleviating photoinhibition in photosystem I, *Physiol Plant.* 161 (2017) 56–74.
69. S. Wada, D. Takagi, C. Miyake, A. Makino, Y. Suzuki, Responses of the photosynthetic electron transport reactions stimulate the oxidation of the reaction center chlorophyll of photosystem I, P700, under drought and high temperatures in rice, *Int J Mol Sci.* 20 (2019) 2068.
70. Y. Alzahrani, A. Kuşvuran, H.F. Alharby, S. Kuşvuran, M.M. Rady, The defensive role of silicon in wheat against stress conditions induced by drought, salinity or cadmium, *Ecotoxicol Environ Saf.* 154 (2018). <https://doi.org/10.1016/j.ecoenv.2018.02.057>.

Table

Table 1 is available in the Supplementary Files section.

Figures

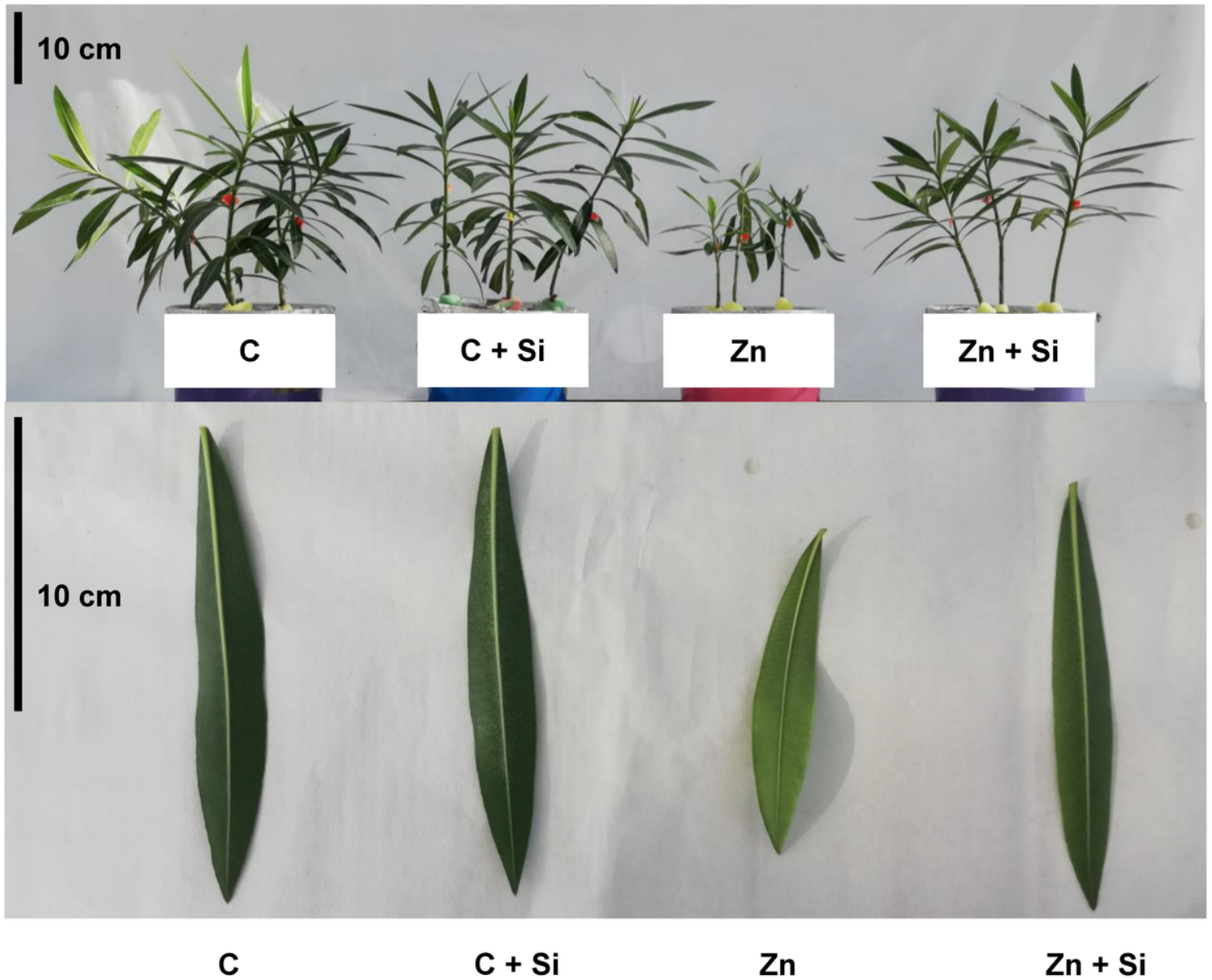


Figure 1

Phenotype of Oleander (*N. oleander*) plants, subjected to zinc toxicity combined with or without silicon application. Four groups of 7-month-old plants were subjected to varying concentrations of silicon and zinc during the final 45 days of cultivation. The two treatments 'C' and 'C + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a basal concentration of zinc (0.76 μ M). The treatments 'Zn' and 'Zn + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a toxic concentration of zinc (1800 μ M).

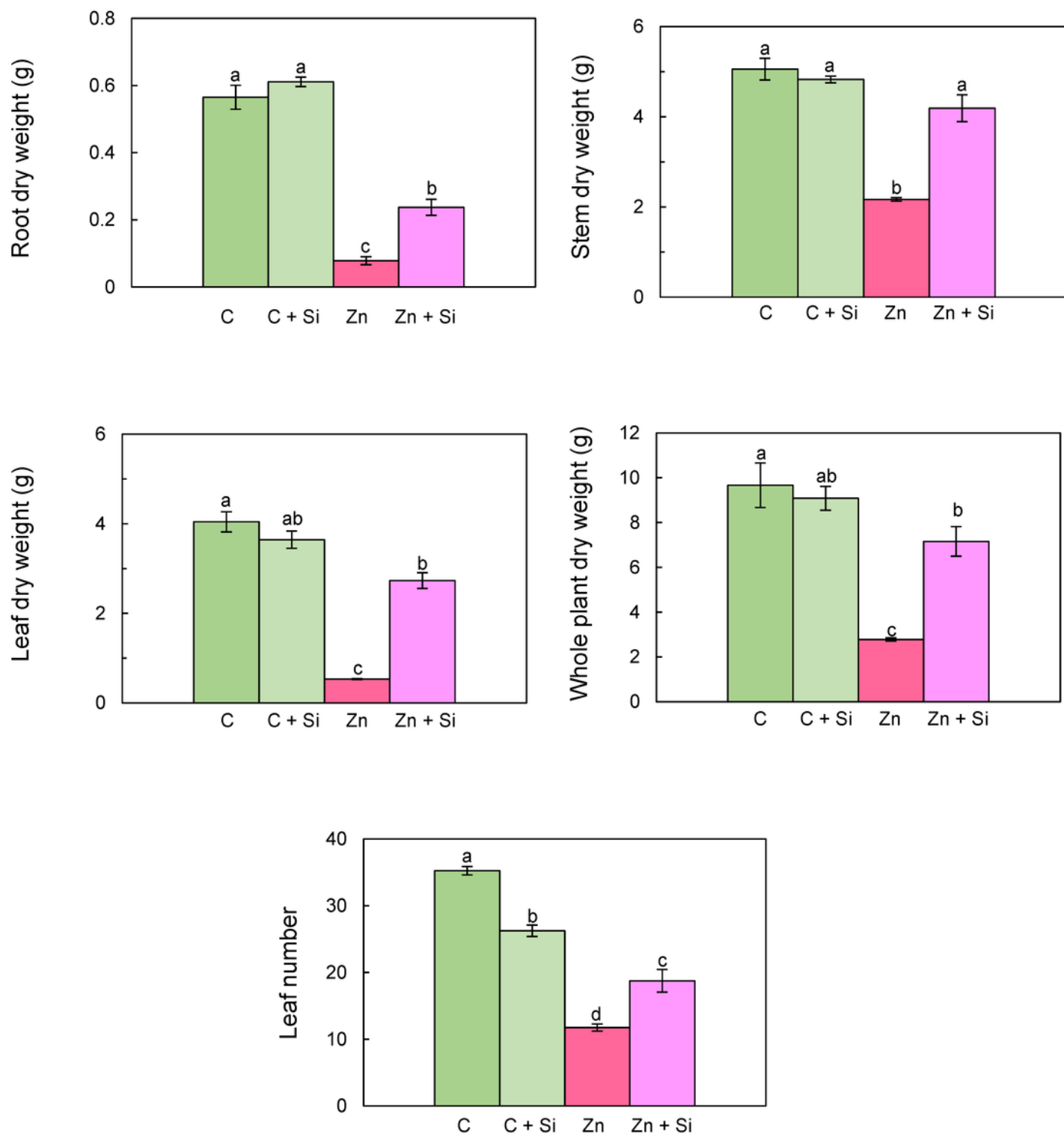


Figure 2

Dry weight of leaves, stem, root and whole plant, and leaf number under zinc toxicity combined with or without silicon application. Four groups of 7-month-old plants were subjected to varying concentrations of silicon and zinc during the final 45 days of cultivation. The two treatments 'C' and 'C + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a basal concentration of zinc (0.76 μ M). The treatments 'Zn' and 'Zn + Si' represent plants exposed to two

concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a toxic concentration of zinc (1800 μ M). Individual plant samples were taken and the data presented represents the average of 4 replicates. Error bars indicate standard errors, and values that share a letter are not significantly different at a 0.05 threshold (multiple mean comparisons - Duncan's test).

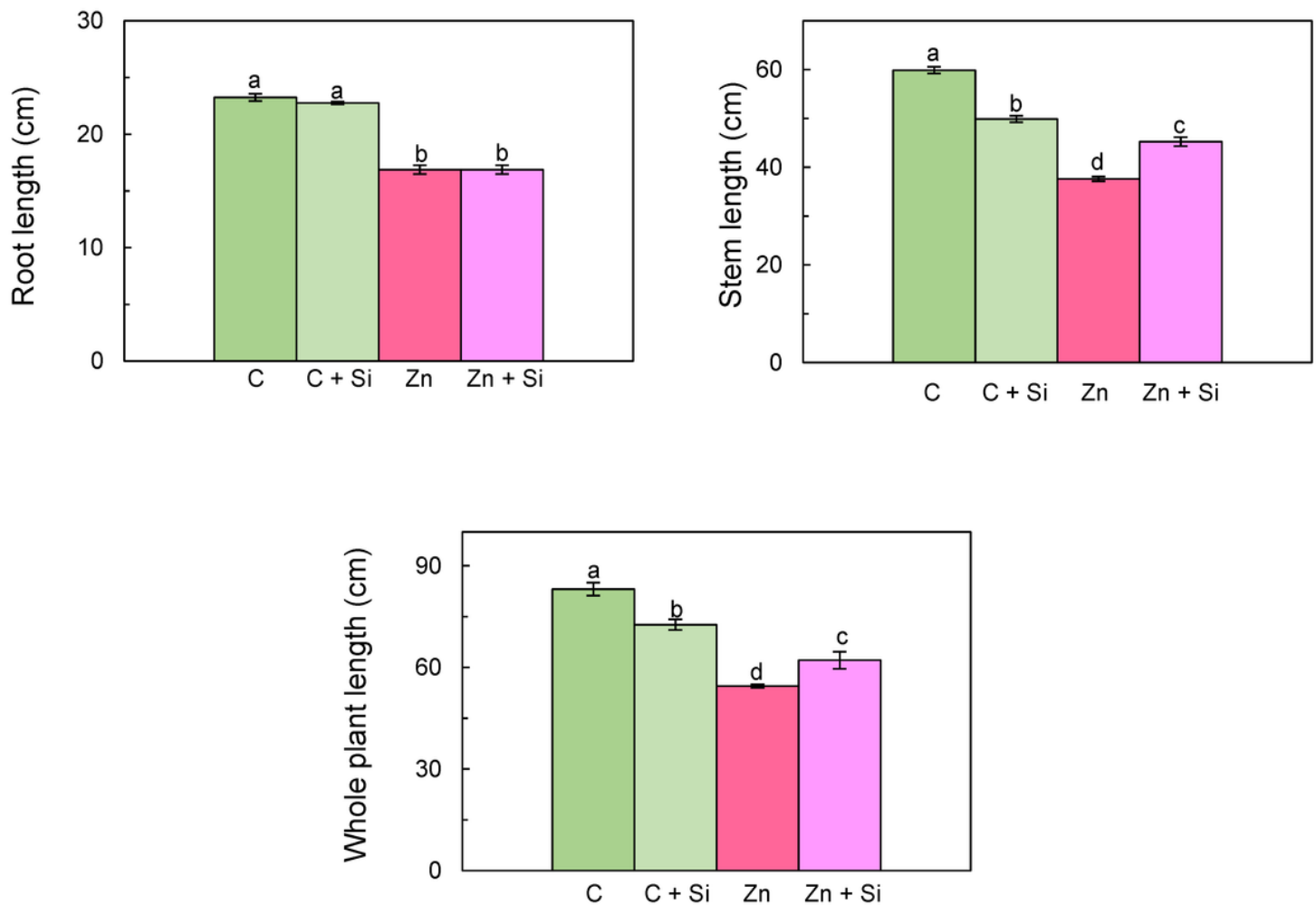


Figure 3

Root, stem, and whole plant length under zinc toxicity combined with or without silicon application. Four groups of 7-month-old plants were subjected to varying concentrations of silicon and zinc during the final 45 days of cultivation. The two treatments 'C' and 'C + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a basal concentration of zinc (0.76 μ M). The treatments 'Zn' and 'Zn + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a toxic concentration of zinc (1800 μ M). Individual plant samples were taken and the data presented represents the average of 4 replicates. Error bars indicate standard errors, and values that share a letter are not significantly different at a 0.05 threshold (multiple mean comparisons - Duncan's test).

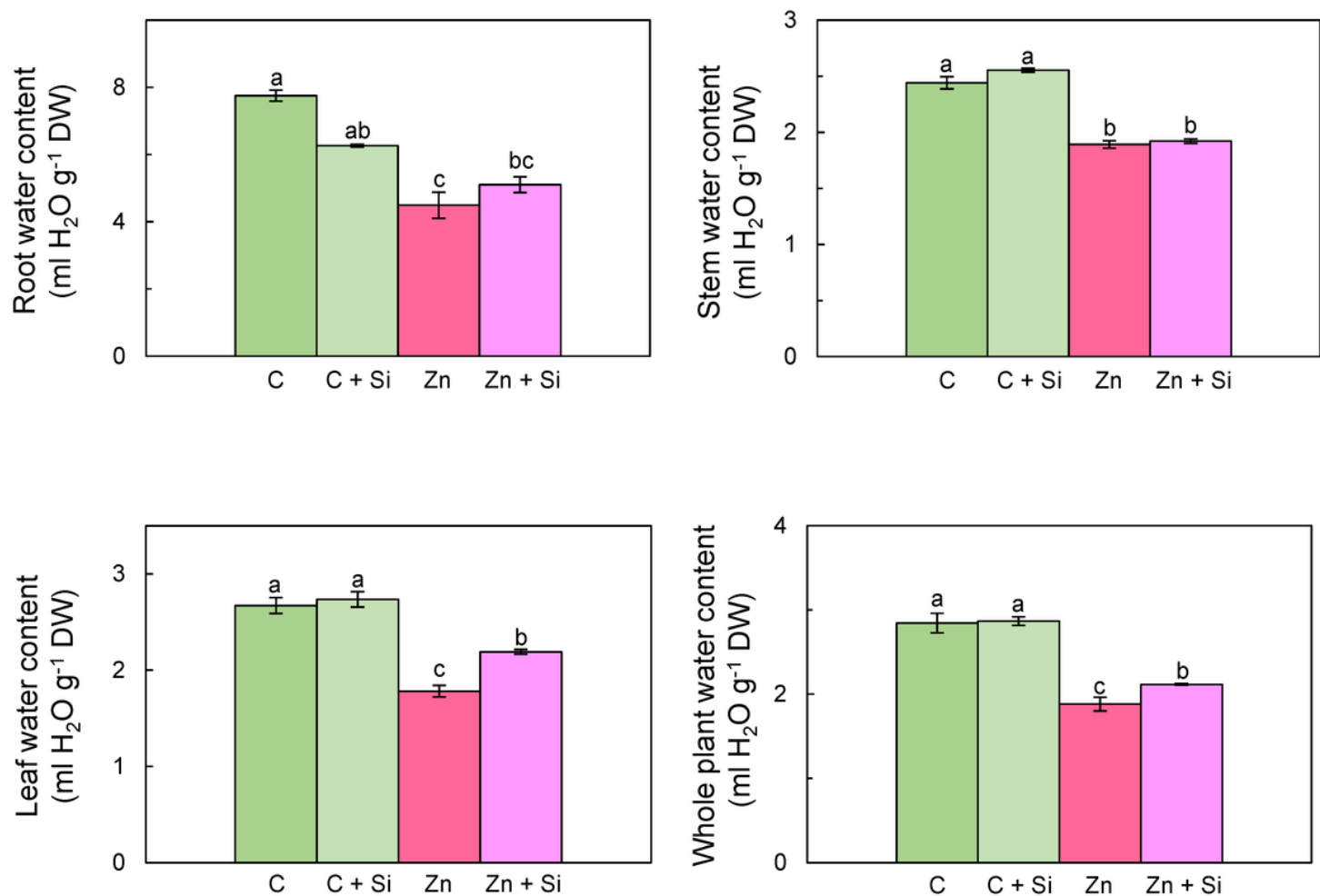


Figure 4

Root, stem, leaf, and whole plant water content under zinc toxicity combined with or without silicon application. Four groups of 7-month-old plants were subjected to varying concentrations of silicon and zinc during the final 45 days of cultivation. The two treatments 'C' and 'C + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a basal concentration of zinc (0.76 μ M). The treatments 'Zn' and 'Zn + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a toxic concentration of zinc (1800 μ M). Individual plant samples were taken and the data presented represents the average of 4 replicates. Error bars indicate standard errors, and values that share a letter are not significantly different at a 0.05 threshold (multiple mean comparisons - Duncan's test).

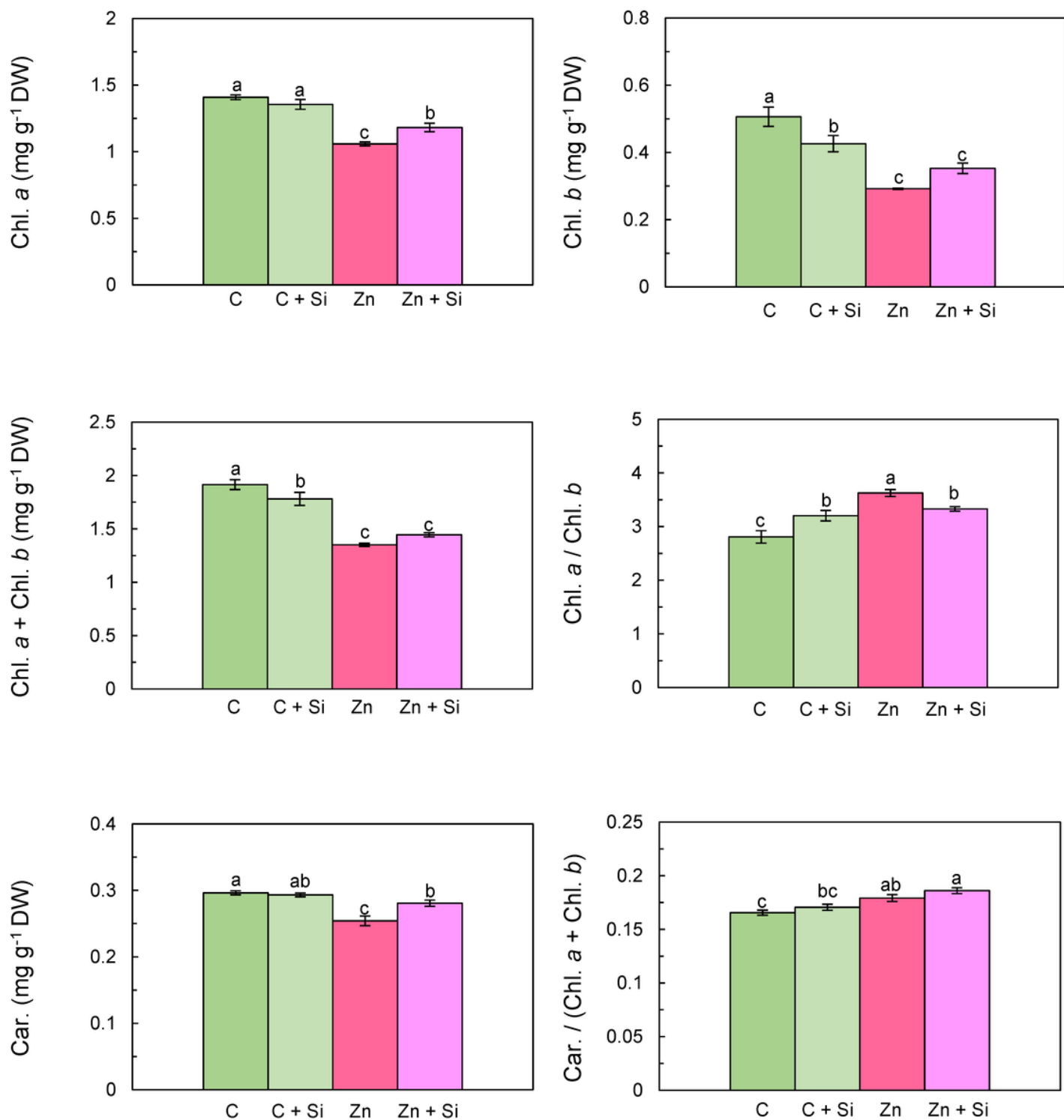


Figure 5

Photosynthetic pigment content under zinc toxicity combined with or without silicon application. Chl. *a*: chlorophyll *a* content; Chl. *b*: chlorophyll *b* content. Car.: carotenoid content. Four groups of 7-month-old plants were subjected to varying concentrations of silicon and zinc during the final 45 days of cultivation. The two treatments 'C' and 'C + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a basal concentration of zinc (0.76 μM). The treatments 'Zn' and 'Zn + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si,

respectively) in the presence of a toxic concentration of zinc (1800 μM). Individual plant samples were taken and the data presented represents the average of 4 replicates. Error bars indicate standard errors, and values that share a letter are not significantly different at a 0.05 threshold (multiple mean comparisons - Duncan's test).

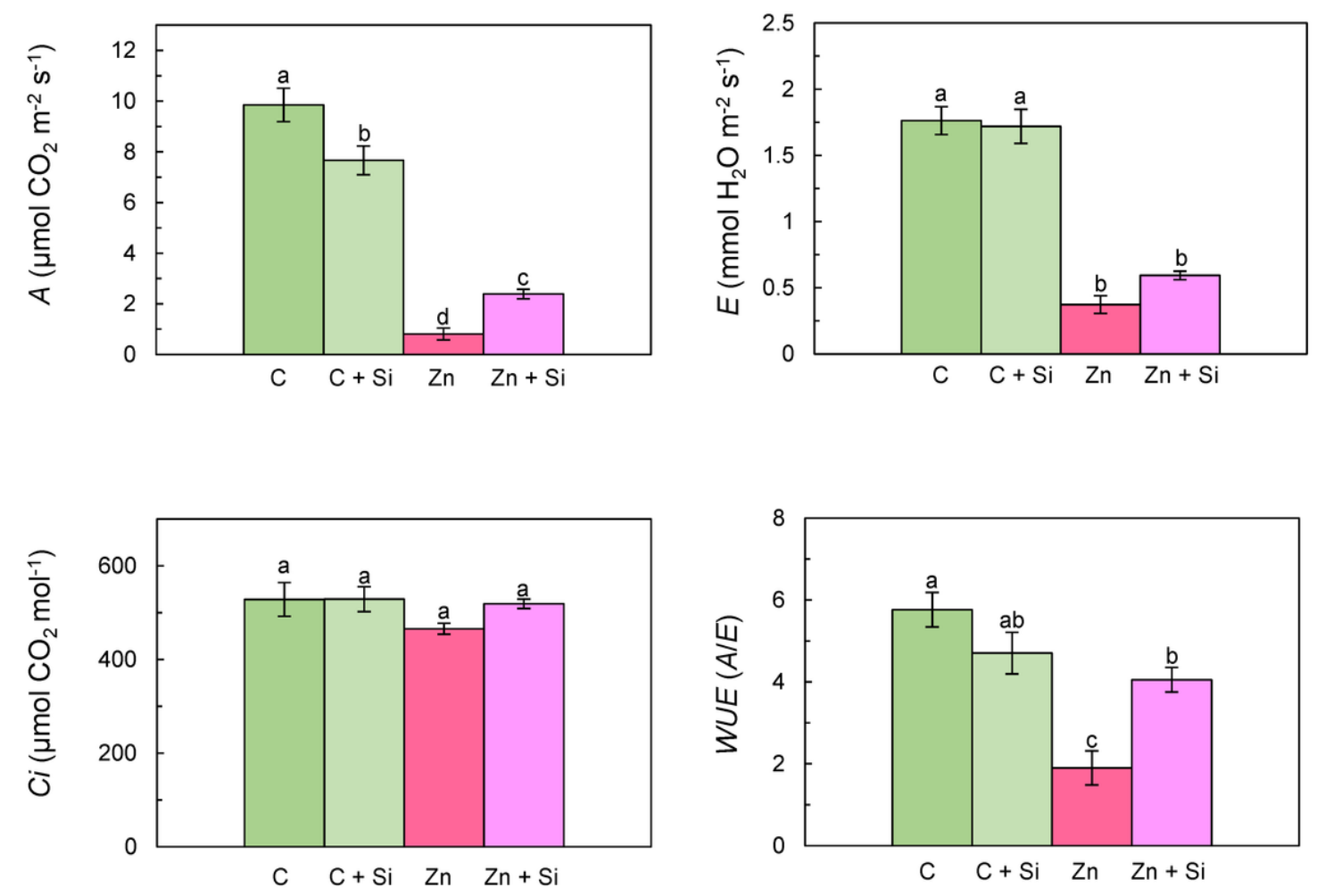


Figure 6

Effect of silicon on photosynthetic gas exchange parameters in leaves of *N. oleander* plants treated with toxic concentrations of zinc. A: net CO₂ assimilation rate; E: transpiration rate; Ci: internal concentration of CO₂; WUE: Water Use Efficiency.

Four groups of 7-month-old plants were subjected to varying concentrations of silicon and zinc during the final 45 days of cultivation. The two treatments 'C' and 'C + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a basal concentration of zinc (0.76 μM). The treatments 'Zn' and 'Zn + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a toxic concentration of zinc (1800 μM). Individual plant samples were taken and the data presented represents the average of 12 replicates. Error bars

indicate standard errors, and values that share a letter are not significantly different at a 0.05 threshold (multiple mean comparisons - Duncan's test).

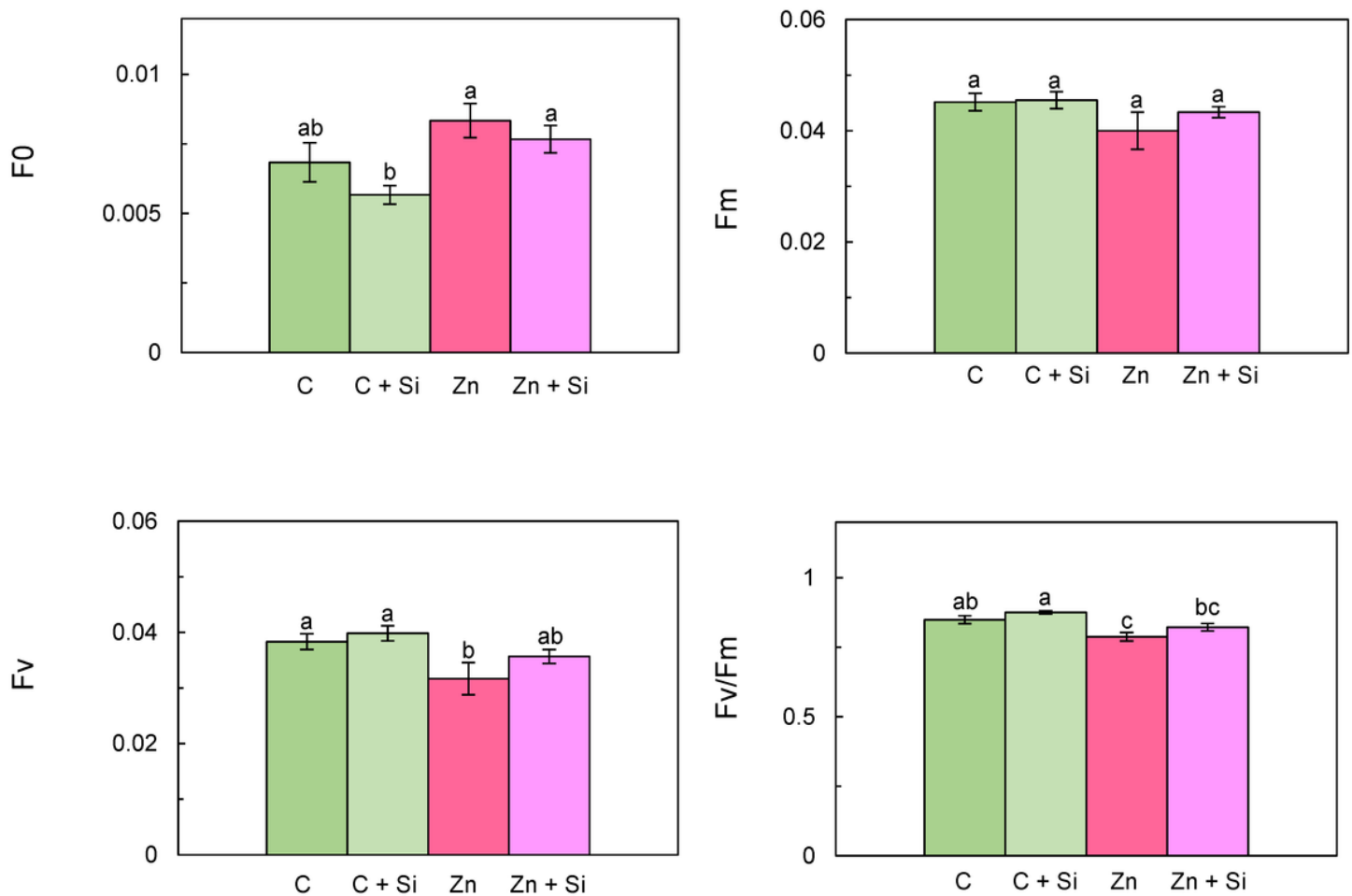


Figure 7

Basic fluorescence parameters of PSII for dark-adapted leaves of *N. oleander* plants under zinc toxicity combined with or without silicon application. F₀: Minimal fluorescence yield of dark-adapted samples with all PSII centres open; F_m: Maximal fluorescence yield of dark-adapted sample with all PSII centres closed; F_v: Variable fluorescence of dark-adapted sample; F_v/F_m: Maximal photosystem II (PSII) quantum yield of dark-adapted samples ((F_m - F₀)/F_m). Four groups of 7-month-old plants were subjected to varying concentrations of silicon and zinc during the final 45 days of cultivation. The two treatments 'C' and 'C + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a basal concentration of zinc (0.76 μM). The treatments 'Zn' and 'Zn + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a toxic concentration of zinc (1800 μM). Individual plant samples were taken and the data presented represents the average of 6 replicates. Error bars indicate standard errors, and values that share a letter are not significantly different at a 0.05 threshold (multiple mean comparisons - Duncan's test).

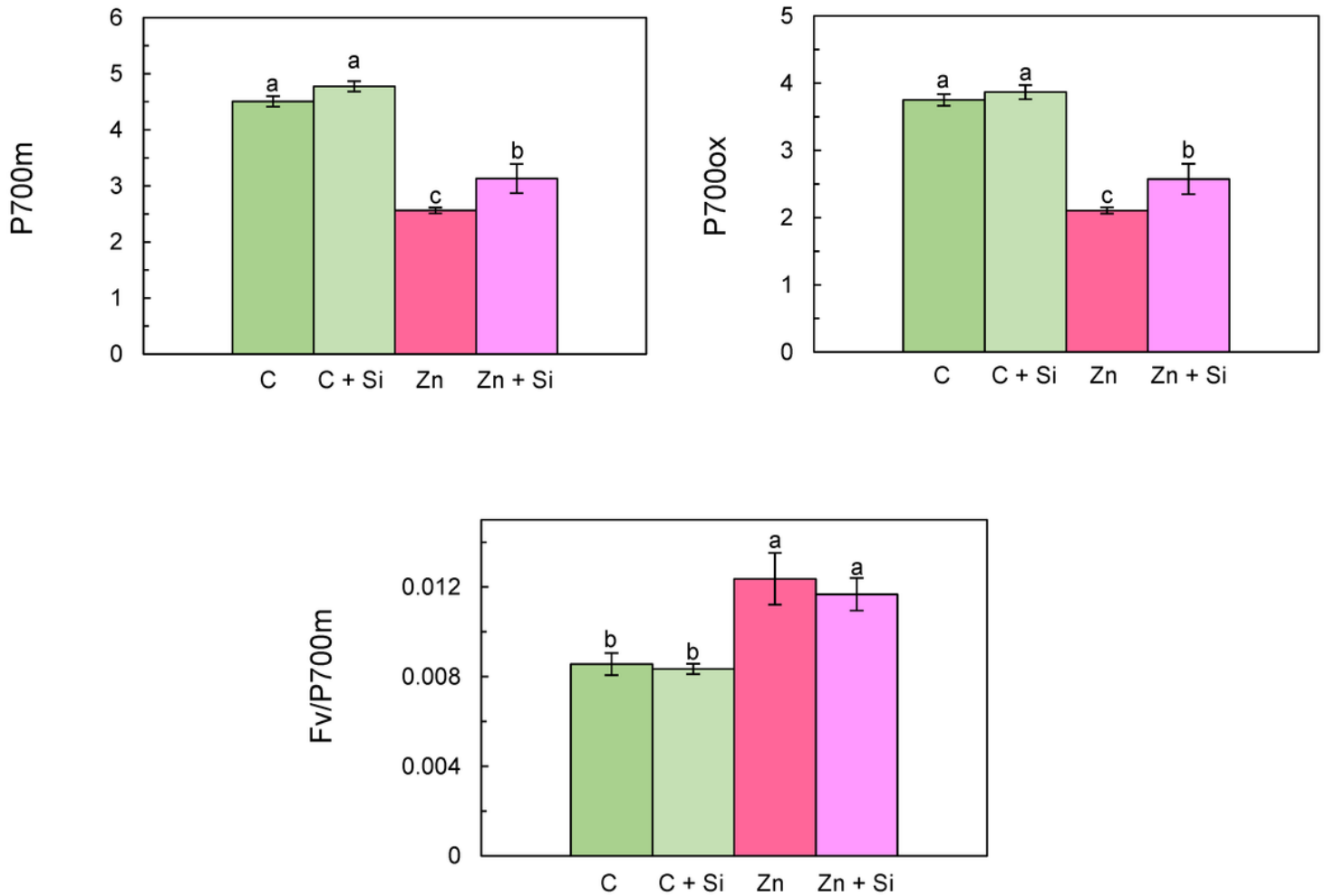


Figure 8

Basic parameters of PSI for dark-adapted leaves of *N. oleander* plants under zinc toxicity combined with or without silicon application. P700m: maximal fluorescence yield of dark-adapted sample with all PSI centres closed. P700ox: Oxidized PSI. Fv: Variable fluorescence of dark-adapted sample (PSII parameter). Fv/P700m: Variable fluorescence of dark-adapted sample/maximal fluorescence yield of dark-adapted sample with all PSI centres closed. Four groups of 7-month-old plants were subjected to varying concentrations of silicon and zinc during the final 45 days of cultivation. The two treatments 'C' and 'C + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a basal concentration of zinc (0.76 μ M). The treatments 'Zn' and 'Zn + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a toxic concentration of zinc (1800 μ M). Individual plant samples were taken and the data presented represents the average of 6 replicates. Error bars indicate standard errors.

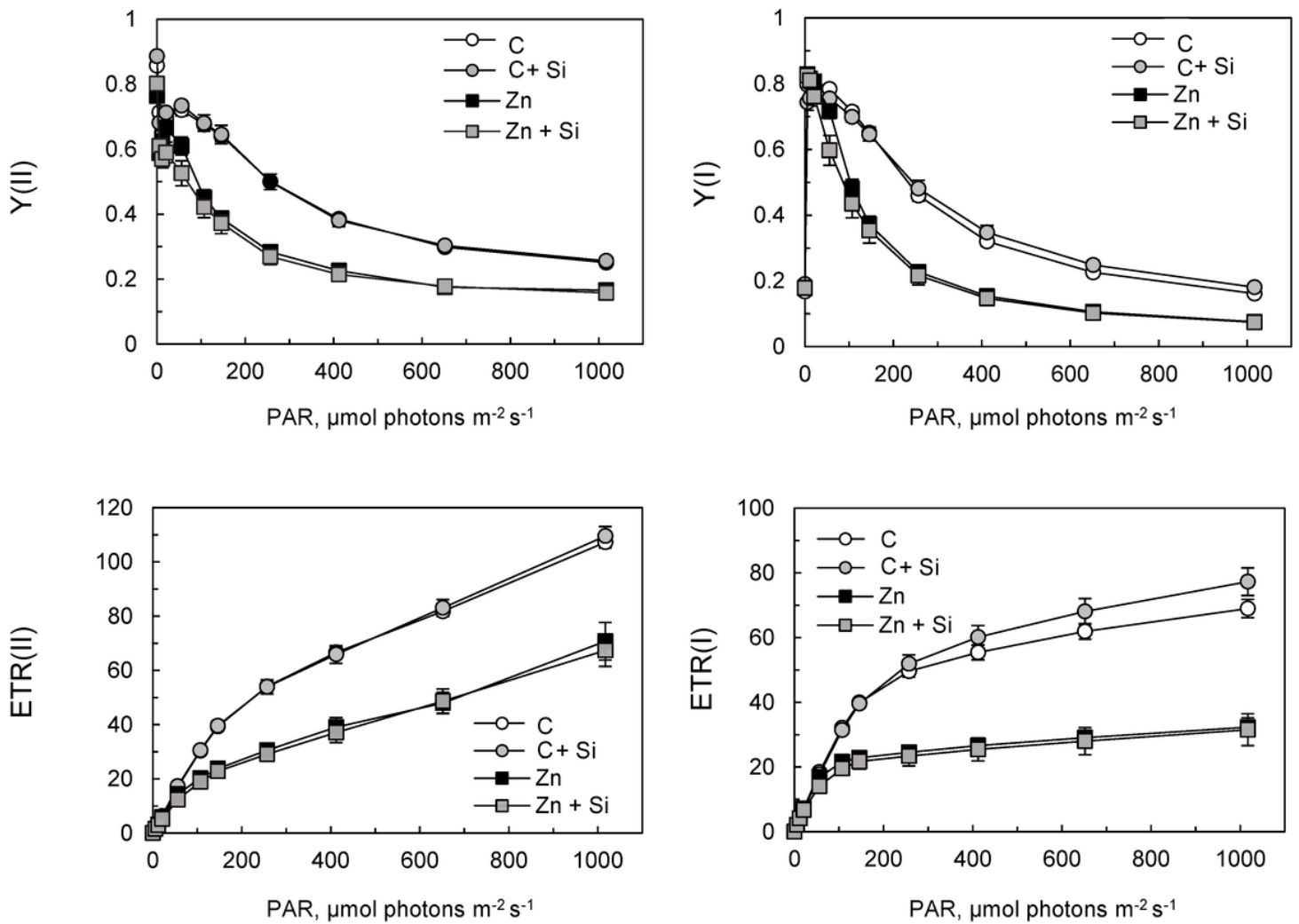


Figure 9

Quantum yield of photochemical energy conversion (Y) and Electron Transfer Rate (ETR) of PSI and PSII for light-adapted leaves of *N. oleander* plants under zinc toxicity combined with or without silicon application. Y(II): quantum yield of photochemical energy conversion in PSII; ETR(II): electron transfer rate (II); Y(I): quantum yield of photochemical energy conversion in PSI; ETR(I): electron transfer rate (I). Four groups of 7-month-old plants were subjected to varying concentrations of silicon and zinc during the final 45 days of cultivation. The two treatments 'C' and 'C + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a basal concentration of zinc (0.76 μM). The treatments 'Zn' and 'Zn + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a toxic concentration of zinc (1800 μM). Individual plant samples were taken and the data presented represents the average of 6 replicates. Error bars indicate standard errors.

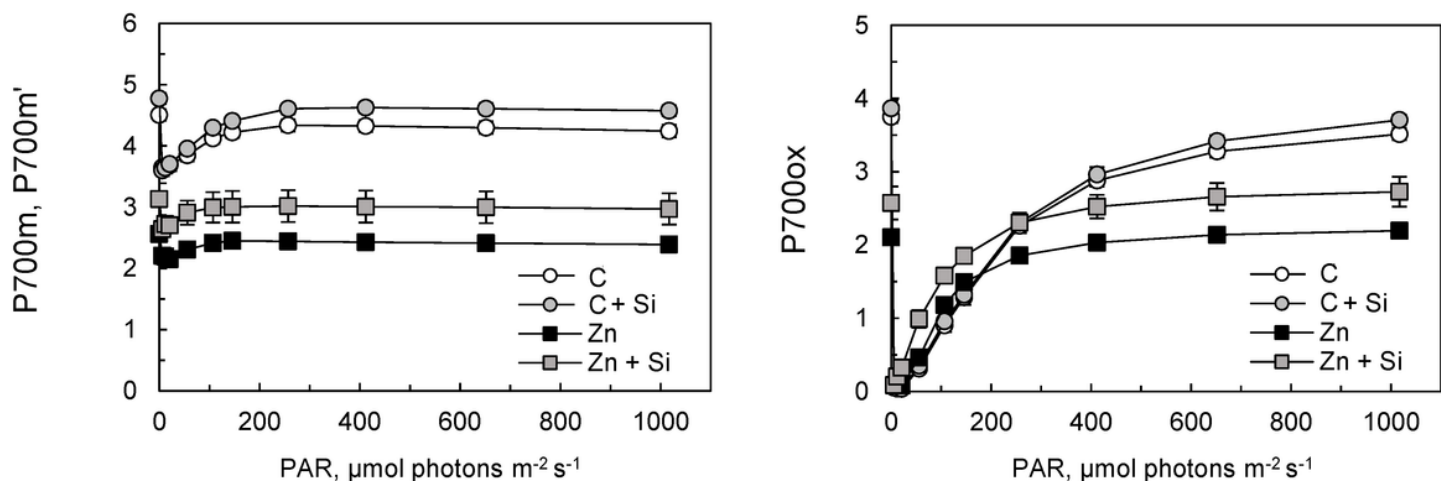


Figure 10

Maximal fluorescence yield (P700m, P700m') and oxidized PSI (P700ox) for leaves of *N. oleander* plants under zinc toxicity combined with or without silicon application. Four groups of 7-month-old plants were subjected to varying concentrations of silicon and zinc during the final 45 days of cultivation. The two treatments 'C' and 'C + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a basal concentration of zinc (0.76 μM). The treatments 'Zn' and 'Zn + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a toxic concentration of zinc (1800 μM). Individual plant samples were taken and the data presented represents the average of 6 replicates. Error bars indicate standard errors.

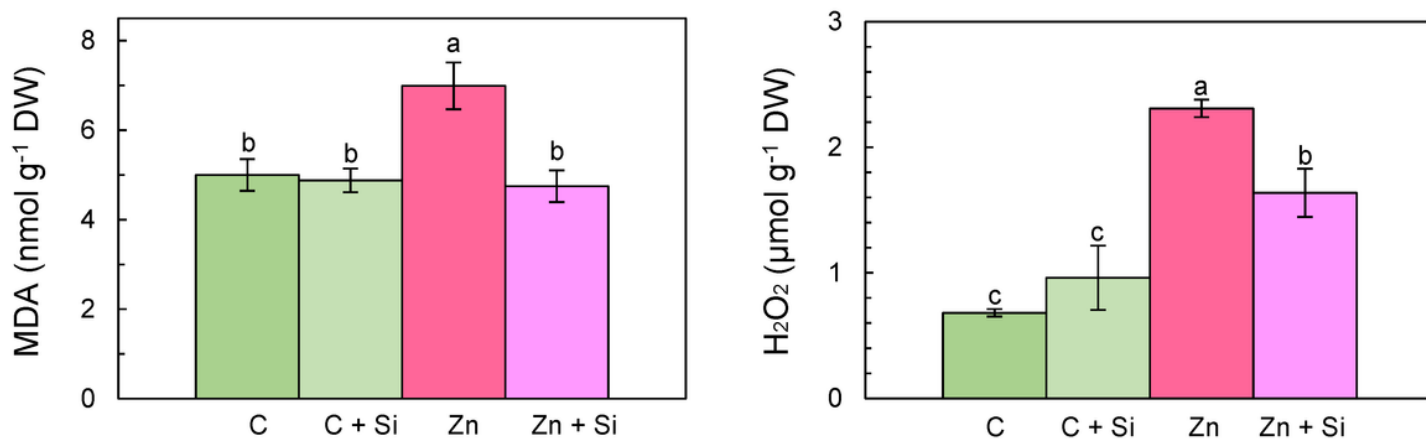


Figure 11

Malondialdehyde (MDA) content and hydrogen peroxide (H_2O_2) content in leaves of *N. oleander* plants subjected to zinc toxicity, with or without silicon application. Four groups of 7-month-old plants were subjected to varying concentrations of silicon and zinc during the final 45 days of cultivation. The two treatments 'C' and 'C + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a basal concentration of zinc (0.76 μM). The treatments 'Zn' and 'Zn + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a toxic concentration of zinc (1800 μM).

Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a toxic concentration of zinc (1800 μ M). Individual plant samples were taken and the data presented represents the average of 4 replicates. Error bars indicate standard errors, and values that share a letter are not significantly different at a 0.05 threshold (multiple mean comparisons - Duncan's test).

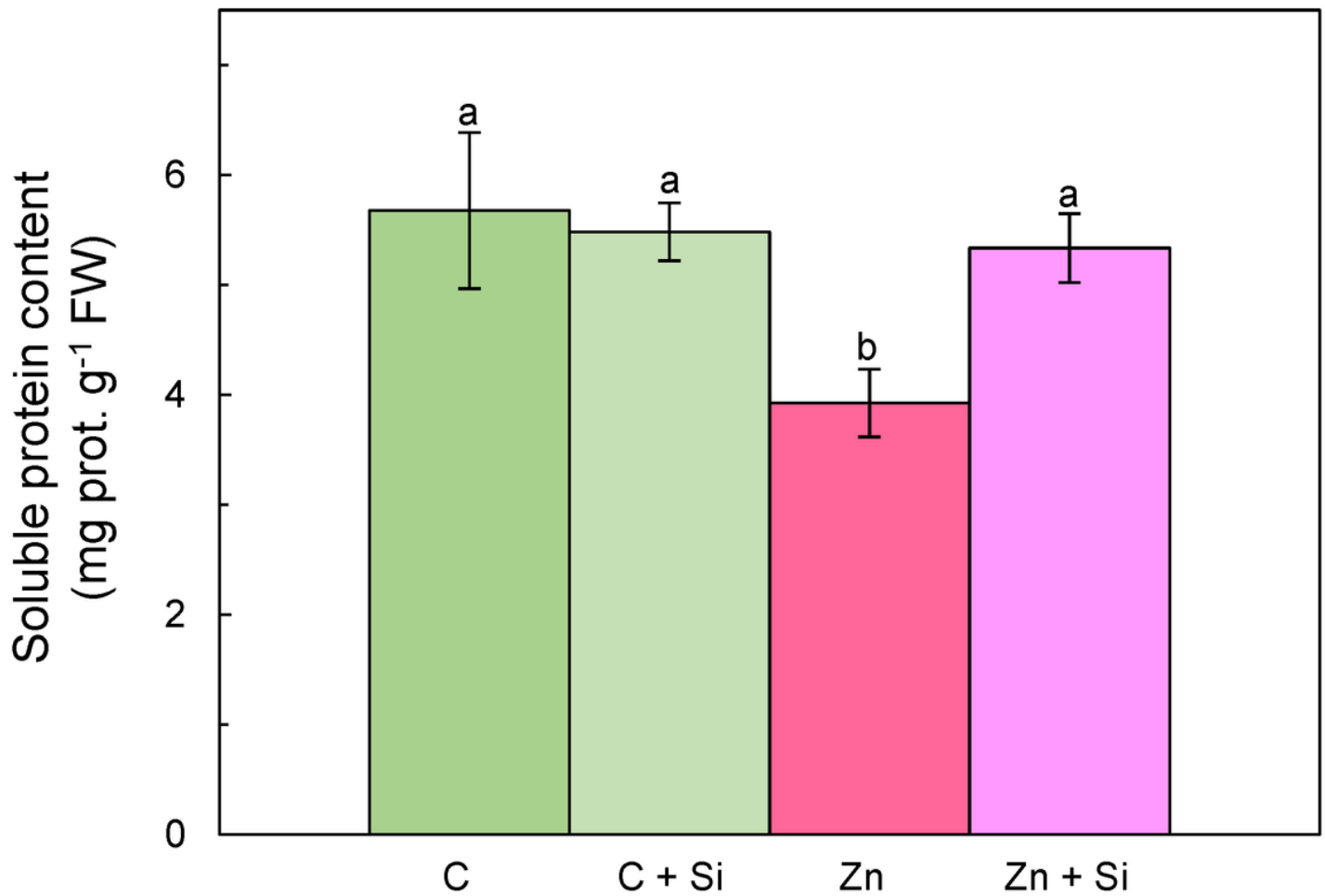


Figure 12

Soluble protein content in leaves of *N. oleander* plants subjected to zinc toxicity, with or without silicon application. Four groups of 7-month-old plants were subjected to varying concentrations of silicon and zinc during the final 45 days of cultivation. The two treatments 'C' and 'C + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a basal concentration of zinc (0.76 μ M). The treatments 'Zn' and 'Zn + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a toxic concentration of zinc (1800 μ M). Individual plant samples were taken and the data presented represents the average of 4 replicates. Error bars indicate standard errors, and values that share a letter are not significantly different at a 0.05 threshold (multiple mean comparisons - Duncan's test).

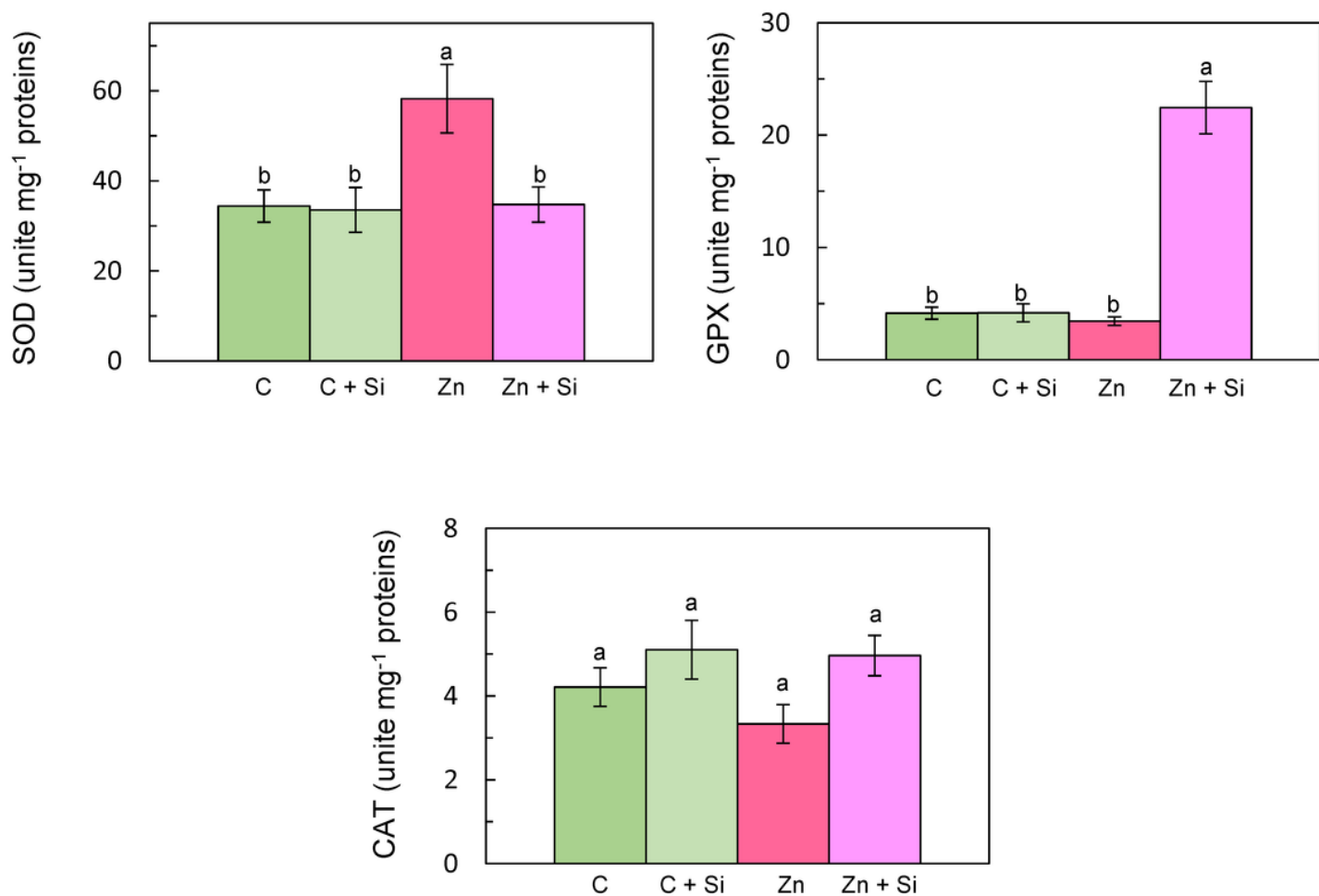


Figure 13

Superoxide dismutase (SOD), guaiacol peroxidase (GPX), and catalase (CAT) activities in leaves of *N. oleander* plants exposed to zinc toxicity, with or without silicon application. Four groups of 7-month-old plants were subjected to varying concentrations of silicon and zinc during the final 45 days of cultivation. The two treatments 'C' and 'C + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a basal concentration of zinc (0.76 μ M). The treatments 'Zn' and 'Zn + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a toxic concentration of zinc (1800 μ M). Individual plant samples were taken and the data presented represents the average of 4 replicates. Error bars indicate standard errors, and values that share a letter are not significantly different at a 0.05 threshold (multiple mean comparisons - Duncan's test).

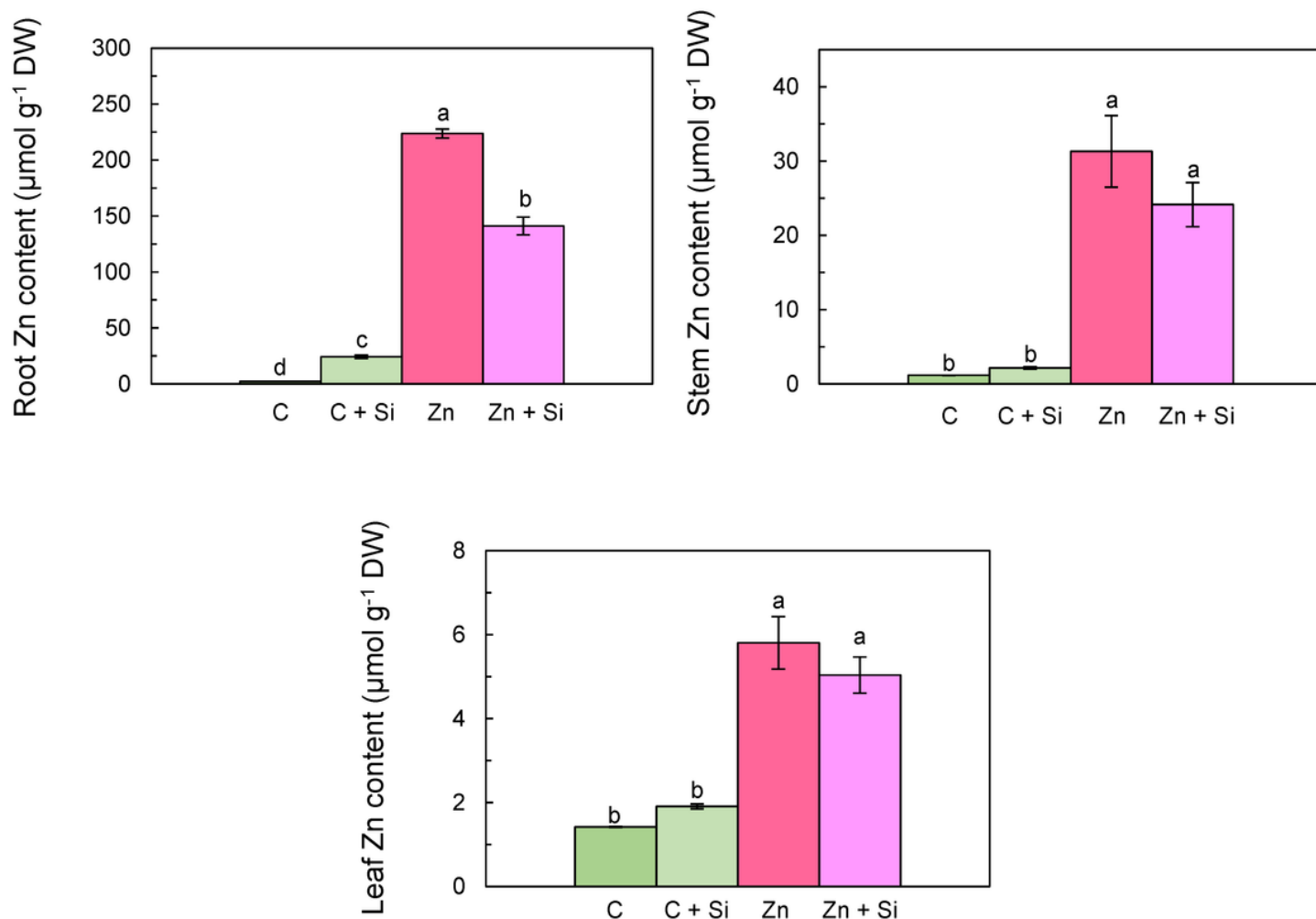


Figure 14

Root, stem, and leaf zinc content ($\mu\text{mol g}^{-1} \text{ DW}$) in *N. oleander* plants subjected to zinc toxicity, with or without silicon application. Four groups of 7-month-old plants were subjected to varying concentrations of silicon and zinc during the final 45 days of cultivation. The two treatments 'C' and 'C + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a basal concentration of zinc ($0.76 \mu\text{M}$). The treatments 'Zn' and 'Zn + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a toxic concentration of zinc ($1800 \mu\text{M}$). Individual plant samples were taken and the data presented represents the average of 4 replicates. Error bars indicate standard errors, and values that share a letter are not significantly different at a 0.05 threshold (multiple mean comparisons - Duncan's test).

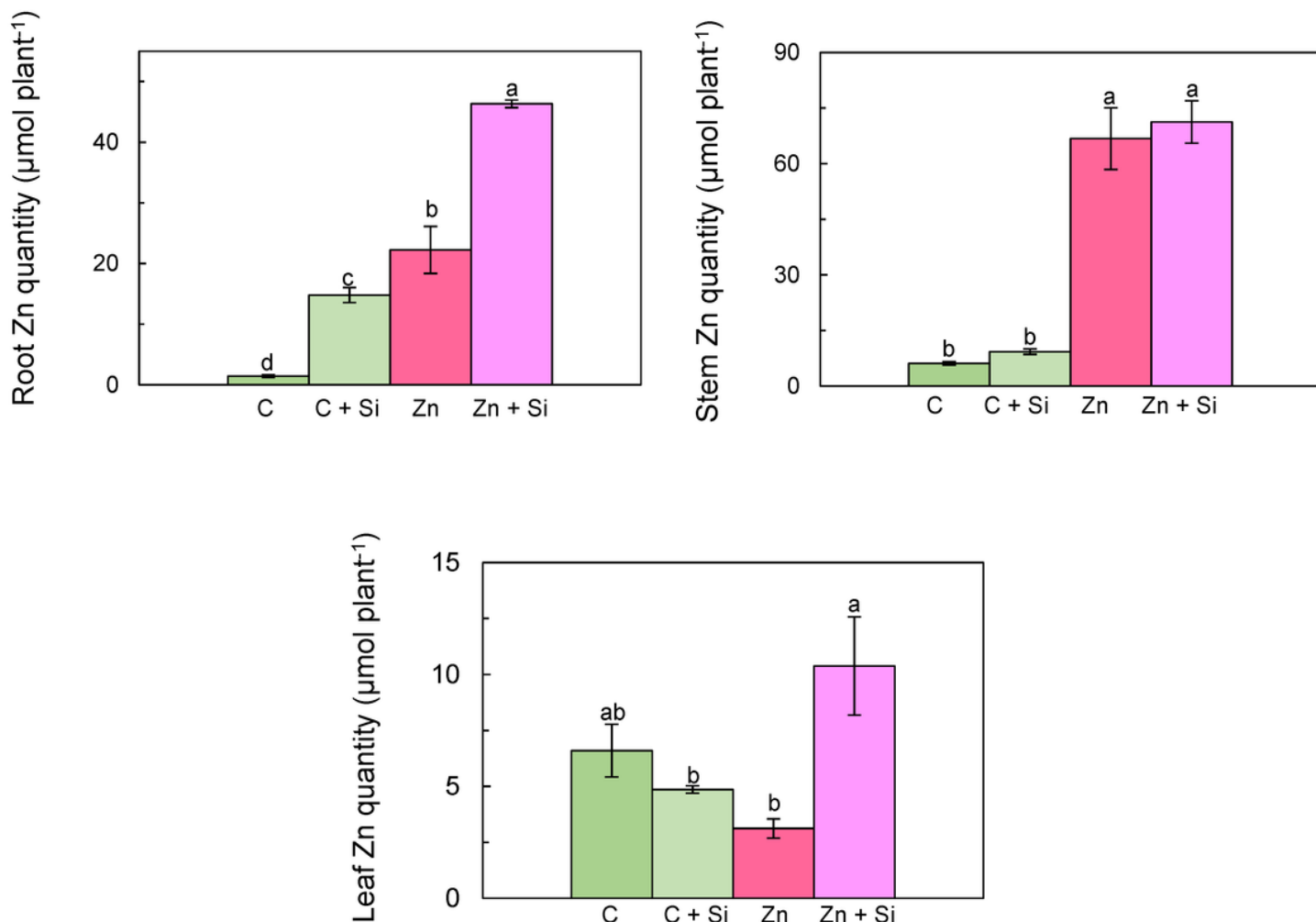


Figure 15

Root, stem, and leaf zinc quantity ($\mu\text{mol plant}^{-1}$) in *N. oleander* plants subjected to zinc toxicity, with or without silicon application. Four groups of 7-month-old plants were subjected to varying concentrations of silicon and zinc during the final 45 days of cultivation. The two treatments 'C' and 'C + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a basal concentration of zinc (0.76 μM). The treatments 'Zn' and 'Zn + Si' represent plants exposed to two concentrations of silicon (0 mM and 0.5 mM Si, respectively) in the presence of a toxic concentration of zinc (1800 μM). Individual plant samples were taken and the data presented represents the average of 4 replicates. Error bars indicate standard errors, and values that share a letter are not significantly different at a 0.05 threshold (multiple mean comparisons - Duncan's test).

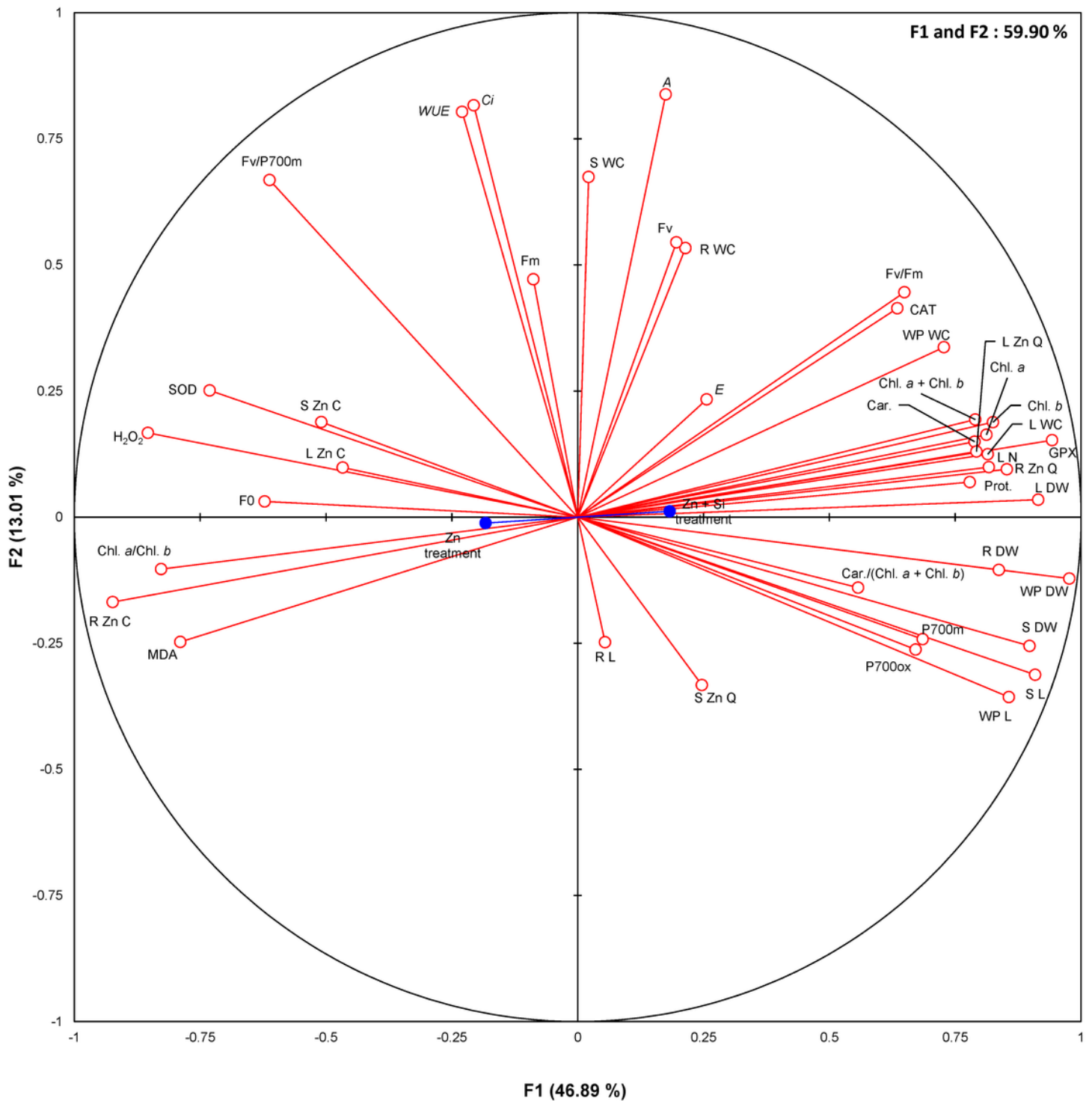


Figure 16

The analytical parameters are represented by circles (•). The different analyzed treatments (Zn and Zn + Si) are indicated by squares (■). These parameters and zinc treatments are projected onto the F1-F2 principal factorial plane, which explains 59.9% of the variation. R DW: root dry weight; S DW: stem dry weight; L DW: leaf dry weight; WP DW: whole plant dry weight; L N: leaf number; R L: root length; S L: stem length; WP L: whole plant length; R WC: root water content; S WC: stem water content; L WC: leaf water

content; WP WC: whole plant water content; Chl. *a*: chlorophyll *a* content; Chl. *b*: chlorophyll *b* content; Chl. *a* + Chl. *b*: chlorophyll *a* content + chlorophyll *b* content; Chl. *a*/Chl. *b*: chlorophyll *a* content/chlorophyll *b* content; Car.: carotenoid content; Car./(Chl. *a* + Chl. *b*): carotenoid content./(chlorophyll *a* content + chlorophyll *b* content); *A*: net CO₂ assimilation rate; *E*: transpiration rate; *C_i*: internal concentration of CO₂; *WUE*: Water Use Efficiency; *F₀*: Minimal fluorescence yield of dark-adapted samples with all PSII centres open; *F_m*: Maximal fluorescence yield of dark-adapted sample with all PSII centres closed; *F_v*: Variable fluorescence of dark-adapted sample; *F_v*/*F_m*: Maximal photosystem II (PSII) quantum yield of dark-adapted samples; *P700m*: maximal fluorescence yield of dark-adapted sample with all PSI centres closed. *P700ox*: Oxidized PSI; *F_v*/*P700m*: variable fluorescence of dark-adapted sample/maximal fluorescence yield of dark-adapted sample with all PSI centres closed; *Prot.*: soluble protein content; *MDA*: malondialdehyde content; *H₂O₂*: hydrogen peroxide content; *SOD*: superoxide dismutase activity; *GPX*: guaiacol peroxidase activity; *CAT*: catalase activity; *R Zn C*: root zinc content; *S Zn C*: stem zinc content; *L Zn C*: leaf zinc content; *R Zn Q*: root zinc quantity; *S Zn Q*: stem zinc quantity; *L Zn Q*: leaf zinc quantity.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table01.docx](#)