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Toxicity of Zinc Chloride Modulates Chlorophyll, Oxidative Stress and TCA Cycle Associated Organic Acids Exudation in Hydroponically Grown Maize Seedlings

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Abstract

Zinc (Zn) is an essential trace element; however, its excess can lead to toxicity, thereby disrupting plant physiology and rhizosphere interactions. This hydroponic research analyzed the consequence of increasing Zn concentrations (Zn1–Zn3) on maize (*Zea mays* L.) seedlings, focusing on growth, photosynthesis, oxidative stress, and root exudation responses. Growth was boosted with a modest Zn supply (Zn1), with root and shoot lengths rising 19.8% and 38.3%, respectively, in evaluation to the control. In contrast, higher Zn levels suppressed shoot length by 25.4% and reduced both fresh and dry biomass. Photosynthetic pigments were adversely affected, with chlorophyll *a*, *b*, and total chlorophyll decreasing by 1.9%, 0.8%, and 1.6%, respectively, while carotenoids declined by 0.06–0.08% under Zn stress. Lipid peroxidation intensified, as indicated by a 1.9–2.8% increase in MDA content, and ROS accumulation rose, with $O_2^{\bullet -}$ levels in leaves by 2.4–5.5% at Zn2–Zn3. Zn stress also significantly altered root exudates composition, for example, lactic acid secretion surged under Zn1 (+ 8697%) but decreased sharply under Zn3 (− 3224%), while citric (+ 58%) and malic (+ 54%) acids increased at Zn1, and oxalic acid consistently declined (1109–158%). These changes, along with a 4–5% reduction in rhizosphere pH, suggest that Zn-induced organic acid exudation serves as a detoxification mechanism. Overall, seedlings exhibited a dual response, where moderate Zn levels enhanced growth and exudation-mediated tolerance, whereas excessive Zn induced oxidative stress, pigment loss, and metabolic disruption, underscoring root exudate modulation as a key indicator of Zn stress resilience in maize.

Introduction

A significant contributor to soil pollution is the excessive buildup of heavy metals, which is mostly caused by human activities including mining, the disposal of toxic waste, and the application of fertilizers and pesticides in agriculture (Palm et al., 2017; Zhang et al., 2020). Zinc (Zn) is unique among heavy metals in that it can be both a necessary micronutrient and a possible toxicity when present in excess. In addition to being a cofactor for many enzymes, zinc is easily absorbed by plants and is essential for the biosynthesis and metabolism of chlorophyll, lipid, carbohydrate, protein, vitamin E, cell wall, RNA production and DNA protection, and hormone regulation (Hussein and Abou-Baker, 2018; Rai et al., 2021; Šimon et al., 2021). However, the bioavailability of Zn determines whether its effects are beneficial or harmful. At adequate levels, it promotes plant growth and productivity, whereas excessive concentrations surpass plant requirements and induce toxicity (Šimon et al., 2021). High Zn levels negatively affect plant growth, physiology, and nutrient balance, while increasing Zn accumulation in tissues (Šimon et al., 2021; Ying et al., 2019). In the end, zinc poisoning affects photosynthesis, produces chlorosis, interferes with cellular functions, changes the integrity and permeability of the plasma membrane, and upsurges the production of reactive oxygen species (ROS) (Ghoto et al., 2022).

Zinc's phytotoxicity has been shown in soil-grown alfalfa plants linked to *Sinorhizobium meliloti*, where exposure to all measured concentrations of zinc dramatically decreased the production of dry biomass and plant growth (Du et al., 2016). Though susceptibility varies by species, similar toxic reactions have been observed in rice (*Oryza sativa* L.), tomato (*Solanum lycopersicon*), and bean (*Phaseolus vulgaris*).

In most cases, Zn stress was associated with alterations in chlorophyll and carotenoid contents, impaired photosynthetic performance, and the induction of oxidative stress markers. According to these results, Zn^{2+} ions that are liberated from their corresponding compounds exhibit preferential toxicity in plants by interfering with important physiological and biochemical functions (Ghoto et al., 2022; García-Gómez et al., 2017).

Disturbances in the water and nutrient transport routes within plants have been identified as the reasons behind zinc phytotoxicity (Lin and Xing, 2007). Due to the redox activity of heavy metals, one of the most well-known mechanisms of toxicity is the increased creation of ROS (Du et al., 2016). ROS can be harmful to lipids, proteins, nucleic acids, and organelles. Examples of highly reactive ROS include superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and hydroxyl radicals ($OH^{\bullet}$). Experimental studies confirmed that Zn can penetrate plant tissues, stimulate the intracellular production of ROS, and thus induce oxidative stress, leading to cellular imbalances and growth inhibition (Repka et al., 2016; Ahmed et al., 2017).

In addition to offering insight into the interactions between plants, soil, and microbes under stress, root exudates are a significant physiological response of plants to their rhizosphere environment. The root tips, which are distinguished by a high metabolic activity and undifferentiated meristematic cells that facilitate the diffusion of metabolites into the surrounding soil, are the primary source of these chemicals (Puhalsky et al., 2023). Most root exudates are composed of low molecular weight metabolites (LMWM), which include organic acids, sugars, phenols, amino acids, and specialized compounds like phytosiderophores. When exposed to heavy metals, plants frequently rearrange their metabolic systems to release more organic acids and amino acids to promote growth and reduce stress (Feng et al., 2021).

Corn is an important cereal crop that is grown on various types of soil around the world; however, soils contaminated with heavy metals (HM) pose serious threats to its growth and productivity (Junli et al., 2016). Even though zinc toxicity has been extensively documented in several crop species, little is known about how excessive zinc specifically affects root exudate activity, especially in maize. The current work examined the physiological and exudation reactions of maize seedlings to excessive zinc stress in hydroponic settings to close this gap. Hydroponic culture was selected to provide a uniform growth medium and to eliminate confounding effects of soil components, thereby allowing precise evaluation of Zn-induced changes. The study's particular goals were to: (i) Assess how zinc stress affects the growth and biomass of maize seedlings. (ii) Evaluate how zinc affects the production of ROS, malondialdehyde (MDA) levels, and chlorophyll content. (iii) Identify the modifications in root exudation patterns, paying special attention to organic acids with low molecular weights.

Materials and Methods

Plant Type, Growth Environment, and Zinc Treatments

The Hubei Academy of Agricultural Sciences in China provided the maize (*Zea mays.* L) seeds. The seeds were surface sterilized with 70% ethanol for 5 minutes, then 10% (v/v) H_2O_2 for 15 minutes, and finally washed with distilled water. Following a 24-hour soak in distilled water, the sterilized seeds were allowed to germinate on damp filter paper in Petri dishes at 28°C in a dark environment. After three days, homogeneous seedlings with radicle lengths of about 2 cm were chosen and placed on black Styrofoam sheets above plastic boxes (top: 19.5 × 13 cm; bottom: 17.5 × 11 cm) with half-strength Hoagland nutritional solution (Hoagland and Arnon, 1950). In a controlled growing chamber with a temperature range of 25–27°C, 50–70% relative humidity, and a light/dark photoperiod of 12/12 hours with a light intensity of 800–1000 µmol m^{-2} s^{-1}, each treatment box held 15 seedlings. The seedlings were placed in full-strength Hoagland solution after three days of growing, and they were subjected to Zn treatment using $ZnCl_2$ at four different concentrations: 0 (control, CK), 100 (Zn1), 200 (Zn2), and 500 (Zn3) mg L^{-1}, respectively. Treatments were applied for 7 days under non-aerated hydroponic conditions. Nutrient solutions were renewed every 3 days. Each treatment was replicated three times. The Zn concentrations that were applied were chosen based on Wei et al. (2007) and correspond to normal levels of Zn-contaminated rice seedlings growth (Šimon et al., 2021).

Assessment of Seedling Growth and Biomass

After being under zinc treatment for seven days, maize seedlings were chosen at random to evaluate the growth. The roots of the seedlings were properly cleaned under flowing distal water after they were carefully plucked. Fifteen seedlings from each treatment were used for the measurements of fresh weights, dry weights, and root and shoot lengths. Fresh and dry weights (g) were measured with an electronic balance (Model BS 223S, Sartorius, Germany), and root and shoot lengths (cm) were measured with a hand scale. Dry biomass was created by oven-drying samples for 72 hours at 65°C. Per plant, all values were reported (Ghoto et al., 2022).

Photosynthetic Pigments Determination

Using the Ghoto et al. (2023) approach, 0.3 g of freshly cut maize seedling leaves were dissolved in 10 mL of 80% acetone to extract the photosynthetic pigments. After 48 hours of sporadic shaking in the dark, the samples were vortexed and centrifuged for 10 minutes at 12,000 rpm. A UV–VIS spectrophotometer (Varian Cary 50, Varian, Palo Alto, USA) was used to measure the absorbance of the supernatant at 663, 645, and 470 nm. Arnon's equations (Arnon, 1949) were used to determine the concentrations of pigments content.

$$Chlorophyll\ a = [12.7\,(OD_{663}) - 2.69\,(OD_{645})] \times \left(\frac{v}{1000} \times\ wt.\,(g)\right)$$

$$Chlorophyll\ b = [22.9\,(OD_{645}) - 4.68\,(OD_{663})] \times \left(\frac{v}{1000} \times\ wt.\,(g)\right)$$

$$Total\ chlorophyll = [20.02\,(OD_{645}) + 9.02\,(OD_{663})] \times \left(\frac{v}{1000} \times\ wt.\,(g)\right)$$

$$Caratenoids = [4.7\,(OD_{645}) - 0.27\,(Chla + Chlb)] \times \left(\frac{v}{1000} \times\ wt.\,(g)\right)$$

The value of content was stated as mg/g fresh weight.

Assessment of Lipid Peroxidation

The method of Ghoto et al. (2020) was used to quantify lipid peroxidation in terms of thiobarbituric acid reactive substances (TBARS), with minor adjustments made by Ghoto et al. (2023). After sample combining 0.2 g of root and 0.2 g leaf separately was homogenized in 1 mL of phosphate buffer saline (pH 7.8) at ice-cold temperatures. After centrifuging the homogenate for 15 minutes at 4°C at 10,000 g, 0.4 mL of the supernatant was combined with 0.65 mL of 0.5% thiobarbituric acid (TBA) made in 20% trichloroacetic acid (TCA). After 20 minutes of incubation at 95°C in a water bath, the reaction mixture was cooled to room temperature. The absorption of the supernatant was measured at 532 nm, 600 nm, and 450 nm after centrifugation at 10,000 g for 15 minutes at 4°C (Ghoto et al., 2023). The corresponding extinction coefficient was used to determine the amount of malondialdehyde (MDA).

$$MDA\ (\mu\,M) = 06.45(OD532 - OD600) - 0.56OD450$$

Measurement of Hydrogen Peroxide and Superoxide Radicals

According to Ghoto et al. (2023), hydrogen peroxide (H_2O_2) was analyzed using spectrophotometry. Samples of roots and leaves (0.2 g) were homogenized in 1 ml of 5% TCA and then centrifuged for 15 minutes at 12000 rpm and 4°C. The mixture of the reactive solution contains 0.6 ml of supernatant, 0.5 ml of potassium phosphate buffer (10 mM, pH 7.0), 0.4 ml of 5% TCA, and 0.5 ml of potassium iodide (KI) at 1 M, respectively. After incubation in the dark for one hour, the absorption was measured at 390 nm, and the concentration of H_2O_2 was determined using a standard curve made with known quantities of H_2O_2 (mother solution at 10 µM).

The content of superoxide radicals ($O_2^{\bullet-}$) was determined with a slight modification as described by Ghoto et al. (2023). After combining 0.2 g of the sample from roots and leaves with 1 ml of phosphate buffer (50 mM, pH 7.8) in a mortar and pestle in an ice bath, the samples were centrifuged for 15 minutes at 12,000 rpm and 4°C. The 0.25 ml supernatant was combined with 1 ml of 7 mM α-naphthylamine and 1 ml of 17 mM p-aminobenzene sulfonic acid after reacting for an hour with 0.75 ml of 1 mM hydroxylamine hydrochloride. The reaction mixture was kept at 25°C for 20 minutes, and a spectrophotometer was used to measure the sample's optical density at 530 nm. In place of the supernatant, $NaNO_2$ was utilized for the standard curve.

Collecting root exudate and measuring pH

Maize seedlings of uniform growth were carefully taken out of the hydroponic system after being exposed to zinc for seven days in full-strength Hoagland solution. After carefully washing the roots for two minutes under running tap water to get rid of any remaining ions and nutrient solution, they were briefly cleaned for one minute with sterile distilled water to reduce contamination. Four seedlings from each of the three replicates were moved to 50 mL sterilized plastic vials (9.5 cm in diameter by 11.5 cm in height) that held 40 mL of distilled water that allowed the seedlings' whole root system to be submerged. Vials were covered with aluminum foil to keep roots dark and prevent light-induced changes in exudation. They were then incubated in a growth chamber at 25–26°C, humidity 50–70%, under a 12-hour light/12-hour dark photoperiod, with light intensity 800–1000 µmol m^{-2} s^{-1} for 24 hours (Valentinuzzi et al., 2015; Ghoto et al., 2023). Following the specified time passe, the roots were meticulously extracted and rinsed with 10 mL more deionized water to guarantee 100% recovery of root exudates, yielding a final volume of 50 mL. The pH of the gathered root exudates was determined at room temperature using a pH meter (ORION 3 Star, USA). A rotating evaporator kept at 40°C was used to condense the collected solution to around 2 mL (Ghoto et al., 2023). Following their passage by 0.22 µm sterile syringe filters (Minisart, Sartorius, Göttingen, Germany), the concentrated exudates (including organic acids) were placed in a 1.5 mL red glass vial and kept at -20°C until they could be further examined using high-performance liquid chromatography (HPLC).

Analysis and Measurement of Organic Acids in Root Exudates

The analysis of the composition and concentration of organic acids in the collected root exudates was performed using HPLC (Shimadzu LC-2010AHT, Kyoto, Japan), equipped with a solid ion exclusion column (Thermo Scientific ODS Hypersil, 250 × 4.6 mm). The mobile phase consisted of solution A, adjusted to a pH of 2.4 using 25 mM KH_2PO_4, as well as phase B, which was methanol. An aliquot of 20 µL of each sample was injected at a flow rate of 1.0 mL/min, and detection occurred at a wavelength of 210 nm over a period of 10 minutes. The identification of organic acids was carried out by comparing the retention times of the samples with those of eight standards that were used to create calibration curves. According to Ghoto et al. (2020), the retention times for the following acids were as follows: fumaric acid (6.6 min), citric acid (5.7 min), acetic acid (4.6 min), lactic acid (4.3 min), malic acid (3.7 min), formic acid (3.3 min), tartaric acid (3.0 min), and oxalic acid (2.6 min).

Statistical analysis

Version 9 of GraphPad Prism and Microsoft Excel 2021 were used to analyze the data. The mean ± standard error (SE) for each concentration was calculated using three biological replicates ($n = 3$). The statistical significance between the treatment groups was assessed using Dunnett's multiple comparison test to evaluate the differences compared to the control.

Results

Zinc-Induced Modulation of Maize Seedlings Growth and Biomass Accumulation

As determined by the fresh and dry weight, as well as the lengths of the roots and above-ground parts, different concentrations of zinc have had a varying effect on the growth and biomass of maize seedlings (Ghoto et al., 2022). The length of maize seedling roots showed a significant increase of 19.8% under treatment Zn1 compared to the control (CK). However, root length decreased by 11.0% and 8.7% under Zn2 and Zn3 treatments, respectively (Fig. 1A). Similar trends were seen in shoot length, which rose 38.3% at Zn1 but decreased 19.0% and 25.4% at Zn2 and Zn3, respectively, in comparison to the control (Fig. 1B). Root fresh weight exhibited a slight but significant increase of 0.52% under the Zn1 treatment compared to the control (CK). In contrast, it decreased by 0.25% and 0.16% under Zn2 and Zn3 treatments, individually (Fig. 1C). In comparison to the control (CK), the fresh weight of the shoots rose by 2.54% with the Zn1 treatment, whereas it decreased by 1.1% and 0.8% under Zn2 and Zn3 treatments, respectively (Fig. 1D). Comparing the Zn1 treatment to the control, the root dry weight rose by 0.03%, but under the Zn2 and Zn3 treatments, it fell by 0.1% (Fig. 16). Similarly, shoot dry weight increased by 0.14% under Zn1 treatment, but decreased by 0.8% and 0.6% under Zn2 and Zn3 treatments, respectively (Fig. 1F). Moderate zinc supplementation (Zn1) significantly promoted maize seedlings growth and biomass accumulation, while higher zinc concentrations (Zn2 and Zn3) inhibited these parameters compared to the control.

Zinc-Mediated Alterations in Chlorophyll and Carotenoids

As an essential pigment for photosynthesis, chlorophyll's content is frequently used to determine whether a plant is stressed or poisonous (Ma et al., 2015). The current study examined the effects of zinc on the composition of photosynthetic pigments by measuring the last two leaves of maize seedlings on the seventh day of exposure to zinc stress (Fig. 2A–F). As shown in Fig. 2A, chlorophyll *a* content increased by 2.3% under Zn1 treatment compared to the control (CK), whereas it decreased by 1.9% and 1.1% under Zn2 and Zn3 treatments, respectively. In comparison to the control (CK), the chlorophyll *b* concentration rose by 1.05% with Zn1 treatment and fell by 0.8% and 0.4% under Zn2 and Zn3 treatments, respectively, as illustrated in Fig. 2B. As shown in Fig. 2C, total chlorophyll content increased by 2% under Zn1 treatment, whereas it decreased by 1.6% and 1.3% under Zn2 and Zn3 treatments, respectively, compared with the control (CK). As shown in Fig. 2D, carotenoid content increased by 0.15% under Zn1 treatment, while it decreased by 0.08% and 0.06% under Zn2 and Zn3 treatments, respectively, compared with the control (CK). In comparison to the control (CK), the chlorophyll *a*/*b* ratio rose by 3.7% with Zn1 treatment and fell by 2.8% and 2.9% under Zn2 and Zn3 treatments, respectively, as illustrated in Fig. 2E. As shown in Fig. 2F, the chlorophyll/carotenoid ratio increased by 17.9% and 20.8% under Zn1 and Zn2 treatments, respectively, whereas it decreased by 9.7% under Zn_3 treatment compared with the control (CK). Overall, moderate Zn supplementation (Zn1)

improved photosynthetic pigments, while higher levels (Zn2–Zn3) suppressed them, suggesting a threshold beyond which Zn becomes detrimental to photosynthetic efficiency.

Alterations in Lipid Peroxidation and ROS under Zinc Stress

The amount of malondialdehyde (MDA) in maize seedlings treated with varying concentrations of zinc was used to indicate membrane lipid peroxidation (Fig. 3A, B). In roots, MDA levels significantly increased by 1.9% and 2.8% under Zn2 and Zn3 treatments, respectively, whereas no significant change was observed at Zn1 compared with the control (CK) (Fig. 3A). In contrast, leaf MDA content decreased significantly by 2.7% under Zn1 treatment, with no significant changes at Zn2 and Zn3 relative to CK (Fig. 3B).

Among ROS, H_2O_2 and $O_2^{\bullet-}$ were measured in maize seedlings after zinc treatments (Fig. 3C–F). When associated with the control (CK), the H_2O_2 levels in roots significantly dropped with Zn1, Zn2, and Zn3 treatments by 40%, 113%, and 127%, respectively (Fig. 3C). In leaves, a significant reduction in H_2O_2 was observed only under Zn1 (Fig. 3D). For $O_2^{\bullet-}$, root levels declined slightly (2.6%) under Zn1, with no significant changes at Zn2 and Zn3 (Fig. 3E). By contrast, leaf $O_2^{\bullet-}$ levels increased by 2.4% and 5.5% under Zn2 and Zn3, respectively, relative to CK (Fig. 3F). Moderate Zn (Zn1) alleviated oxidative damage, while excess Zn (Zn2–Zn3) induced ROS production.

Effects of Zn on Maize Seedlings Root Exudates and Rhizosphere pH

Root exudates from maize seedlings exposed to Zn were analyzed using HPLC. Six organic acids like oxalic, fumaric, formic, lactic, malic, and citric, were identified and quantified based on their retention times and peak areas. Figures 4–5 indicate the amounts of various organic acids in the root exudates.

The pH of exudation reduced by 5%, 4.4%, 4% at Zn1, Zn2, Zn3, respectively, due to changes in the exudate compositions in comparison to the CK (Fig. 4A). While in root exudations, a moderate Zn (Zn1) enhanced the exudation of several organic acids, whereas higher Zn (Zn2–Zn3) suppressed most of them. These changes, along with the consistent decline of oxalic acid and slight increase of fumaric acid, contributed to a reduction in root exudate pH. Oxalic acid declined significantly across all Zn treatments, with reductions of 1109%, 658%, and 158% at Zn1, Zn2, and Zn3, respectively (Fig. 4B). Opposite to oxalic acid a slight increase of 1.1%, 1.4%, and 1.5% in fumaric acid was observed after Zn1, Zn2, and Zn3 treatments, respectively (Fig. 4C).

Comparing Zn1 and Zn3 to the control (CK), formic acid exudation rose by 123.2% and decreased by 42.8%, respectively (Fig. 5A). Lactic acid showed the most pronounced response, rising by 8697.4% at Zn1 and then decreasing by 3224.4% at Zn3 (Fig. 5B). Malic acid increased by 54% under Zn1 but decreased by 16% under Zn3 relative to CK (Fig. 5C). Citric acid content was also enhanced, showing a 58% increase under Zn1 (Fig. 5D).

Discussion

Effect of Zn Concentration on Maize Seedlings Biomass

Zinc (Zn) toxicity is becoming more widely recognized as a serious environmental problem, mostly because of industrialization and human activity that cause the accumulation in soils and in water (Małecki et al., 2016). Although zinc is the second most abundant transition metal and a necessary micronutrient for normal metabolism and plant growth at concentrations below 5 μg g^{-1} (Broadley et al., 2007; Ait et al., 2004), excessive zinc uptake can cause cellular and structural disruptions that lower crop productivity (Ghoto et al., 2022; Šimon et al., 2021). Excessive Zn-induced stunted growth, chlorosis, impaired root development, and reduced membrane polarization (Rizvi and Khan, 2018; Kaya et al., 2018). Moreover, high zinc levels frequently result in nutrient imbalances by preventing the uptake or translocation of vital elements like iron, manganese, copper, and phosphorus (Kaya et al., 2000; Wang et al., 2009), which eventually lowers the biomass of plants (Liu et al., 2016; Šimon et al., 2021).

In the current study, maize seedlings were affected by zinc stress in two different ways. In comparison to the control, shoot and root biomass were moderately stimulated at lower Zn levels (Zn1), indicating a possible growth-promoting function of Zn when administered in trace amounts. Similar stimulatory effects of Zn have been reported previously, where mild Zn stress activated defense mechanisms that maintained plant integrity against environmental stressors (Liu et al., 2014). Plant development may be aided by zinc ions, which may function as activators of enzymes involved in cytokinin metabolism (Nyitrai et al., 2003). Nevertheless, maize seedlings showed notable decreases ($P < 0.05$) in biomass and pigments contents at higher concentrations (Zn2 and Zn3), along with obvious signs of phytotoxicity. These findings confirm that Zn toxicity is concentration and exposure-dependent, with prolonged exposure severely inhibiting plant survival in hydroponic (Šimon et al., 2021). Collectively, our results confirm that while low concentrations of Zn may exert a stimulatory effect, excessive Zn accumulation in the growth medium is highly toxic to maize, resulting in substantial reductions in growth, biomass, and physiological functions.

Zinc Toxicity on Chlorophyll and Photosynthetic Efficiency in Maize Seedlings

Chlorophyll directly aids in the movement of electrons and the absorption of light, reduced photosynthetic efficiency and prevented plant growth are the outcomes of any change in its stability or content (Ghoto et al., 2022; Šimon et al., 2021). Under heavy metal stress, particularly the toxicity of zinc (Zn), the metabolism of chlorophyll is closely linked to antioxidant defense, hormonal regulation, and detoxification pathways, which are essential for the survival of plants (Wang et al., 2022). In this study, maize seedlings exposed to different concentrations of Zn showed a concentration-dependent response in various plant pigments after 7 days of exposure. A moderate supply of Zn (Zn1 and Zn2) has promoted the accumulation of pigments compared to the control, indicating an adaptation mechanism that may protect the photosynthetic apparatus. In contrast, exposure to excessive Zn (Zn3) has led to a

significant decrease in pigment content, indicating severe phytotoxicity caused by excessive Zn (Fig. 2). This biphasic response is consistent with previous findings that moderate Zn can stimulate the biosynthesis of chlorophyll, while toxic levels promote the breakdown of pigments (Zhou et al., 2018; Cui & Zhao, 2011).

The reduction in photosynthetic pigments under high Zn stress is associated with several biochemical and physiological disruptions. High quantities of zinc can destabilize pigment-protein complexes and decrease the activity of enzymes involved in the biosynthesis of chlorophyll by substituting Zn^{2+} or other heavy metals for the key Mg^{2+} ion (Manios et al., 2003; Ding et al., 2006; Uruç Parlak, 2016). Additionally, zinc toxicity upsets the balance of nutrients. For example, toxic Zn reduces the amount of magnesium that was transferred to shoots but increased its accumulation in maize roots, and Mn concentrations dropped in both roots and shoots, indicating nutritional imbalance and competitive inhibition (Šimon et al., 2021). Mn depletion probably reduces photosynthetic activity and root barrier protection against Zn uptake since it is essential for photosystem II water-splitting and the development of Fe plaque on root surfaces (Liu et al., 2010). Moreover, Zn stress alters photosynthetic membrane proteins, accelerating chlorophyll degradation and further diminishing photosynthetic performance (Chen et al., 2017). Hence, understanding the precise mechanisms of Zn uptake, distribution, and detoxification within various plant species is essential for developing targeted biotechnological and agronomic strategies to optimize crop performance in diverse environmental contexts (Šimon et al., 2021).

Malondialdehyde (MDA) Accumulation as an Indicator of Zinc-Induced Oxidative Stress

It is commonly acknowledged that malondialdehyde (MDA), a byproduct of membrane lipid peroxidation, is a trustworthy indicator of oxidative stress and plant stress tolerance (Šimon et al., 2021). In the current investigation, maize seedlings exposed to high quantities of zinc (Zn) showed a marked rise in MDA levels, especially in the roots, which confirmed increased membrane instability and lipid peroxidation under Zn stress (Fig. 3A-B). Strong proof of Zn-induced oxidative damage was shown by the 3–5% rise in MDA content in both roots and leaves at the highest Zn concentration (Zn3) as compared to the control (Fig. 3A-B). Comparable responses have been observed in maize roots under Zn toxicity (Cui & Zhao, 2011) and in the microalga *Spirulina platensis-S5* (Kocaman, 2023), where MDA accumulation correlated positively with antioxidant enzyme activities under metal ion stress. Such findings reinforce the notion that MDA is a central indicator of Zn-induced cellular injury and oxidative imbalance in plants.

Interestingly, organ-specific variations were observed in maize seedlings. MDA levels at lower Zn exposure (Zn1) did not differ substantially from the control at the roots, suggesting that maize roots effectively tolerated moderate Zn stress through efficient detoxification and antioxidant defense mechanisms (Fig. 3A). However, at Zn2 and Zn3, MDA levels rose significantly in roots while remaining comparatively stable in leaves, indicating that oxidative stress is initiated primarily at the root level (Fig. 3A-B). This heightened sensitivity of roots is attributed to their direct contact with Zn-rich hydroponic solution, resulting in localized Zn accumulation, membrane disruption, and an excess of ROS.

In contrast, leaves displayed relative tolerance, likely due to systemic detoxification and compartmentalization strategies. These organ-specific differences highlight roots as the initial and primary target of Zn phytotoxicity (Gupta et al., 2011).

Mechanistically, the increased MDA accumulation reflects Zn-mediated destabilization of the plasma membrane, which serves as the first physiological barrier to metal entry. An excess of Zn ions interacts with the lipids and proteins of the membrane, causing lipid peroxidation, an ion imbalance, and a deterioration of membrane reliability (Liptáková et al., 2013). Concurrently, Zn stress stimulates ROS overproduction, particularly hydrogen peroxide (H_2O_2), which exacerbates oxidative injury by disrupting lipid bilayers, altering protein conformation, and damaging DNA bases (Pitzschke et al., 2006). These biochemical alterations are further coupled with reduced chlorophyll content and impaired antioxidant enzyme regulation, providing strong evidence that Zn toxicity accelerates premature senescence in maize (Cui & Zhao, 2011). Collectively emphasizing the integration of physiological, biochemical, and organ-specific reactions, the MDA not only serves as a biomarker but also as a functional indicator to evaluate Zn-induced oxidative stress and the dynamics of lipid peroxidation.

Effect of Zn on H_2O_2 and $O_2^{\bullet-}$

In the case of Zn toxicity, where hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\bullet-}$) are important indicators of oxidative imbalance, ROS are among the first biochemical markers of metal-induced stress (Ghoto et al., 2023). The results of our study showed that maize seedlings exposed to high concentrations of Zn had significantly higher levels of H_2O_2 and $O_2^{\bullet-}$ than the control forms (Fig. 3C-F). In general, plants maintain a fragile balance between the production and retention of ROS; however, disturbances caused by Zn disrupt this balance, leading to cellular oxidative stress. As postulated by Shao et al. (2008) and Chen et al. (2017), the reduction in the activities of antioxidant enzymes documented in our results suggests an inadequacy of maize seedlings to fully detoxify the excessive burden of ROS, exacerbating oxidative damage. Such results are modulated by various factors, including the plant's genotype, the duration of Zn exposure, and the concentration, but the general mechanism remains consistent in research: Zn-related stress induces uncontrolled ROS production, undermining cellular integrity (Rao & Sresty, 2000; Morina et al., 2010; Kaya et al., 2018).

Due to its considerable diffusibility and comparatively lengthy half-life, hydrogen peroxide (H_2O_2) is a particularly dangerous ROS in tissues under zinc stress. It not only oxidizes biomolecules but also traverses membranes, disseminating oxidative signals beyond the site of its production (Kocaman, 2023). Our findings indicated that the H_2O_2 concentration in Zn-stressed maize shoots except in Zn1 was elevated relative to control levels, whereas roots exhibited a decline following a 7-day exposure period (Fig. 3C-D), corroborating the findings of Islam et al. (2014). This tissue-specific distribution suggests that photosynthetic tissues are more susceptible to Zn-induced ROS than subterranean parts, potentially attributable to the higher metabolic rates observed in leaves. Furthermore, it has been demonstrated that Zn interferes with photosynthesis by substituting magnesium (Mg^{2+}) in chlorophyll, leading to impaired

pigment synthesis and restricting nutrient uptake in roots, thereby exacerbating oxidative imbalance (Yang et al., 2011).

As shown by elevated MDA concentrations under Zn stress, lipid peroxidation is also directly linked to the excessive formation of H_2O_2 and $O_2^{\bullet-}$ (Fig. 3). According to Chaoui et al. (1997) and Pitzschke et al. (2006), excessive ROS easily activate lipoxygenases and encourage the peroxidation of unsaturated membrane lipids, destabilizing membranes and increasing ion leakage. This assertion was substantiated in our experiment through the observed increase in electrolyte leakage, a marker indicative of compromised membrane integrity. A comparable trend has been documented in maize, and rice, under conditions of heavy metal stress, where elevated H_2O_2 levels correlate with increased lipid peroxidation and metabolic injury (Liu et al., 2015; Šimon et al., 2021). In maize, the discernible consequences of ROS-mediated membrane disruption encompassed premature leaf senescence, diminished water retention, and impaired enzymatic activities, which collectively restrict photosynthetic efficiency and growth potential.

Organic Acids Exudation and TCA Cycle Modulation under Zinc Stress

Root exudation is influenced by both environmental and developmental factors. While hydroponic systems help isolate zinc effects, they lack soil buffering and microbial mediation. Our findings show root exudation is influenced by zinc dosage and inhibited at higher levels, aligning with energy allocation trade-offs (Montiel-Rozas et al., 2016). Root exudation is central to plant–environment interactions, particularly under metal stress. In this study, maize seedlings exposed to Zn stress exhibited a 4–5% reduction in exudate pH (Fig. 4A), reflecting rhizosphere acidification driven by proton release and LMWOA secretion. At the root–soil interface, this acidification, which has previously been observed in maize and wheat, improves zinc solubility, promotes chelation, and alters microbial and nutrient dynamics (Israr et al., 2016; Ghoto et al., 2022).

After seven days of exposure to Zn, six primary organic acids were detected in the secretions of maize seedlings roots using high-performance liquid chromatography (HPLC): oxalic, fumaric, formic, lactic, malic, and citric acids (Figures. 4 and 5). Their secretion patterns demonstrated strong dose-dependent changes, reflecting Zn-induced reprogramming of carbon metabolism. As observed with maize seedlings, oxalic acid (Fig. 4B) showed a drastic ten-fold decline under Zn stress from CK to 1108.8%, 658.1%, 158.2% in Zn1, Zn2, Zn3, respectively, suggesting diversion of carbon away from oxalate biosynthesis. This reduction contrasts with its well-documented role in Al and Cd tolerance, where enhanced oxalate secretion immobilizes metals (Yang et al., 2020; Qin et al., 2021). With divergence under zinc stress, fumaric acid, a direct tricarboxylic acid (TCA) intermediate, only slightly increased (1.1–1.5%) across treatments (Fig. 4C), most likely due to changed carbon flux rather than direct detoxification (Sun et al., 2020). In line with findings in wheat and rice under heavy metal stress, formic acid (Fig. 5A) secretion rose by 123.2% significantly at Zn1 but decreased by 42.8% at Zn3 as compare with their control. This suggests an early role in metal chelation and redox homeostasis followed by

metabolic suppression at higher Zn levels (Shen et al., 2015). Lactic acid progressively rises at 8697.4% at Zn1 and decreased to 3224.4% at Zn3 (Fig. 5B) as liken to their control, reflecting reduced glycolytic overflow into lactate fermentation, a phenomenon also observed in maize and barley under Cd toxicity (Wu et al., 2015). Malic acid (Fig. 5C) rose by 54% at Zn1 but declined by 15.6% at Zn3 relative to the control, aligning with its established role in Zn and Cd detoxification through rhizosphere chelation and transport, as noted in *Thlaspi caerulescens* and wheat (Kochian et al., 2015; Luo et al., 2014). Similarly, citric acid increased by 58% at Zn1 (Fig. 5D), consistent with its strong chelating capacity to form Zn–citrate complexes that lower Zn bioavailability, corroborating results in maize and hyperaccumulator species (Tu et al., 2020; Dresler et al., 2014).

These metabolite shifts are closely tied to TCA cycle modulation. Citrate, malate, and fumarate are direct TCA intermediates, while oxalate derives from oxaloacetate metabolism, and formate arises from one-carbon fluxes linked to TCA intermediates (Igamberdiev and Eprintsev, 2016). Lactate, although not a classical TCA product, indicates pyruvate overflow under stress-induced anaerobic metabolism (Loreti et al., 2016). Moderate Zn exposure (Zn1) enhanced the exudation of formate, malate and citrate, implying upregulated TCA activity that supports metal chelation and redox balance (Das et al., 2016; Sharma et al., 2016). Conversely, a high Zn concentration (Zn3) inhibited the production of oxalate and lactate, reflecting the inhibition of flow in the TCA flux and glycolytic pathways (Cuypers et al., 2016), which exceeded the plant's metabolic capacity for detoxification (Chiang et al., 2006).

Overall, these results reveal a biphasic strategy in which maize seedlings roots increase the secretion of organic acids at moderate Zn levels to facilitate detoxification but undergo metabolic inhibition at high Zn exposure. This threshold-dependent regulation of metabolites derived from the TCA cycle reflects the responses observed under stress from Cd and Al (Komárková et al., 2022; Qin et al., 2021) and emphasizes the centrality of organic acid metabolism in mediating plant adaptation to heavy metal toxicity.

The detoxification function of organic acids is due to their carboxyl groups, which form stable complexes with Zn^{2+} ions, reducing the activity of free ions and the translocation of Zn to susceptible tissues (Osmolovskaya et al., 2018). The results of our survey regarding oxalic acid and citric acid align with those of Qin et al. (2021), who linked these acids to the availability of Cd in maize and soybean systems. Citrates, with three carboxyl groups, exhibit a strong affinity for multiple cations, whereas oxalate forms insoluble precipitates. This is consistent with research demonstrating the role of oxalate and malate in aluminum tolerance in corn and wheat (Kochian, 1995) and the detoxification of Cd in mangrove and legume species (Bao et al., 2011). Our results support the idea that the exudation of oxalic and citric acid represents a conserved cross-detoxification mechanism with specific modulation by Zn.

Figure 6 *The simplified schematic representation, on the left (Zn1), a maize root is shown with the label "Enhanced exudation". Below the root, colored circles represent the organic acids secreted at moderate Zn levels: formic, lactic, malic, and citric acids. This indicates that moderate Zn stimulates the TCA cycle and enhances organic acid secretion for detoxification. In the center, a simplified TCA cycle is drawn,*

highlighting citrate, malate, and fumarate as key intermediates. This connects root metabolism with exudation. On the right (Zn3), another maize root is shown with the label "Inhibited exudation". Only oxalic acid is represented (gray circle), indicating a strong suppression of this metabolite under high Zn stress. At the bottom, a red arrow runs from Zn1 to Zn3, symbolizing the shift from enhanced to inhibited organic acid secretion as Zn concentration increases. In short, the diagram captures the biphasic pattern: moderate Zn boosts citrate, malate, formate, and lactate exudation, whereas high Zn suppresses oxalate and overall exudation.

Conclusion

The negative effects of excess zinc on maize seedlings are emphasized in this study, which also highlights how plants respond to zinc-induced oxidative stress. High exposure to zinc reduced vegetative biomass and chlorophyll content, while causing oxidative damage, as evidenced by the increased accumulation of ROS and MDA. These disturbances led to membrane destabilization and reduced photosynthetic efficiency, threatening the growth and dynamics of the seedlings. In response, maize seedlings released root exudates containing organic acids such as formic acid, lactic acid, malic acid, citric acid, oxalic acid, and fumaric acid in the rhizosphere. These acids are important for detoxification, chelation of zinc, and nutrient availability. This response, combining physiological adjustments and rhizospheric modifications via root exudates, reflects a coordinated tolerance mechanism that enables maize to mitigate Zn toxicity. These findings deepen our understanding of Zn plant interactions and provide a foundation for improving crop resilience under zinc-contaminated conditions. Our results establish a framework linking Zn toxicity, oxidative stress, and root exudation as an integrated plant defense strategy.

Declarations

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Ethical approval. There are neither humans nor animals involved in this effort.

Consent to participate This is not applicable

Consent for publication This is not applicable

Competing interest. Ghoto K and Zheng HL declare that we have no competing interest of interest.

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Figures

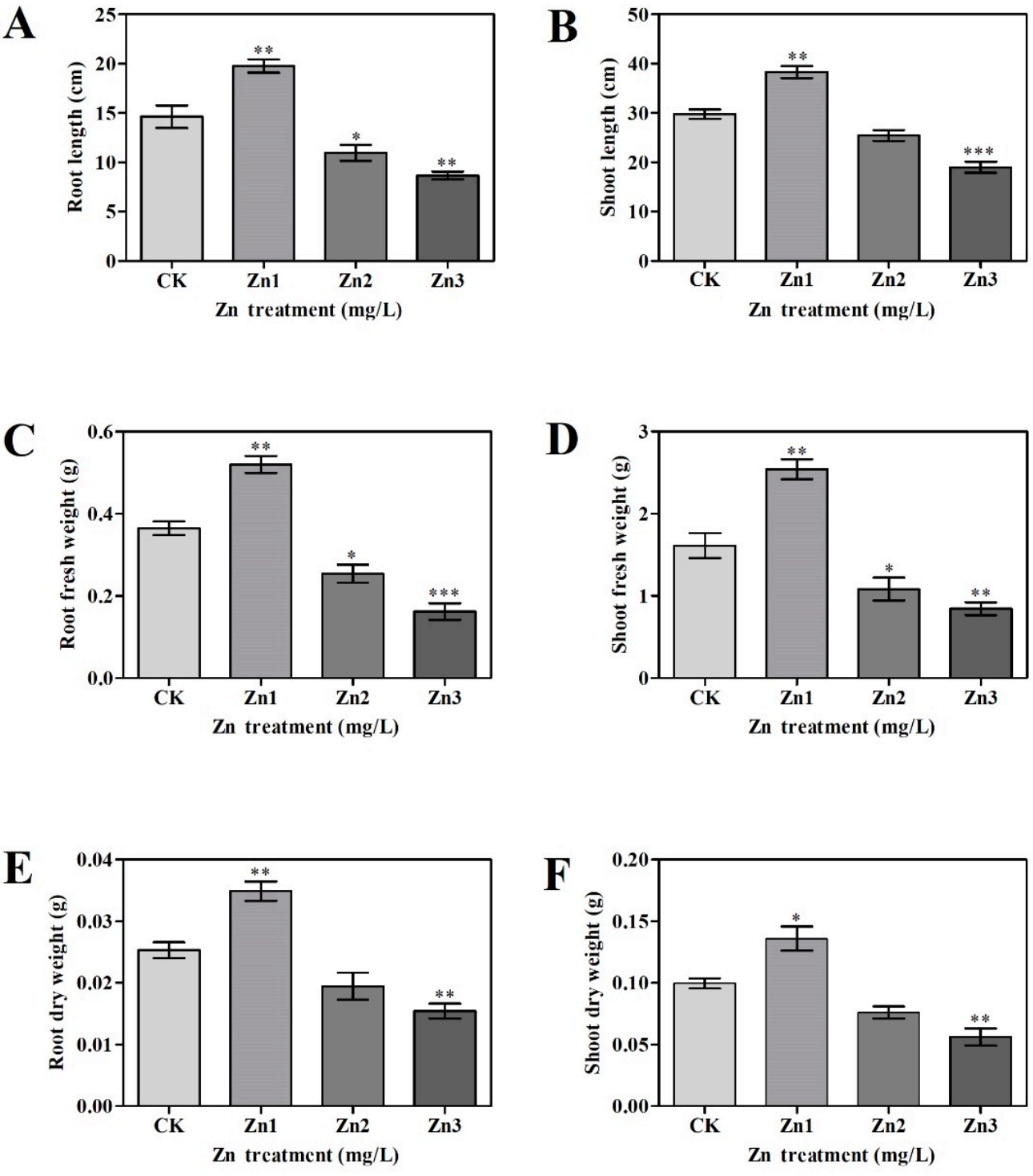


Figure 1

Effects of Zn on the growth of maize seedlings. (A) Root length, (B) Shoot length, (C) Fresh weight of roots, (D) Fresh weight of shoots, (E) Dry weight of roots, and (F) Dry weight of shoots. The data are presented as mean ± SE of three independent replications (*n* = 15). *, **, and *** marked on the columns indicate significant differences between the control (CK) and the treatments at a given concentration of Zn in a hydroponic medium at levels of 5%, 1%, and 0.1%, respectively, according to Dunnett's multiple comparison test.

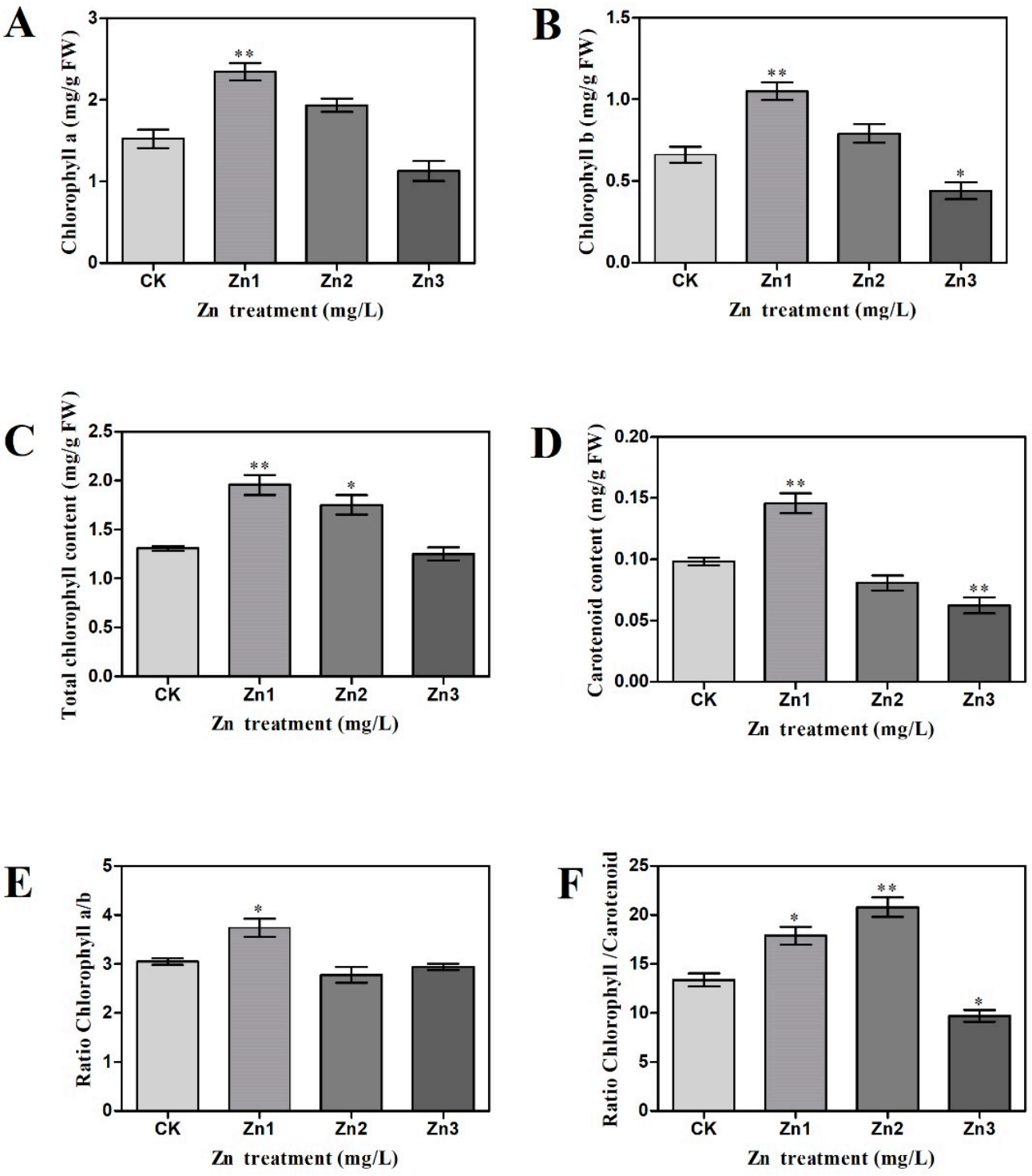


Figure 2

Effects of Zn on the chlorophyll contents of maize seedlings. (A) Chlorophyll *a* content, (B) Chlorophyll *b* content, (C) Total chlorophyll content, (D) Carotenoid content, (E) Chlorophyll *a*/*b* ratio, and (F) Chlorophyll/carotenoid ratio. The data are presented as mean ± SE of three independent replications (*n* = 3). *, ** and *** indicated on the bars denote significant differences between CK and the treatments with a given Zn concentration in a hydroponic medium at levels of 5%, 1%, and 0.1%, respectively, according to the Dunnett multiple comparison test.

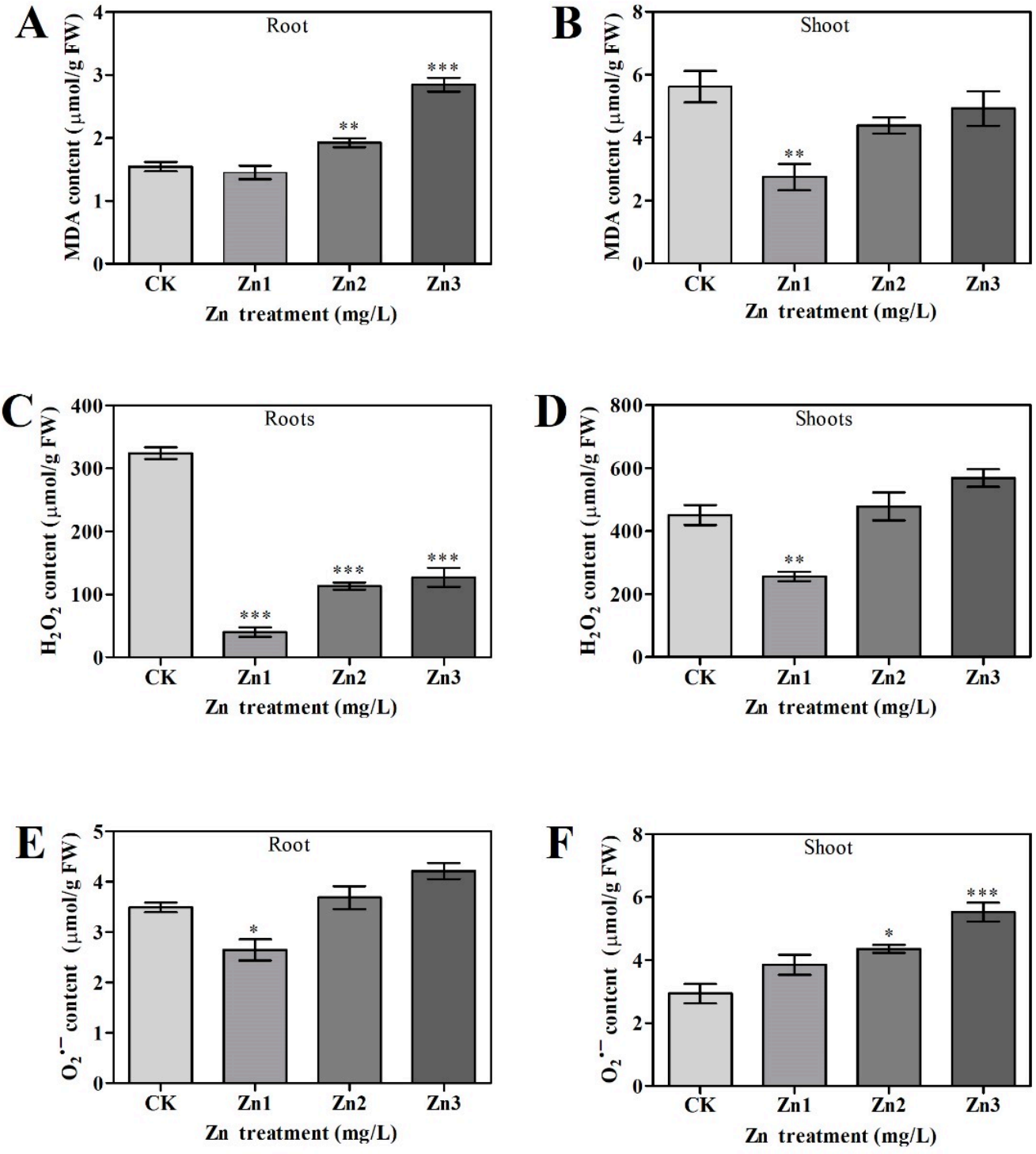


Figure 3

Effects of Zn on the lipid peroxidation and reactive oxygen species (ROS) contents in maize seedlings. (A) MDA content in the roots, (B) MDA content in the leaves, (C) H_2O_2 content in the roots, (D) H_2O_2 content in the leaves, (E) $O_2^{\bullet-}$ content in the roots, and (F) $O_2^{\bullet-}$ content in the leaves. *, **, and *** indicated on the columns denote significant differences between CK and the treatments with a given Zn concentration in a hydroponic medium at levels of 5%, 1%, and 0.1%, respectively, according to the Dunnett multiple comparison test.

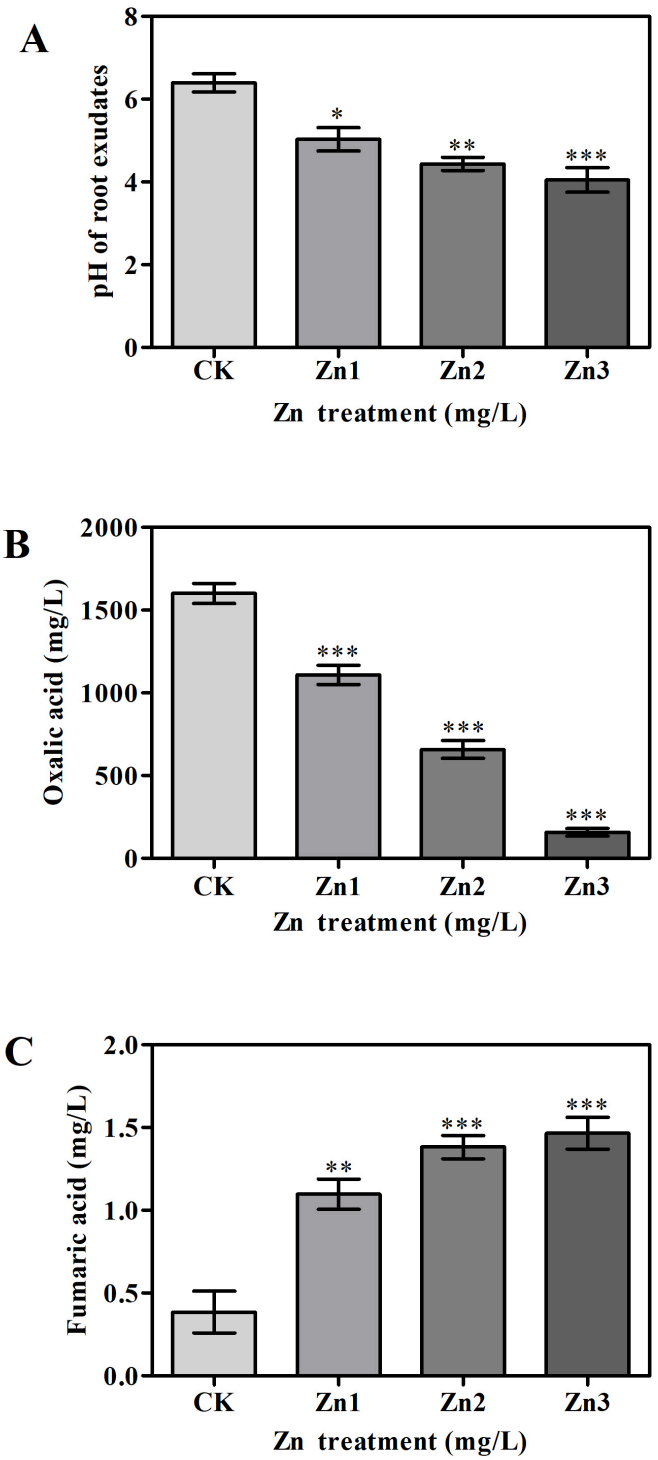


Figure 4

Effects of Zn on the pH value and the contents of organic acids in the root exudates of maize seedlings. (A) pH, (B), Oxalic acid and (C) Fumaric acid. The data are means ± SE of three repetitions (*n* = 3). *, **, and *** indicated on the pillars show significant differences between the CK and the treatments with a given Zn concentration in a hydroponic medium at levels of 5%, 1%, and 0.1%, respectively, according to the Dunnett multiple comparison test.

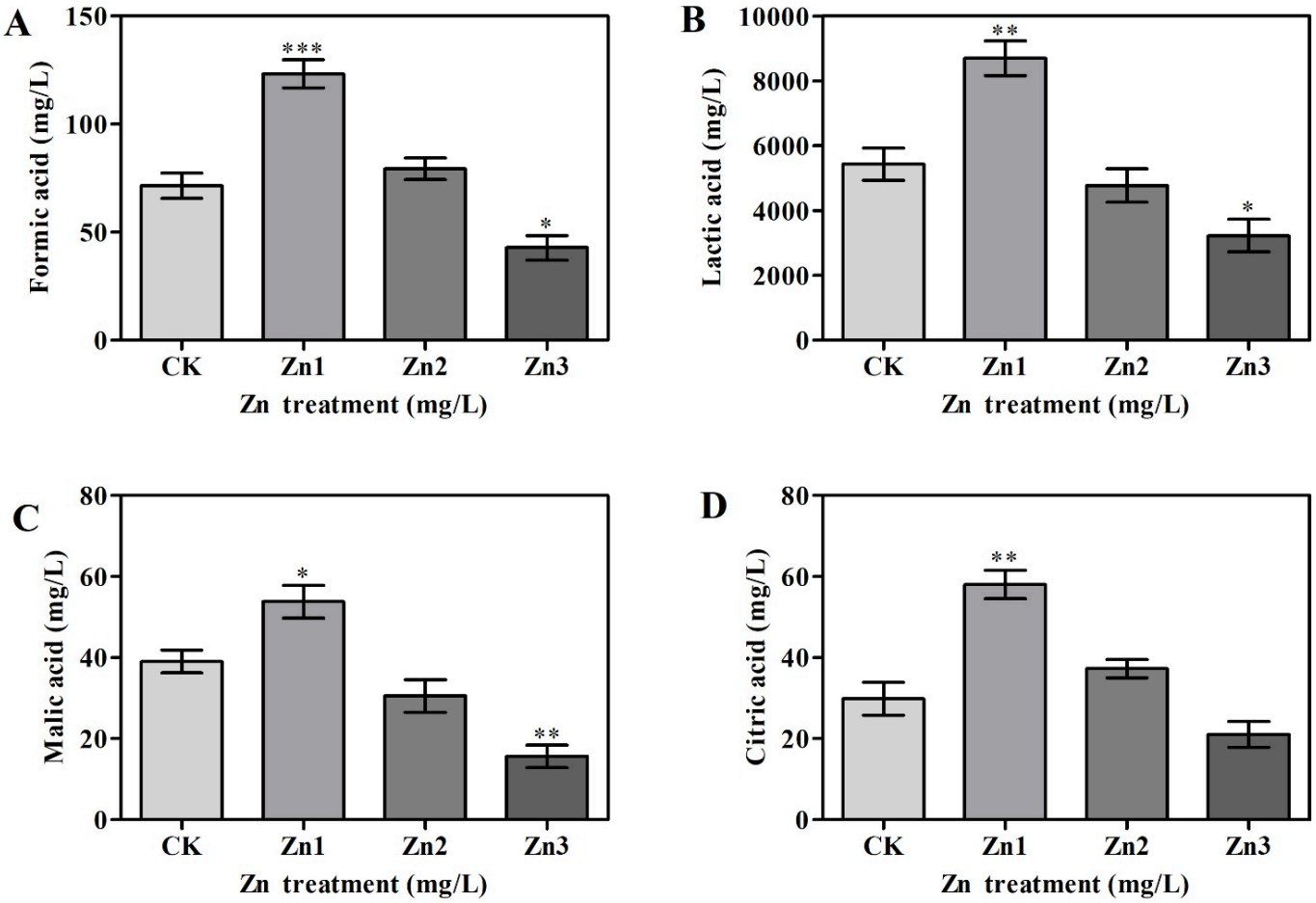


Figure 5

Effects of Zn on the contents of organic acids in the root exudates of maize seedlings. (A) Formic acid, (B) Lactic acid, (C) Malic acid, and (D) Citric acid. The data are means ± SE derived from three replicates (n = 3). *, **, and *** marked on the pillars indicate significant differences between CK and the treatments with a given concentration of Zn in a hydroponic medium at levels of 5%, 1%, and 0.1%, respectively, according to Dunnett's multiple comparison test.

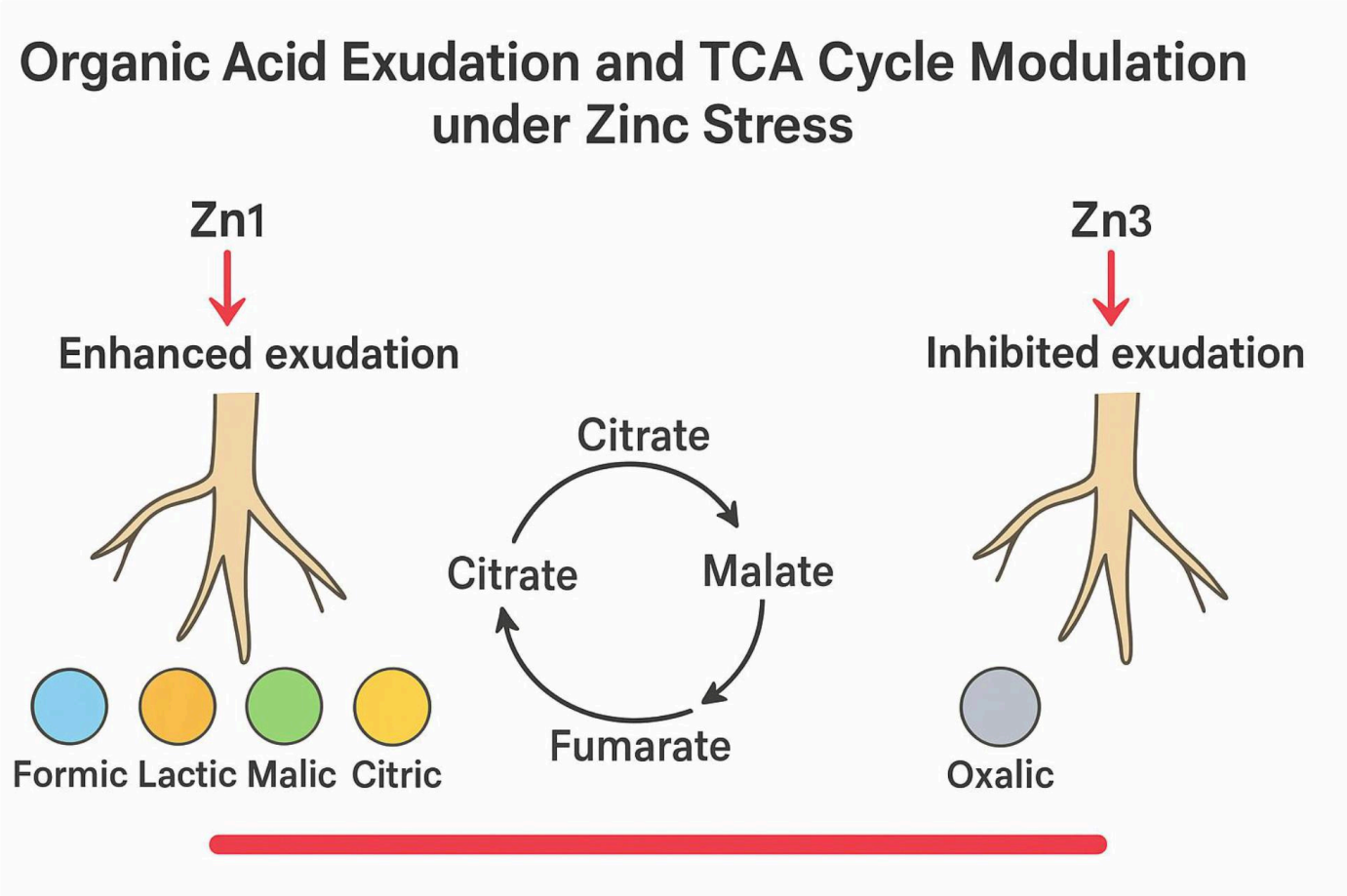


Figure 6

Organic acids exudation and TCA cycle modulation under zinc stress.

Supplementary Files

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- GraphicalAbstract.png