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Unraveling Zinc Toxicity Thresholds in Soil and Leaves of Field-grown Soybean and Their Impacts on the Antioxidant Defense System, Nutritional Status, and Physiological Performance

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Abstract

Information regarding zinc (Zn) toxicity thresholds in soil and leaves of soybean grown under corrective Zn fertilization in tropical clayey Oxisols remains scarce under field conditions. This study aimed to determine critical Zn toxicity levels in soil and the diagnostic leaf and to evaluate their effects on the nutritional status, physiological performance, antioxidant defense system, and grain yield of soybean. The experiment evaluated the residual effect of Zn rates (0, 12, 24, 48, and 96 kg ha^{-1}) applied and incorporated into the soil in a previous maize crop, using a randomized complete block design with four replications. Zinc application increased Zn availability in the soil and Zn concentration in the diagnostic leaf without affecting the nutritional status of other nutrients. Excessive Zn availability induced oxidative stress and only partially activated antioxidant defense mechanisms, as evidenced by increases in superoxide dismutase activity and ascorbic acid concentration, whereas carotenoids, phenolic compounds, flavonoids, and anthocyanins showed no significant contribution to stress mitigation. Consequently, high Zn levels reduced photosystem II efficiency, net photosynthetic rate, sucrose concentration, and grain yield. The critical Zn toxicity levels were 13 mg dm^{-3} in soil and 76 mg kg^{-1} in the diagnostic leaf. The negative effects of Zn toxicity on soybean were more closely associated with oxidative stress and insufficient antioxidant protection than with nutritional imbalances or disturbances in amino acid metabolism. These findings provide the first field-based critical Zn toxicity thresholds for soybean cultivated in a tropical clayey Oxisol and establish important criteria for the safe management of corrective Zn fertilization in tropical agricultural systems.

INTRODUCTION

Zinc (Zn) deficiency is one of the major nutritional constraints to global agricultural production, particularly in tropical regions characterized by highly weathered soils, such as Oxisols [1], which exhibit high acidity, low organic matter content, and a high capacity to adsorb Zn onto iron and aluminum oxides [2,3]. Under these conditions, Zn availability may be insufficient to meet crop demand, thereby limiting agricultural productivity, especially in sensitive crops such as soybean [4], due to the essential role of Zn in enzyme activation, antioxidant defense, and gas exchange processes [5]. Therefore, soil Zn fertilization, particularly through broadcast application followed by incorporation, represents an effective strategy because of its residual effect resulting from the gradual release of Zn over successive cropping cycles. This residual effect allows Zn to remain available for subsequent crops, such as soybean, thereby contributing to improved crop productivity [6,7].

In corrective fertilization systems, Zn rates applied to the soil, particularly through broadcast application followed by incorporation, tend to be higher due to the strong adsorption of this micronutrient onto soil colloids, a process that is further intensified in tropical soils after liming. Increases in clay content and soil pH enhance Zn retention by iron and aluminum oxides in Oxisols, thereby reducing its availability to plants [8]. Under these conditions, clayey Oxisols impose greater challenges for Zn management, requiring strategies that ensure an adequate supply of the micronutrient while preventing toxic effects. Excessive Zn availability may disrupt nutritional homeostasis and reduce both photosystem II efficiency and CO_2 assimilation [9].

Although previous studies have evaluated the immediate effects of Zn application on crops such as maize [10,11], information regarding its residual effects in crop succession systems remains limited, particularly for soybean, one of the most important agricultural crops in tropical regions. Critical Zn toxicity levels in soil and plants are still poorly understood for soybean under field conditions. Existing studies have mainly been conducted under greenhouse or pot conditions [1,12], where evaluations are restricted to early plant growth stages and root development is constrained, which does not adequately represent actual field conditions. This knowledge gap hampers the interpretation of soil and leaf analyses, as well as the development of more efficient and safer Zn fertilization recommendations for soybean production systems.

For soybean, the adequate Zn concentration range in the diagnostic leaf for crops grown under tropical field conditions is 20–50 mg kg^{-1} [13], and this range has remained unchanged for more than 25 years [14], highlighting the limited advancement of research on Zn nutrition in this crop. This knowledge gap is even more pronounced regarding Zn toxicity thresholds, for which field-based studies are still lacking. Existing information is restricted to reports suggesting that leaf Zn concentrations close to 72 mg kg^{-1} may be considered excessive for soybean; however, these values were not established through experimental validation under field conditions [15].

There is evidence that excessive Zn concentrations in plants can induce oxidative stress and physiological disturbances that impair the photosynthetic apparatus [5]. However, little is known about the antioxidant defense responses and nutritional homeostasis of soybean grown under field conditions. Studies conducted under pot conditions indicate that excessive Zn may cause antagonistic interactions with other nutrients, particularly Fe[16], leading to important nutritional deficiencies. Nevertheless, under field conditions in Oxisols, which naturally contain high Fe concentrations, and where plants develop a more extensive root system, these adverse effects may be attenuated. The lack of such information, together with the absence of well-established critical Zn toxicity levels in soil and plants, hinders the interpretation of soil and leaf analyses, as well as the development of safer and more efficient Zn fertilization recommendations.

Therefore, we hypothesized that: (i) residual Zn in the soil resulting from corrective fertilization may alter Zn uptake, the acquisition of other nutrients, and the physiological performance and grain yield of soybean grown in succession; and (ii) elevated Zn concentrations in soil and leaves may impair physiological processes, particularly the photosynthetic rate, primarily due to oxidative stress associated with an insufficient antioxidant defense system rather than nutritional imbalances involving nutrients other than Zn. To elucidate soybean responses to Zn toxicity under field conditions and establish critical toxicity thresholds in soil and plants, this study was conducted to evaluate the effects of Zn application incorporated into a clayey Oxisol on the nutritional status, physiological and biochemical parameters, and grain yield of soybean.

Understanding the response mechanisms of soybean under Zn toxicity in field conditions will be valuable for elucidating how this crop copes with oxidative stress and its impacts on plant development, contributing to the development of future strategies aimed at increasing tolerance to Zn toxicity and improving the sustainability of production systems. Furthermore, establishing critical Zn toxicity thresholds will enable more accurate interpretation of soil and leaf analyses, supporting more reliable diagnoses for soybean and guiding safer and more efficient Zn management practices.

RESULTS

Soil Zn application promoted a linear increase in the available Zn concentration in the soil ($Y = 0.1372x + 1.518$; $R^2 = 0.98$; $F = 60.51$; $p < 0.01$), indicating an increase of 0.1372 mg dm^{-3} of soil Zn for each kg ha^{-1} of Zn applied.

Soil Zn application did not affect the accumulation of N, P, K, Ca, Mg, B, Mn, or Cu in the soybean shoot. However, it promoted a quadratic increase in Zn accumulation and a linear decrease in Fe accumulation (Fig. 1).

Soil Zn application did not affect the leaf concentrations of N, P, K, Ca, Mg, B, Fe, Mn, or Cu in soybean; however, it promoted a linear increase in leaf Zn concentration (Fig. 2).

Grain Zn concentration increased linearly with increasing Zn application rates, whereas the concentrations of N, P, K, Ca, Mg, Fe, Mn, and Cu were not significantly affected by Zn fertilization (Fig. 3).

Zn application promoted important changes in the antioxidant metabolism and organic compound profile of soybean plants (Fig. 4). A linear increase was observed in malondialdehyde (MDA) content, superoxide dismutase (SOD) activity, and ascorbic acid concentration with increasing Zn application rates. Total phenolic compounds showed a quadratic response to Zn application, increasing up to a rate of 75 kg ha^{-1} Zn. In contrast, sucrose concentration exhibited a

decreasing quadratic response, indicating a progressive reduction with increasing Zn fertilization. On the other hand, total soluble sugars and total free amino acids were not significantly affected by Zn application rates and remained relatively constant across treatments.

Residual Zn fertilization promoted a linear reduction in the maximum quantum efficiency of photosystem II (Fv/Fm), without affecting the contents of pheophytin a, pheophytin b, carotenoids, flavonoid index, or anthocyanin index in soybean plants (Fig. 5).

Soil Zn application exerted a quadratic effect on net photosynthetic rate (A), with a maximum response at a Zn rate of 46 kg ha^{-1}, as well as on transpiration rate (E). In contrast, stomatal conductance (gs) and intercellular CO_2 concentration (Ci) were not affected by the applied Zn rates (Fig. 5).

The maximum estimated soybean grain yield was 3,666 kg ha^{-1}, achieved at a Zn application rate of 46 kg ha^{-1} (Fig. 6a), corresponding to a soil Zn concentration of 7.24 mg dm^{-3} (Fig. 6b) and a diagnostic leaf Zn concentration of 53 mg kg^{-1} (Fig. 6c). A 5% reduction in maximum grain yield, considered the critical Zn toxicity threshold, was observed at a Zn application rate of 78 kg ha^{-1} (Fig. 6a), corresponding to a soil Zn concentration of 13 mg dm^{-3} (Fig. 6b) and a diagnostic leaf Zn concentration of 76 mg kg^{-1} (Fig. 6c).

DISCUSSION

1. Effects of soil-Incorporated zinc on the soil–plant system, zinc interactions with other nutrients, nutritional status, and grain nutrient concentrations in soybean

The dynamics of zinc in soil are governed by simultaneous processes of adsorption, desorption, precipitation, and complexation, which are directly influenced by soil texture, mineralogy, and soil solution chemistry. In tropical soils such as the highly clayey Oxisol evaluated in this study, the predominance of crystalline Fe oxides (hematite and goethite), Al oxides (gibbsite), and 1:1 clay minerals such as kaolinite strongly influences Zn retention and availability. Despite the agronomic relevance of Zn fertilization in tropical production systems, studies evaluating the application of high Zn rates approaching 100 kg ha^{-1}, supplied as a soluble source and incorporated into the 0–20 cm soil layer, remain scarce. Consequently, limited information is available regarding the behavior and dynamics of Zn under these conditions, particularly in highly weathered tropical soils characterized by a high density of reactive mineral surfaces.

Under these conditions, soil Zn application promoted a linear increase in DTPA-extractable Zn concentrations even after two consecutive cropping seasons involving maize and soybean. The results showed that each kilogram of Zn applied increased soil-extractable Zn by 0.1372 mg dm^{-3}, corresponding to the slope of the regression equation. Consequently, the application of 96 kg ha^{-1} Zn increased DTPA-extractable Zn by 13.17 mg dm^{-3}, equivalent to 26.34 kg ha^{-1} of recovered Zn in the 0–20 cm soil layer. This corresponds to a DTPA recovery efficiency of approximately 27%, indicating that a substantial proportion of the applied Zn was converted into forms not readily extracted by this method. Comparable recovery efficiencies have been reported in previous studies using the DTPA extractant in soils with different physicochemical characteristics. Recovery values ranged from approximately 20% in tropical soils [17] to 31% in clayey Vertisols [18]. These results suggest that, despite the increase in extractable Zn, a considerable fraction of the applied micronutrient was retained by soil constituents through adsorption and fixation processes, particularly in highly weathered soils rich in Fe and Al oxides.

The relatively low Zn extraction efficiency in the soil, as well as the limited recovery of the element by plants, can be attributed to the high adsorption capacity of this micronutrient, particularly in highly clayey Oxisols, combined with a series of reactions that reduce its availability in the soil. Among the main mechanisms involved, the ability of Zn^{2+} to form inner-sphere complexes with surface hydroxyl groups (OH^-) present on Fe and Al oxides and 1:1 clay minerals is particularly important. These interactions establish covalent-type bonds that markedly reduce Zn activity in the soil

solution [1,19]. Furthermore, these processes favor the formation of low-lability compounds, such as $Zn(OH)_2$, $ZnCO_3$, and $Zn_3(PO_4)_2$, which are only sparingly available and are poorly recovered by the DTPA extractant [20].

This study provided an opportunity to elucidate the relationship between increasing soil Zn availability, Zn uptake, and its potential antagonistic interactions with other nutrients, thereby determining whether such effects are agronomically relevant to the nutritional status of soybean grown in a tropical Oxisol. The results showed that increasing soil Zn availability enhanced Zn accumulation in soybean plants while reducing Fe uptake (Fig. 1h). Similar responses have been reported in soybean grown under controlled conditions, where excessive Zn supply reduced Fe accumulation due to antagonistic interactions among metals and competition for transport systems involved in nutrient uptake [16,21–23]. In contrast, other studies have reported reductions in K, Ca, and Mg uptake under excessive Zn supply, which have been attributed to alterations in plasma membrane H^+-ATPase activity and consequent impairment of nutrient acquisition processes [24].

However, most previous studies investigating the effects of excess Zn on nutrient uptake were conducted under controlled conditions, making it difficult to determine whether these interactions are sufficiently severe to induce agronomically significant nutritional disorders under field conditions. The present study helps clarify this issue. Despite the substantial increase in plant Zn concentrations, no significant changes were observed in the leaf concentrations of other nutrients, with the exception of Zn itself. Therefore, although excess Zn reduced Fe accumulation, this effect was not sufficient to induce nutritional deficiency or compromise the overall nutritional status of soybean plants. These findings indicate that the negative effects associated with elevated Zn supply were not driven by nutrient imbalance, but rather by direct physiological and biochemical responses to Zn excess.

These findings reinforce the hypothesis that, in clayey tropical Oxisols, the high Zn adsorption capacity reduces the agronomic significance of antagonistic interactions between this micronutrient and other nutrients, even under conditions of elevated Zn availability in the soil. A substantial proportion of the applied Zn interacts with soil colloids, particularly Fe and Al oxides, decreasing its immediate availability and promoting its gradual release into the soil solution [25]. Furthermore, under field conditions, the greater development of the root system and the increased exploration of the soil volume contribute to maintaining nutrient uptake and nutritional homeostasis in plants [26,27]. Consequently, although Zn excess may alter the uptake dynamics of specific nutrients, these effects are often insufficient to induce nutritionally relevant deficiencies or substantially affect plant nutritional status in highly weathered tropical soils.

Another important aspect associated with Zn fertilization is its effect on grain nutritional quality, particularly in light of growing global concerns regarding Zn deficiency in human diets. Over recent decades, declines in the nutritional quality of staple crops have been reported worldwide, contributing to micronutrient deficiencies, especially Zn deficiency, which affects approximately 20% of the global population [28,29]. This scenario has stimulated considerable research efforts aimed at increasing Zn concentrations in edible plant products through agronomic biofortification strategies. In the present study, Zn fertilization increased grain Zn concentrations, and the optimum Zn rate for maximum grain yield (46 kg ha^{-1}) resulted in a grain Zn concentration of 35 mg kg^{-1}. This concentration is nutritionally relevant because agronomic biofortification programs generally target grain Zn concentrations ranging from 40 to 50 mg kg^{-1} to substantially improve dietary Zn intake and contribute to meeting human nutritional requirements [30].

However, information regarding grain Zn concentrations that may become detrimental to grain quality or pose risks to food safety remains limited. From a food safety perspective, even the highest Zn rate evaluated in this study (96 kg ha^{-1}), which resulted in a grain Zn concentration of 41 mg kg^{-1}, would provide approximately 8.2 mg of Zn through the consumption of 200 g of soybean grains, a quantity considerably higher than typical daily human consumption. This intake remains well below the Tolerable Upper Intake Level (UL) of 40 mg Zn day^{-1} established for adults [31]. These results indicate that, even under Zn fertilization rates sufficiently high to induce toxicity symptoms in soybean plants, grain Zn

concentrations remained within levels considered safe for human consumption. Furthermore, the increased Zn accumulation in the grains did not compromise the nutritional quality of the grain by reducing the concentrations of other essential nutrients. Overall, the findings demonstrate that soil Zn fertilization substantially enhances grain Zn concentration while maintaining adequate concentrations of other nutrients and preserving food safety standards for human consumption [28,32]. Therefore, under the conditions of this study, soil application alone was sufficient to improve grain Zn enrichment, with no apparent need for complementary foliar Zn applications.

2. Soybean responses to zinc toxicity: antioxidant defense mechanisms and physiological impacts

Most studies on Zn toxicity in soybean plants have been conducted under controlled conditions using pots with or without soil. These studies have shown that the major reductions in plant growth caused by excess Zn are mainly associated with nutritional effects resulting from antagonistic interactions with other nutrients [21,23,33], which, as demonstrated in the present study, have little agronomic relevance in field-grown soybean. In addition, few studies have evaluated the antioxidant responses of soybean plants [34] and gas exchange parameters [35] under Zn toxicity in field conditions. Under this cultivation system, the antioxidant mechanisms, both enzymatic and non-enzymatic, that are most responsive to Zn-induced stress in soybean plants remain poorly understood, since these responses are highly species-specific. A better understanding of these mechanisms may support future studies aimed at increasing soybean tolerance to environments with high Zn availability and other adverse conditions.

Zn application, particularly at the highest rates, promoted substantial Zn uptake by soybean plants and intensified oxidative stress, as evidenced by the increased malondialdehyde (MDA) concentrations (Fig. 4a), indicating enhanced lipid peroxidation and damage to cellular membranes. Excess Zn is known to stimulate the production of reactive oxygen species (ROS), leading to oxidative damage to cellular structures and metabolic impairment [9,36]. In response to this stress condition, plant species activate distinct antioxidant defense mechanisms involving both enzymatic and non-enzymatic components. Among antioxidant enzymes, superoxide dismutase (SOD) plays a central role in the cellular defense system against oxidative stress and is considered the first line of defense against reactive oxygen species (ROS). This metalloenzyme catalyzes the dismutation of the superoxide radical ($O_2^{\bullet -}$) into H_2O_2 and molecular O_2, thereby reducing intracellular oxidative potential [37]. In plants, different SOD isoforms operate in distinct cellular compartments, with Cu/Zn-SOD being particularly important for the detoxification of ROS generated during photosynthesis [38].

The results showed that oxidative stress induced by excess Zn in soybean activated the first enzymatic antioxidant defense line, as evidenced by the increased activity of superoxide dismutase (SOD) (Fig. 4b), which acts as a protective mechanism against the accumulation of reactive oxygen species (ROS). This response indicates that soybean plants were able to perceive the oxidative imbalance caused by excess Zn and activate antioxidant pathways aimed at limiting cellular damage. However, despite the increase in antioxidant activity, the elevated MDA concentrations observed in the plants indicate that these defense mechanisms were not sufficient to completely prevent oxidative damage. This finding suggests that ROS production exceeded the cellular detoxification capacity, resulting in membrane lipid peroxidation and oxidative injury even in the presence of enhanced SOD activity [39,40].

However, the non-enzymatic antioxidant defense system is often considered more effective in mitigating plant toxicity because it encompasses a wide range of antioxidant compounds capable of directly scavenging reactive oxygen species and maintaining cellular redox balance[4]. The findings of the present study suggest that soybean plants exhibit a relatively limited capacity to strengthen their non-enzymatic antioxidant defense system under Zn-induced stress, with the response being largely restricted to a single compound. This was evidenced by the fact that Zn stress increased only leaf ascorbic acid concentrations (Fig. 4c), whereas the other non-enzymatic antioxidants evaluated were not consistently stimulated. Ascorbic acid plays a key role in neutralizing reactive oxygen species and attenuating cellular damage [41]. Moreover, it is a central component of the ascorbate–glutathione cycle, contributing to ROS detoxification, cellular redox

homeostasis, and membrane protection under stress conditions [42], representing an important adaptive strategy to mitigate oxidative damage [43].

In contrast, although phenolic compounds increased at moderate Zn rates, their accumulation stabilized at toxic Zn levels (> 78 kg ha^{-1} Zn), suggesting a limited contribution of these metabolites under severe stress conditions. This finding is noteworthy because phenolic compounds are recognized as effective antioxidants involved in ROS scavenging and cellular protection [44,45]. Furthermore, Zn toxicity did not significantly affect the concentrations of other antioxidant compounds evaluated, including flavonoids and anthocyanins (Figs. 4d, 4e, and 4f). It is possible that under more severe toxicity conditions, the stabilization or lack of further accumulation of these secondary metabolites reflects metabolic constraints that limit the plant's ability to sustain high rates of antioxidant biosynthesis due to the physiological and energetic costs imposed by excess Zn. These results reinforce the hypothesis that, although soybean plants activate specific antioxidant defense mechanisms in response to Zn toxicity, their effectiveness becomes limited under severe stress conditions. Consequently, the persistence of oxidative damage, as evidenced by the increased MDA concentrations, suggests that the antioxidant response was insufficient to fully counteract ROS overproduction. From a physiological perspective, these findings indicate that increasing the capacity and diversity of antioxidant compound synthesis through breeding strategies or biotechnological approaches may represent a promising avenue for improving soybean tolerance to environments with excessive Zn availability.

The linear reduction in the maximum quantum efficiency of photosystem II (Fv/Fm) (Fig. 5a) observed at the highest Zn rates indicates impairment of the photochemical apparatus in soybean plants. Fv/Fm is widely recognized as one of the most sensitive indicators of photosystem II integrity, and reductions in this parameter are commonly associated with photoinhibition and oxidative damage to thylakoid membranes, resulting in impaired photosynthetic electron transport [46]. These findings further support the evidence that the antioxidant defense system of soybean plants exposed to Zn toxicity was only partially effective in mitigating oxidative stress. Consequently, the persistence of oxidative damage likely contributed to the reduction in net photosynthetic rate (A) (Fig. 5g), possibly through structural and functional damage to chloroplasts, affecting both photochemical and biochemical processes involved in photosynthesis [47,48]. In contrast, stomatal conductance (gs) (Fig. 5h), internal CO_2 concentration (Ci) (Fig. 5i), and transpiration rate (E) (Fig. 5j) were not significantly affected, even under elevated Zn concentrations. These results indicate that the reduction in photosynthetic performance was predominantly associated with biochemical and photochemical limitations of the photosynthetic apparatus rather than with stomatal restrictions. This demonstrates that the limitations observed in photosynthesis were more closely associated with biochemical and photochemical alterations of the photosynthetic apparatus than with stomatal restrictions [49,50].

The physiological alterations induced by excess Zn also affected primary metabolism, as evidenced by the reduction in sucrose concentrations (Fig. 4e). The decrease in sucrose observed in this study is directly related to the lower photosynthetic carbon assimilation, since sucrose is the main carbohydrate transported in plants and its synthesis depends on the supply of photoassimilates generated through photosynthesis [51]. Thus, the reduction in net photosynthetic rate (A) (Fig. 5g), resulting from photochemical impairment of photosystem II (Fig. 5a) and oxidative stress induced by excess Zn, reduced the availability of photoassimilates required for sucrose synthesis [52]. Simultaneously, the activation of defense mechanisms likely increased the metabolic demand for carbon and energy, thereby reducing the allocation of assimilates to sink organs [53]. As a consequence, the lower availability of sucrose may have contributed to the reduction in soybean yield (Fig. 7a), demonstrating that Zn toxicity compromises not only photosynthetic performance but also carbon metabolism and yield formation[39].

Overall, the results demonstrate that excess Zn triggers substantial metabolic reprogramming in soybean plants, characterized by the activation of both enzymatic and non-enzymatic antioxidant defense systems. Although these mechanisms contributed to mitigating oxidative stress, they were insufficient to fully prevent the physiological damage

caused by Zn toxicity. On the other hand, excess Zn did not compromise the nutritional homeostasis of the other nutrients evaluated, but significantly reduced photosynthetic performance and carbon assimilation. These findings reinforce the dual role of Zn in plant metabolism: essential at moderate concentrations, with optimal responses observed at approximately 46 kg ha^{-1} Zn, yet potentially toxic when present at excessive levels.

3. Zinc toxicity effects on soybean yield and the establishment of critical soil and leaf zinc levels

Grain yield represents the outcome of the balance between the beneficial effects of zinc at optimal concentrations, including enhanced enzymatic activity, membrane integrity, and photosynthetic efficiency, and the toxic effects associated with excessive Zn supply, which induces oxidative stress and reduces carbon assimilation [54]. Therefore, establishing critical Zn toxicity thresholds in soybean is essential for accurate nutritional diagnosis and for providing a scientific basis for corrective fertilization recommendations, thereby contributing to more sustainable crop production systems.

In the present study, the Zn rate of 46 kg ha^{-1} provided the maximum grain yield of 3,666 kg ha^{-1} and corresponded to optimum Zn concentrations of 8.2 mg dm^{-3} in the soil and 53 mg kg^{-1} in the leaves. However, there are data indicating that soil Zn concentrations (DTPA-extractable) above 1.2 mg dm^{-3} are considered high ([55], and that adequate leaf Zn concentrations range from 20 to 50 mg kg^{-1} [14], meaning that concentrations above these values would not be expected to result in soybean yield responses to Zn fertilization. This reinforces that these standards need to be updated for clayey tropical Oxisols.

Regarding the critical soil Zn toxicity level, grain yield reduction was associated with a soil Zn concentration of 13 mg dm^{-3}, representing, to our knowledge, the first field-based study to establish a Zn toxicity threshold for soybean under tropical conditions. Although previous studies have reported higher critical values, reaching up to 34 mg kg^{-1} of DTPA-extractable Zn in Oxisols [1], these investigations were conducted under controlled pot conditions and may not adequately represent the complexity of field-grown cropping systems [56,57]. Furthermore, the lower critical toxicity level observed in the present study may also be related to the high Zn adsorption capacity of the clayey Oxisol evaluated, which contains approximately 57% clay [58]. This finding highlights that critical Zn availability thresholds are strongly influenced by soil physicochemical properties, particularly texture, mineralogy, and the adsorption capacity of the soil for this micronutrient [59].

Another important finding of this study was the establishment of a critical leaf Zn toxicity level of 76 mg kg^{-1} for field-grown soybean. This value is higher than the leaf Zn concentrations currently considered adequate for soybean under field conditions, which range from 20 to 50 mg kg^{-1} [14] and from 48 to 63 mg kg^{-1} [60]. However, it remains lower than the concentrations reported as toxic for soybean plants grown under controlled conditions during the early stages of development, including values of 140 mg kg^{-1} [12] and 187 mg kg^{-1} [1] both determined using the shoot dry matter of plants cultivated in pots. Nevertheless, these toxicity thresholds obtained under pot conditions should be interpreted with caution, since factors such as cultivation environment, sampled plant tissue (leaf or whole shoot), and plant developmental stage can substantially influence the observed Zn concentrations. Therefore, such values cannot be directly extrapolated to field-grown soybean, a limitation that has been widely recognized in the literature [61].

Overall, this study provides the first field-based evidence of critical Zn toxicity thresholds in soybean, establishing values of 13 mg dm^{-3} of Zn in the soil and 76 mg kg^{-1} of Zn in the leaves for soybean cultivated in a clayey tropical Oxisol under a corrective fertilization system. The relative tolerance of soybean to these elevated Zn concentrations may be attributed to several factors. First, the low recovery efficiency of applied Zn by plants, resulting from the chemical properties of the Oxisol, combined with fertilizer incorporation into the 0–20 cm soil layer, likely enhanced Zn adsorption onto soil colloids, thereby reducing its availability to plants, as discussed previously. In addition, the observed effects occurred under residual Zn conditions, since soybean cultivation was preceded by a maize crop. This succession likely attenuated the

immediate effects of high Zn availability on soybean plants, reducing the intensity of toxicity symptoms compared with situations involving direct Zn application immediately before soybean cultivation. Another factor that may have contributed to the relatively high tolerance observed was the maintenance of adequate Fe nutritional status, even under elevated Zn concentrations in the soil. This finding is particularly relevant because adequate Fe supply has been reported to alleviate Zn toxicity in soybean by reducing the negative effects associated with Zn–Fe antagonism and maintaining essential physiological processes [21].

Overall, the identification of critical Zn toxicity levels provides valuable information for improving nutritional diagnosis and supporting a more rational management of this micronutrient in soybean production systems. The establishment of these thresholds may contribute to maximizing Zn use efficiency while reducing the risks of phytotoxicity in high-productivity tropical environments. Future research should focus on validating these critical levels under field conditions across a wider range of soil types, particularly considering the strong influence of soil Zn adsorption capacity on micronutrient availability. Such studies will help refine diagnostic criteria and expand the scientific basis for Zn management recommendations in tropical agricultural systems.

MATERIALS ANAD METHODS

a. Experimental Design

The experiment was conducted under field conditions at the experimental area of the School of Agricultural and Veterinary Sciences, São Paulo State University (UNESP/FCAV), located in Jaboticabal, São Paulo State, Brazil (21°15'3.13" S, 48°17'8.88" W; 546 m above sea level), during the 2024/2025 growing season, from November 17, 2024, to March 8, 2025, using the soybean cultivar TMG 7063 IPRO. The experiment was arranged in a randomized complete block design consisting of five Zn rates applied to the soil (0, 12, 24, 48, and 96 kg ha^{-1} Zn) with four replications. Each experimental plot consisted of nine soybean rows spaced 0.45 m apart and 18 m in length. The evaluation area comprised the five central rows, excluding 1 m from each end of the plot as border rows. To minimize the risk of contamination among adjacent plots, a 4 m buffer zone was maintained between experimental units.

b. Soil Preparation and Zn Treatment application

Increasing Zn rates were applied on December 18, 2023, as zinc sulfate monohydrate (33% Zn and 18% S), broadcast over the soil surface and subsequently incorporated into the 0–20 cm soil layer using a heavy disk harrow, followed by a leveling harrow. Sulfur levels were balanced among treatments to avoid confounding effects. Soil chemical and particle-size analyses were performed according to the methods described by [3] and [62], respectively, using soil samples collected from the 0–20 and 20–40 cm layers (Table 1). The soil was classified as a dystrophic Red Latosol according to the Brazilian Soil Classification System [63], corresponding to an Oxisol in the USDA Soil Taxonomy [64]. Initial soil Zn concentrations were classified as high (> 1.2 mg dm^{-3}, DTPA extraction) according to the interpretation criteria proposed for agricultural crops by [65] and [55].

Table 1
Chemical and particle-size analyses of the soil from the experimental area, Jaboticabal, São Paulo State, Brazil.

Depth	B	Cu	Fe	Mn	Zn	Clay	Silt	Sand
cm			mg dm^{-3}			------	%	-----
0–20	0,42	0,60	13	28,2	2,7	54	21	24
20–40	0,52	0,3	7	30,1	1,2	57	20	23

	pH	O.M.	P	S	Ca	Mg	Na	K	Al	H + Al	SB	CEC	V	m
	$CaCl_2$	g dm^{-3}	mg dm^{-3}		----------mmol$_c$ dm^{-3}----------									--%--
0–20	6,1	22	33	6	32	13	-	3,3	0	25	48,8	73,5	66	0
20–40	6,0	16	17	17	30	13	-	2,7	0	25	46,0	71,7	64	0

OM = organic matter; CEC = cation exchange capacity; V = base saturation; m = aluminum saturation; SB = sum of bases.

c. Climatic Conditions

The climate of the study area was classified as tropical with a dry winter (Aw) according to the Köppen climate classification system [66]. Rainfall data were recorded using a meteorological station installed at the experimental site, and a water balance was calculated throughout the soybean growing season. Total precipitation during the experimental period reached 663 mm (Fig. 7), which is within the range considered adequate for soybean cultivation, whose water requirement generally varies from 400 to 700 mm (EMBRAPA, 2011).

d. Nutritional Analysis of the Diagnstic Leaf, Wholw Plant, and Grains

Leaf sampling was performed at the soybean flowering stage by collecting fifteen leaves per plot. The third recently matured trifoliate leaf from the apex was sampled, following the recommendation of [68]. At full flowering (R2), fifteen whole plants were collected per plot, and grain samples were obtained at harvest. Whole-plant samples were decontaminated by washing with deionized water containing 0.1% neutral detergent, followed by immersion in a 0.3% HCl solution and a final rinse with deionized water. Subsequently, the plant material was dried in a forced-air oven at 65 ± 5°C until constant dry weight was achieved. The diagnostic leaf, whole-plant, and grain samples were then analyzed to determine the concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), copper (Cu), iron (Fe), and zinc (Zn), according to the methods described by [69].

e. Chlorophyll a Fluorescence ans Photosynthetic Pigments

The maximum quantum efficiency of photosystem II (Fv/Fm) was determined in the diagnostic leaf between 07:00 and 09:00 h using a portable chlorophyll fluorometer (OS30P+, Opti-Sciences Inc., USA), following the methodology described by [70]. Carotenoid and pheophytin contents were determined from leaf discs extracted with 80% acetone, and absorbance readings were obtained using a spectrophotometer according to the methodology proposed by [71].

f. Flavonoid and Anthocyanin Indices

Fifty-one days after sowing, flavonoid and anthocyanin indices were evaluated in the middle third of the third fully expanded mature leaf using a Multi-Pigment Meter (MPM-100, Opti-Sciences Inc., USA).

g. Total Phenolics

Total phenolic compounds were quantified using fresh leaf discs collected 51 days after sowing. Determination was performed through a colorimetric reaction using the Folin–Ciocalteu reagent and a 20% Na_2CO_3 solution, following the methodology described by [72]. Absorbance readings were obtained using a spectrophotometer (DU640 model, Beckman, USA) at 765 nm, and the results were expressed based on an appropriate standard curve.

h. Teor de Malondialde√≠do (MDA)

Sixty days after sowing, fresh leaf tissue samples (0.4 g) were immediately frozen in liquid nitrogen (− 80°C), ground in the presence of 20% (w/v) polyvinylpolypyrrolidone (PVPP) and 0.1% trichloroacetic acid (TCA) on ice, and subsequently centrifuged at 11,000 × g and 4°C for 10 min. The supernatant was mixed with 0.5% thiobarbituric acid (TBA) prepared in 20% TCA and incubated at 95°C for 30 min. The samples were then cooled on ice for 10 min and centrifuged again at 11,000 × g and 4°C for 10 min. Absorbance was measured at 535 and 600 nm, and malondialdehyde (MDA) content was calculated using the molar extinction coefficient ($\varepsilon = 1.55 \times 10^5\ M^{-1}\ cm^{-1}$) according to [73].

i. Superoxide Dismutase (SOD) Extraction and Activity Assay

Enzyme extraction from leaf and root tissues was performed according to the methodology of [74]. Samples were homogenized in a chilled mortar and pestle using an extraction buffer containing 100 mM potassium phosphate buffer (pH 7.5), 1 mM EDTA, 3 mM DL-dithiothreitol, and 5% (w/v) insoluble polyvinylpolypyrrolidone (PVPP). The buffer-to-tissue ratio was 3:1 (v/w) for leaves and fruits and 2:1 (v/w) for roots. The homogenate was centrifuged at 10,000 × g for 30 min, and the supernatant was stored at − 80°C for subsequent analysis of superoxide dismutase (SOD; EC 1.15.1.1) activity. Protein concentration was determined according to [74]using bovine serum albumin as the standard. Superoxide dismutase activity was assayed following the method of [75]. The reaction mixture consisted of 50 mM sodium phosphate buffer (pH 7.8), 50 mM methionine, 10 mM EDTA, 1 mM nitroblue tetrazolium chloride, and 0.1 mM riboflavin. The samples were incubated under fluorescent light at 25°C for 5 min. Absorbance was measured at 560 nm, and the results were expressed as U mg^{-1} protein.

j. Ascorbic Acid (AsA)

Ascorbic acid content was determined by titration with 0.02% 2,6-dichlorophenolindophenol following extraction with 0.5% oxalic acid, according to the methodology described by [76].

k. Leaf Gas Exchange

Leaf gas exchange measurements were performed on the diagnostic leaves at 60 days after sowing. Net photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), and intercellular CO_2 concentration (Ci) were measured using an open-system infrared gas analyzer (IRGA) (LCpro-SD, ADC BioScientific Ltd., UK). Measurements were conducted under a photosynthetic photon flux density (PPFD) of 1,500 μmol $m^{-2}\ s^{-1}$, a CO_2 concentration of 400 ppm, and a chamber temperature of 30°C. All measurements were taken between 09:00 and 11:00 h.

l. Grain Yield

Grain yield was determined by harvesting three central rows within the plot's usable area. Grain moisture content was measured, and grain yield was adjusted to a standard moisture content of 14% and expressed in kg ha^{-1}.

m. Nutrient Accumalation

Nutrient accumulation (kg ha^{-1}) was calculated by multiplying shoot dry matter yield (kg ha^{-1}) by nutrient concentration (g kg^{-1}) and dividing the result by 1,000.

n. Determination of Critical Thresholds

Critical zinc (Zn) toxicity thresholds in the soil, diagnostic leaf, and applied Zn rates were determined from response curves fitted using second-order polynomial regression models, according to the methodology described by [4]. Quadratic models were initially fitted to describe the relationships between: (i) Zn rates and grain yield; (ii) soil Zn concentration and grain yield; and (iii) Zn concentration in the diagnostic leaf and grain yield. Toxicity thresholds were defined as the Zn levels associated with a 5% reduction in relative yield after the maximum yield had been reached.

o. Statistical Analysis

Data were initially evaluated for compliance with the assumptions of normality and homogeneity of variance. Normality was assessed using the Shapiro–Wilk test ($p > 0.05$), while homogeneity of variances was verified using Levene's test ($p > 0.05$). Once these assumptions were met, the data were subjected to analysis of variance (ANOVA). For variables showing significant treatment effects ($p < 0.05$), polynomial regression models were fitted to evaluate the responses to Zn rates.

CONCLUSION

High Zn rates induced oxidative stress in soybean plants and only partially activated antioxidant defense mechanisms. Although increases in enzymatic antioxidant activity, represented by superoxide dismutase (SOD), and in ascorbic acid concentration were observed, these responses were insufficient to fully mitigate oxidative damage. Furthermore, other important antioxidant compounds, including phenolics, flavonoids, anthocyanins, and carotenoids, did not contribute significantly to the defense response under Zn toxicity. Consequently, excess Zn reduced photosystem II efficiency, net photosynthetic rate, sucrose concentration, and ultimately soybean grain yield.

The critical Zn toxicity levels established for soybean were 13 mg dm^{-3} of DTPA-extractable Zn in the soil and 76 mg kg^{-1} of Zn in the leaves. The results demonstrated that the reductions in photosynthetic efficiency and grain yield were primarily associated with oxidative stress and an insufficient antioxidant defense response rather than with nutritional antagonism or disturbances in amino acid metabolism.

This study provides the first criteria for interpreting Zn concentrations in soil and leaf tissues for the safe management of corrective Zn fertilization incorporated into tropical soils cultivated with soybean. In addition, it advances the understanding of the physiological mechanisms involved in soybean responses to Zn toxicity under field conditions.

DECLARATIONS

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Author Contribution

J.V.S.S. conducted the experiment, performed data collection, laboratory analyses, statistical analyses, data interpretation, and manuscript writing. R.M.P. contributed to manuscript writing and revision. A.M.S.R. assisted in conducting the experiment, laboratory analyses, and manuscript revision. C.N.S.C. assisted with the physiological analyses. M.F.C. assisted with laboratory analyses and data collection. All authors read, reviewed, and approved the final manuscript.

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Data Availability

Data AvailabilityThe datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

REFERENCES

1. Fageria, N. K. Níveis adequados e tóxicos de zinco na produção de arroz, feijão, milho e soja em solo de cerrado. *Revista Brasileira de Engenharia Agrícola e Ambiental* (2000).
2. Alloway, B. J. Soil factors associated with zinc deficiency in crops and humans. *Environ. Geochem. Health*. **31**, 537–548 (2009).
3. van Raij, B. Os métodos de análise química do sistema IAC de análise de solo no contexto nacional. in *Análise química para avaliação da fertilidade de solos tropicais* 5–39 (2001).
4. de Prado, R. *M. Mineral Nutrition of Tropical Plants* (Springer International Publishing, 2021). 10.1007/978-3-030-71262-4
5. Kaur, H. & Garg, N. Zinc toxicity in plants: a review. *Planta* **253**, 129 (2021).
6. Inocêncio, M. F. Resposta da soja à adubação com zinco em solo com teores acima do nível crítico. *Pesqui Agropecu Bras.* **47**, 1550–1554 (2012).
7. Leite, C. M., de Muraoka, C., Colzato, T. & Alleoni, L. R. M. F. Soil-applied Zn effect on soil fractions. *Sci Agric* **77**, (2020).
8. Abreu, C. A., Lopes, A. S. & Santos, G. Micronutrientes. in Fertilidade do solo (ed Novais, R. F.) 645–736 (Sociedade Brasileira de Ciência do Solo, Viçosa, (2007).
9. CAKMAK, I. Tansley Review No. 111. *New Phytologist* 146, 185–205 (2000).
10. Ritchey, K. D., Cox, F. R., Galrão, E. Z. & Yost, R. S. Zinc availability for corn, sorghum and soybean in a clayey dark-red latosol. *Pesqui Agropecu Bras.* **21**, 215–225 (1986).
11. Galrão, E. Z. Métodos de aplicação de zinco e avaliação de sua disponibilidade para o milho num Latossolo Vermelho-Escuro, argiloso, fase cerrado. *Rev. Bras. Cienc. Solo*. **20**, 283–289 (1996).
12. Borkert, C. M., Cox, F. R. & Tucker, M. R. Zinc and copper toxicity in peanut, soybean, rice, and corn in soil mixtures. *Commun. Soil. Sci. Plant. Anal.* **29**, 2991–3005 (1998).
13. Ambrosano, E. J. et al. Leguminosas e Oleaginosas. in Recomendações de adubação e calagem para o Estado de São Paulo (eds van Raij, B., Cantarella, H., Quaggio, J. A. & Furlani, A.) (1997). M. C.) 189–191 (IAC, Campinas.

14. Quaggio, J. A., Cantarella, H., Rosolem, C. A. & Crusciol, C. A. C. Soja (Glycine max). in Boletim Técnico 100 263–281 (Instituto Agronômico de Campinas (IAC), Campinas, (2022).
15. EMBRAPA. *Tecnologias de Produção de Soja - Região Central Do Brasil 2012 e 2013* (Embrapa Soja, 2011).
16. de Oliveira, N. T. et al. Iron counteracts zinc-induced toxicity in soybeans. *Plant Physiol. Biochem.* **194**, 335–344 (2023).
17. Oliveira, M. F. G., Novais, R. F., Neves, J. C. L., Vasconcellos, C. A. & Alves, V. M. C. Relação entre o zinco disponível, por diferentes extratores, e as frações de zinco em amostras de solos. *Rev. Bras. Cienc. Solo*. **23**, 827–836 (1999).
18. Barman, K. K., Ganeshamurthy, A. N. & Takkar, P. N. Zinc requirement of soybean (Glycine max) - wheat (Triticum aestivum) cropping sequence in some swell-shrink soils. *Indian J. Agricultural Sci.* **68**, 759–761 (1998).
19. Lindsay, W. L. *Chemical Equilibria in Soils* (Wiley, 1991).
20. Nascimento, C. W. A. & Fontes, R. L. F. Correlação entre características de Latossolos e parâmetros de equações de adsorção de cobre e zinco. *Rev. Bras. Cienc. Solo*. **28**, 965–971 (2004).
21. Fontes, R. L. F. & Cox, F. R. Iron deficiency and zinc toxicity in soybean grown in nutrient solution with different levels of sulfur. *J. Plant. Nutr.* **21**, 1715–1722 (1998).
22. Pawlowski, M. L., Helfenstein, J., Frossard, E. & Hartman, G. L. Boron and zinc deficiencies and toxicities and their interactions with other nutrients in soybean roots, leaves, and seeds. *J. Plant. Nutr.* **42**, 634–649 (2019).
23. Wallace, A., Romney, E. M. & Patel, P. M. Zinc induced iron deficiency in soybeans. *Commun. Soil. Sci. Plant. Anal.* **7**, 37–41 (1976).
24. Rout, G. R. & Das, P. Effect of Metal Toxicity on Plant Growth and Metabolism: I. Zinc. *Agronomie* **23**, 3–11 (2003).
25. Silveira, M. L., Alleoni, L. R. F., O'Connor, G. A. & Chang, A. C. Heavy metal sequential extraction methods—A modification for tropical soils. *Chemosphere* **64**, 1929–1938 (2006).
26. Fan, X., Zhou, X., Chen, H., Tang, M. & Xie, X. Cross-Talks Between Macro- and Micronutrient Uptake and Signaling in Plants. *Front Plant. Sci* **12**, (2021).
27. Shanmugam, V. et al. Differential expression and regulation of iron-regulated metal transporters in *Arabidopsis halleri* and *Arabidopsis thaliana* – the role in zinc tolerance. *New Phytol.* **190**, 125–137 (2011).
28. Cakmak, I. & Kutman, U. B. Agronomic biofortification of cereals with zinc: a review. *Eur. J. Soil. Sci.* **69**, 172–180 (2018).
29. Silva, A. B. P., Borges, L. F. S., Lucini, F., Silva, G. N. & Santos, E. F. Technologies in Agronomic Biofortification with Zinc in Brazil: A Review. *Plants* **14**, 1828 (2025).
30. Rehman, R., Latif, M. M., Khan, M. A. & Ahmed, Z. Agronomic Biofortification of Crops with Zinc: A Comprehensive Overview. in *Breeding Zinc Crops for Better Human Health* 153–184Springer Nature Switzerland, Cham, (2025). 10.1007/978-3-031-84342-6_8
31. (IOM), I. of M. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academies Press, Washington, DC, (2001).
32. Cakmak, I. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant. Soil.* **302**, 1–17 (2008).
33. Loneragan, J. F. & Webb, M. J. Interactions Between Zinc and Other Nutrients Affecting the Growth of Plants. in *Zinc in Soils and Plants* 119–134Springer Netherlands, Dordrecht, (1993). 10.1007/978-94-011-0878-2_9
34. Yusefi-Tanha, E., Fallah, S., Rostamnejadi, A. & Pokhrel, L. R. Zinc oxide nanoparticles (ZnONPs) as a novel nanofertilizer: Influence on seed yield and antioxidant defense system in soil grown soybean (Glycine max cv. Kowsar). *Sci. Total Environ.* **738**, 140240 (2020).
35. Andrejić, G., Gajić, G., Prica, M., Dželetović, Ž. & Rakić, T. Zinc accumulation, photosynthetic gas exchange, and chlorophyll a fluorescence in Zn-stressed Miscanthus × giganteus plants. *Photosynthetica* **56**, 1249–1258 (2018).

36. Sharma, P., Jha, A. B., Dubey, R. S. & Pessarakli, M. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *J. Bot.* 1–26 (2012). (2012).
37. Alscher, R. G. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J. Exp. Bot.* **53**, 1331–1341 (2002).
38. Ramana Gopavajhula, V. et al. Modeling and analysis of soybean (Glycine max. L) Cu/Zn, Mn and Fe superoxide dismutases. *Genet. Mol. Biol.* **36**, 225–236 (2013).
39. Broadley, M. R., White, P. J., Hammond, J. P., Zelko, I. & Lux, A. Zinc in plants. *New Phytol.* **173**, 677–702 (2007).
40. Hasanuzzaman, M. et al. Reactive Oxygen Species and Antioxidant Defense in Plants under Abiotic Stress: Revisiting the Crucial Role of a Universal Defense Regulator. *Antioxidants* **9**, 681 (2020).
41. Gill, S. S. & Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **48**, 909–930 (2010).
42. Pandey, S. et al. Abiotic Stress Tolerance in Plants: Myriad Roles of Ascorbate Peroxidase. *Front Plant. Sci* **8**, (2017).
43. Noreen, S. et al. Foliar fertigation of ascorbic acid and zinc improves growth, antioxidant enzyme activity and harvest index in barley (Hordeum vulgare L.) grown under salt stress. *Plant Physiol. Biochem.* **158**, 244–254 (2021).
44. Marreiro, D. et al. Zinc and Oxidative Stress: Current Mechanisms. *Antioxidants* **6**, 24 (2017).
45. Sharma, A. et al. Response of Phenylpropanoid Pathway and the Role of Polyphenols in Plants under Abiotic Stress. *Molecules* **24**, 2452 (2019).
46. Sagardoy, R., Morales, F., López-Millán, A., -F., Abadía, A. & Abadía, J. Effects of zinc toxicity on sugar beet (*Beta vulgaris* L.) plants grown in hydroponics. *Plant. Biol.* **11**, 339–350 (2009).
47. Krupa, Z. & Baszynski, T. Some aspects of heavy metals toxicity towards photosynthetic apparatus: Direct and indirect effects on light and dark reactions. *Acta Physiol. Plant.* **17**, 177–190 (1995).
48. Moustakas, M. et al. Photosystem II Tolerance to Excess Zinc Exposure and High Light Stress in Salvia sclarea L. *Agronomy* **14**, 589 (2024).
49. DiMario, R. J., Clayton, H., Mukherjee, A., Ludwig, M. & Moroney, J. V. Plant Carbonic Anhydrases: Structures, Locations, Evolution, and Physiological Roles. *Mol. Plant.* **10**, 30–46 (2017).
50. Solymosi, K. & Bertrand, M. Soil metals, chloroplasts, and secure crop production: a review. *Agron. Sustain. Dev.* **32**, 245–272 (2012).
51. Taiz, L., Zeiger, E., Moller, I. M. & Murphy, A. *Plant Physiology and Development* (Sinauer Associates, 2017).
52. Tsonev, T. & Lidon, F. J. C. Zinc in Plants – An Overview. in *Zinc in Soils and Crop Nutrition* (ed. Alloway, B. J.) 139–165 (International Zinc Association (IZA), Brussels, Belgium, (2008).
53. Paul, M. J. & Foyer, C. H. Sink regulation of photosynthesis. *J. Exp. Bot.* **52**, 1383–1400 (2001).
54. Hafeez, B. Role of Zinc in Plant Nutrition- A Review. *Am. J. Experimental Agric.* **3**, 374–391 (2013).
55. Boaretto, R. M., Quaggio, J. A., Mellis, E. V. & Cantarella, H. Micronutrientes. in *Recomendações de adubação e calagem para o Estado de São Paulo* (eds. Cantarella, H., Quaggio, J. A., Junior, M., Boaretto, R. M. & Raij, B. van) 121–127 (Instituto Agronômico (IAC), Campinas, (2022).
56. Lončarić, Z. et al. Efficiency of Biofortification with Zn and Se in Soybean: Yield and Overall Mineral Content in Plant. *Appl. Sci.* **14**, 11349 (2024).
57. Martínez Cuesta, N. et al. Zinc fertilization strategies in soybean: plant uptake, yield, and seed concentration. *J. Plant. Nutr.* **46**, 1134–1144 (2023).
58. Jordão, C. P. et al. Adsorption of Zn(II) in Oxisols as affected by selective removal of soil fractions. *Int. J. Environ. Stud.* **67**, 879–897 (2010).
59. Kumar, P., Athokpam, H. S., Singh, R. K. K., Devi, K. N. & Singh, N. O. Estimation of Critical Limits of Zinc in Soil and Plant for Predicting Response of Maize (Zea mays L.) to Zinc and Nutrient Uptake in Acid Soils, Manipur. *Int. J. Curr.*

Microbiol. Appl. Sci. **9**, 2052–2061 (2020).

60. de Souza, H. A. et al. Critical levels and sufficiency ranges for leaf nutrient diagnosis by two methods in soybean grown in the Northeast of Brazil. *Rev Bras. Cienc. Solo* **44**, (2020).
61. Martínez-Cuesta, N. et al. Effect of zinc application strategies on maize grain yield and zinc concentration in mollisols. *J. Plant. Nutr.* **44**, 486–497 (2021).
62. Camargo, O. A., Moniz, A. C., Jorge, J. A. & Valadares, J. M. A. *S. Métodos de Análise Química, Mineralógica e Física de Solos Do Instituto Agronômico de Campinas* (Instituto Agronômico (IAC), 1986).
63. Santos, H. G. et al. *Sistema Brasileiro de Classificação de Solos* (Embrapa, 2018).
64. Staff, S. S. *Keys to Soil Taxonomy* (USDA-Natural Resources Conservation Service, 2014).
65. van Raij, B., Cantarella, H., Camargo, C. E. O., Quaggio, J. A. & Furlani, A. M. C. Introdução. in *Recomendações de adubação e calagem para o Estado de São Paulo* (eds. Raij, B. van, Cantarella, H., Quaggio, J. A. & Furlani, A. M. C.) 1–6 (Instituto Agronômico (IAC), Campinas, (1997).
66. Köppen, W. *Climatologia: Con Un Estudio de Los Climas de La Tierra* (Fondo de Cultura Económica, 1948).
67. EMBRAPA. *Tecnologias de Produção de Soja - Região Central Do Brasil 2012 e 2013* (Embrapa Soja, 2011).
68. Malavolta, E., Vitti, G. C. & de Oliveira, S. A. *Avaliação Do Estado Nutricional Das Plantas: Princípios e Aplicações* (POTAFOS, 1997).
69. Bataglia, O. C., Furlani, A. M. C., Teixeira, J. P. F., Furlani, P. R. & Gallo, J. R. *Métodos de Análise Química de Plantas*. (1983).
70. Lichtenthaler, H. K., Buschmann, C. & Knapp, M. How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio R_{Fd} of leaves with the PAM fluorometer. *Photosynthetica* 43, 379–393 (2005).
71. Lichtenthaler, H. K. [34] Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. in 350–382 (1987). 10.1016/0076-6879(87)48036-1
72. Singleton, V. L. & Rossi, J. A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic*. **16**, 144–158 (1965).
73. Gratão, P. L. et al. Biochemical dissection of diageotropica and Never ripe tomato mutants to Cd-stressful conditions. *Plant Physiol. Biochem.* **56**, 79–96 (2012).
74. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254 (1976).
75. Giannopolitis, C. N. & Ries, S. K. Superoxide Dismutases. *Plant. Physiol.* **59**, 315–318 (1977).
76. Kampfenkel, K., Vanmontagu, M. & Inze, D. Extraction and Determination of Ascorbate and Dehydroascorbate from Plant Tissue. *Anal. Biochem.* **225**, 165–167 (1995).

Figures

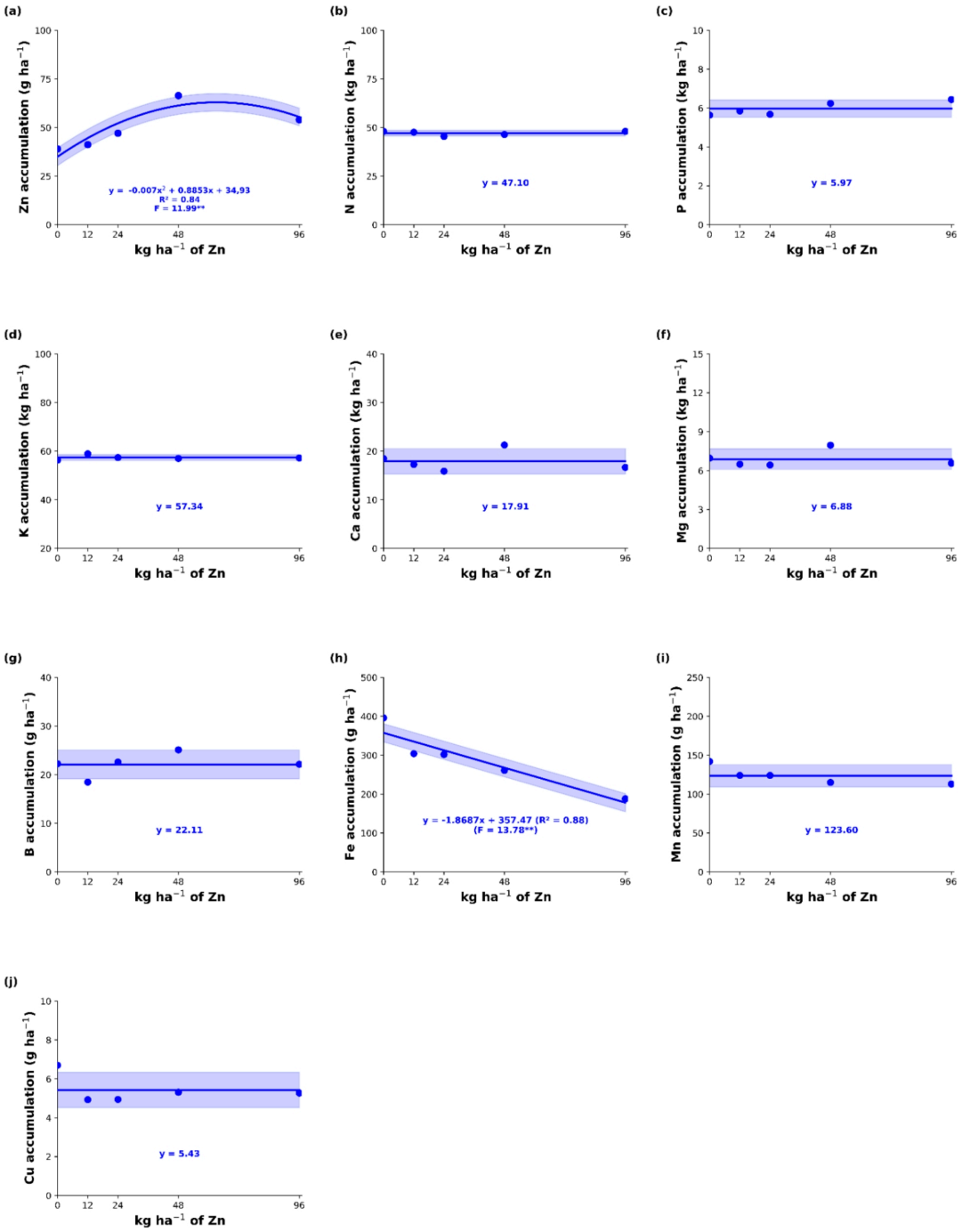


Figure 1

Nutrient accumulation in soybean: Zn (a), N (b), P (c), K (d), Ca (e), Mg (f), B (g), Fe (h), Mn (i), and Cu (j) in response to different Zn application rates. ** and * indicate significance at the 1% and 5% probability levels, respectively, according to the F-test.

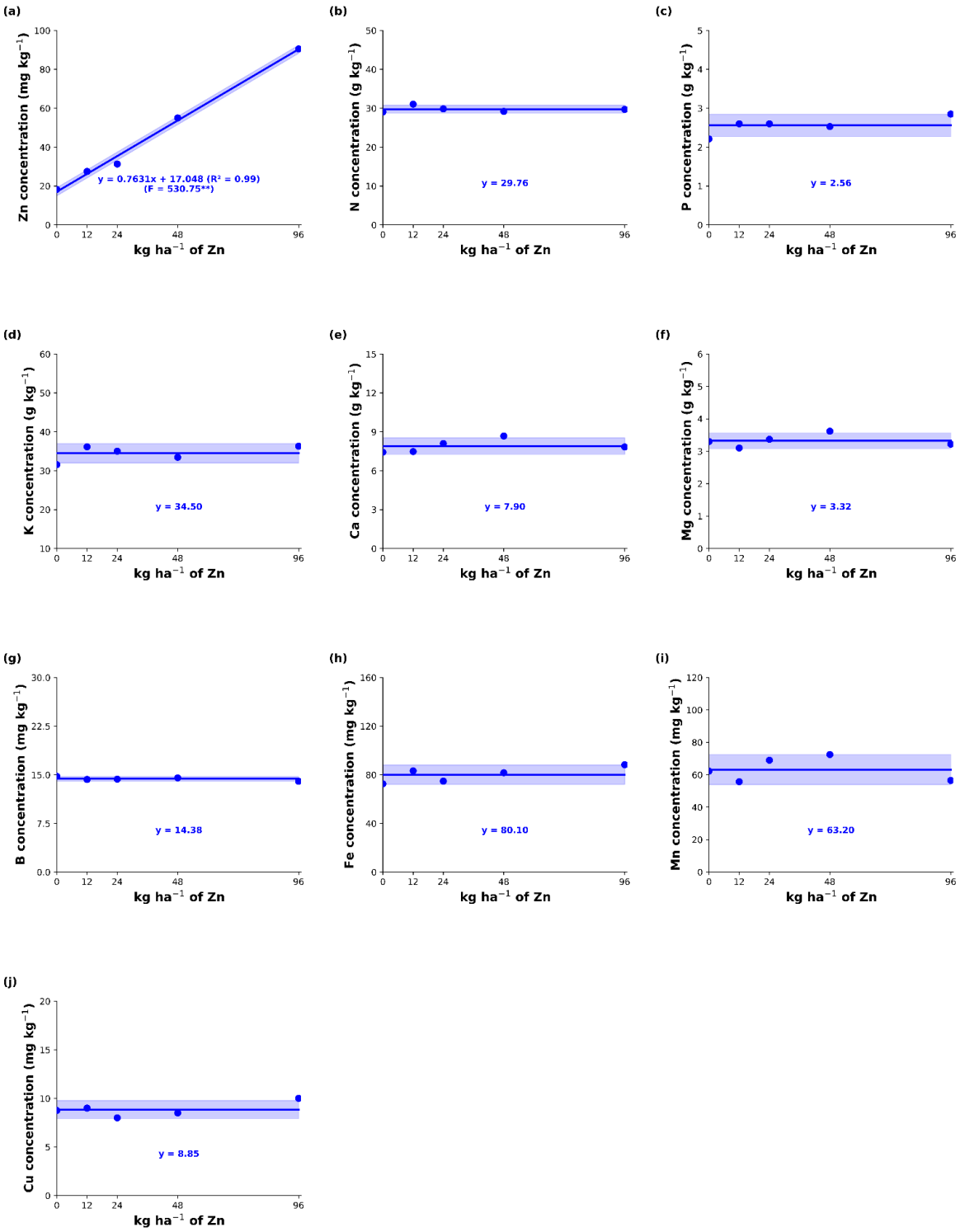


Figure 2

Nutrient concentrations in the diagnostic leaf of soybean: Zn (a), N (b), P (c), K (d), Ca (e), Mg (f), B (g), Fe (h), Mn (i), and Cu (j) in response to different Zn application rates. ** and * indicate significance at the 1% and 5% probability levels, respectively, according to the F-test.

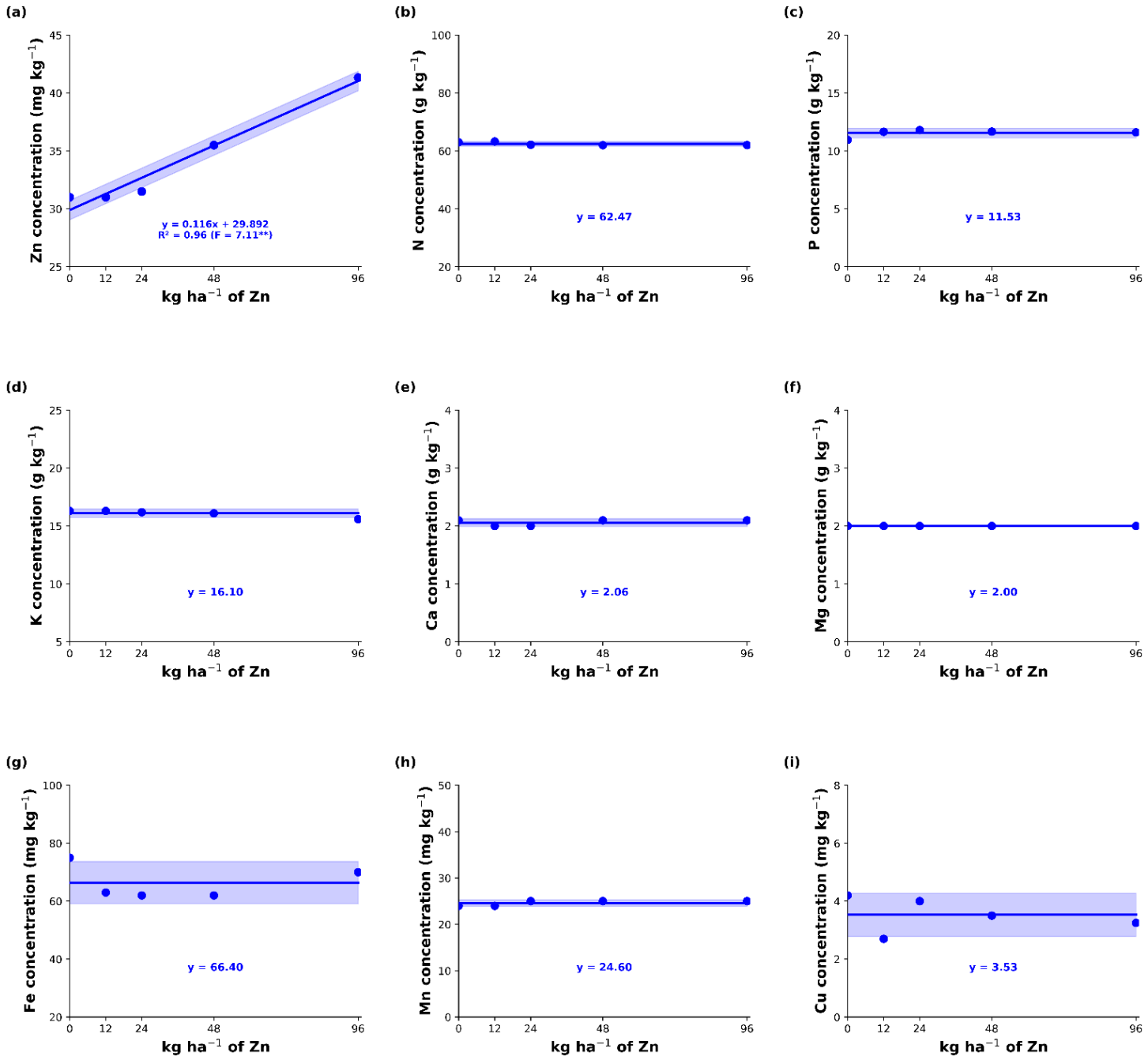


Figure 3

Nutrient concentrations in soybean grains: Zn (a), N (b), P (c), K (d), Ca (e), Mg (f), Fe (g), Mn (h), and Cu (i) in response to different Zn application rates (0, 12, 24, 48, and 96 kg ha⁻¹ Zn). ** and * indicate significance at the 1% and 5% probability levels, respectively, according to the F-test.

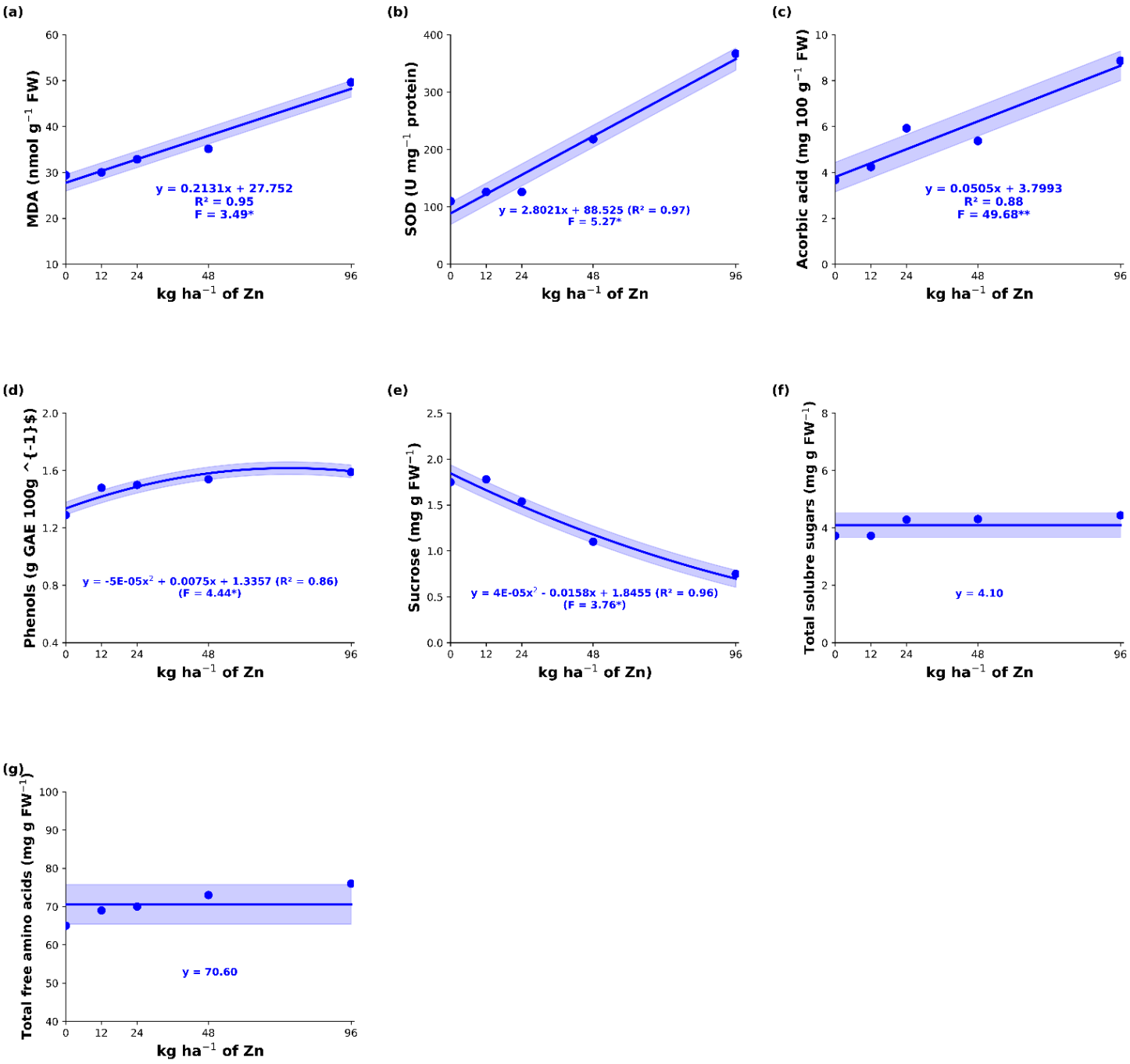


Figure 4

Malondialdehyde (MDA) content (a), superoxide dismutase (SOD) activity (b), ascorbic acid content (c), total phenolic compounds (d), sucrose content (e), total soluble sugar content (f), and total free amino acid content (g) in response to different Zn application rates in soybean. ** and * indicate significance at the 1% and 5% probability levels, respectively, according to the F-test.

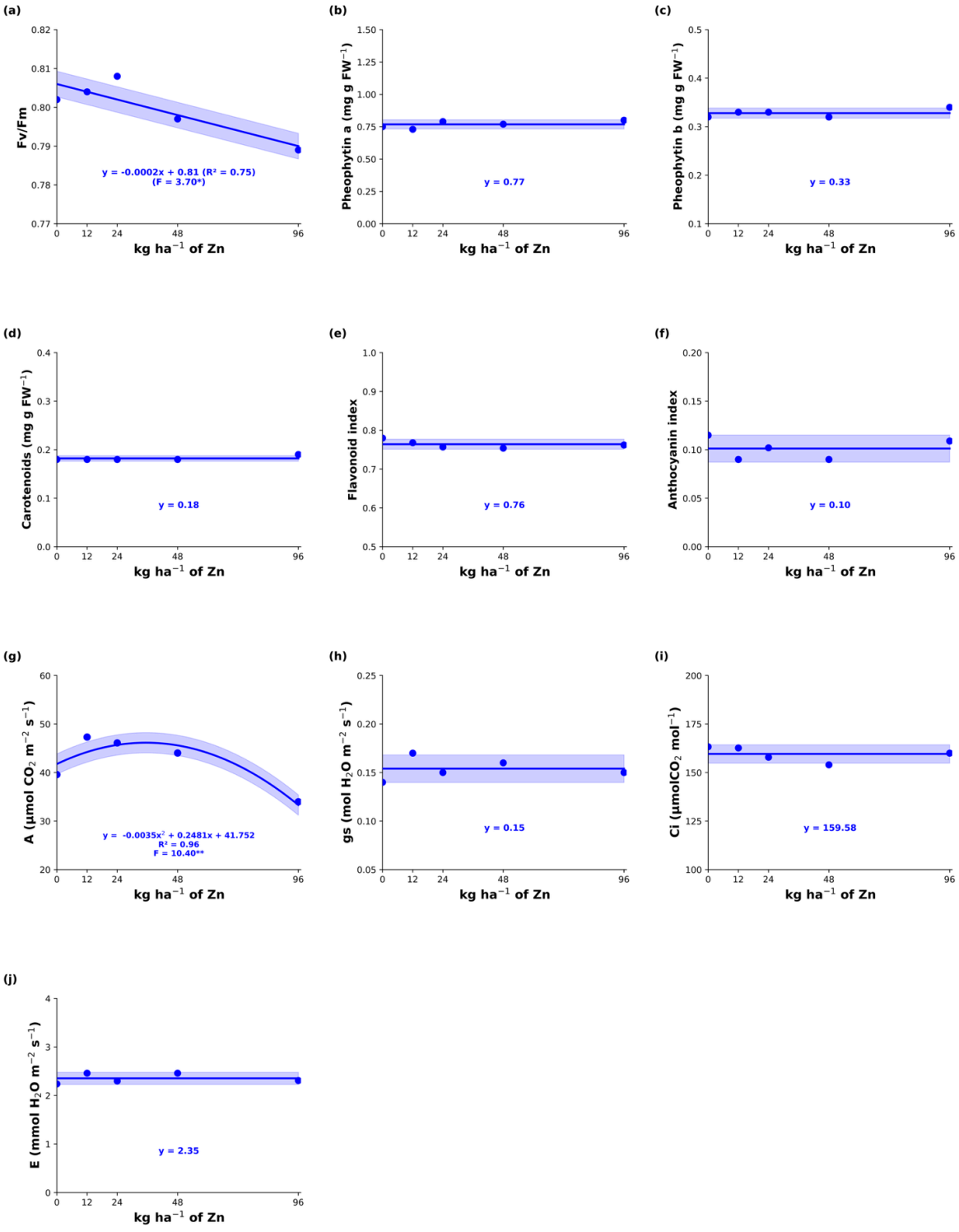


Figure 5

Maximum quantum efficiency of photosystem II (Fv/Fm) (a), pheophytin a (b), pheophytin b (c), carotenoid content (d), flavonoid index (e), anthocyanin index (f), net photosynthetic rate (A) (g), stomatal conductance (gs) (h), intercellular CO_2 concentration (Ci) (i), and transpiration rate (E) (j) in soybean plants subjected to different residual Zn application rates. ** and * indicate significance at the 1% and 5% probability levels, respectively, according to the F-test.

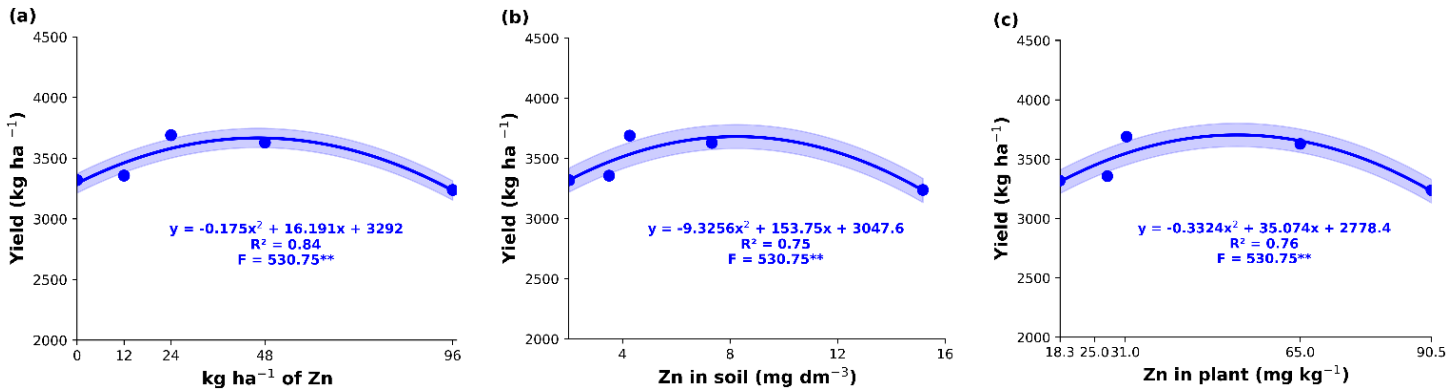


Figure 6

Relationship between soybean grain yield and (a) Zn application rates, (b) soil Zn concentration, and (c) diagnostic leaf Zn concentration. ** and * indicate significance at the 1% and 5% probability levels, respectively, according to the F-test.

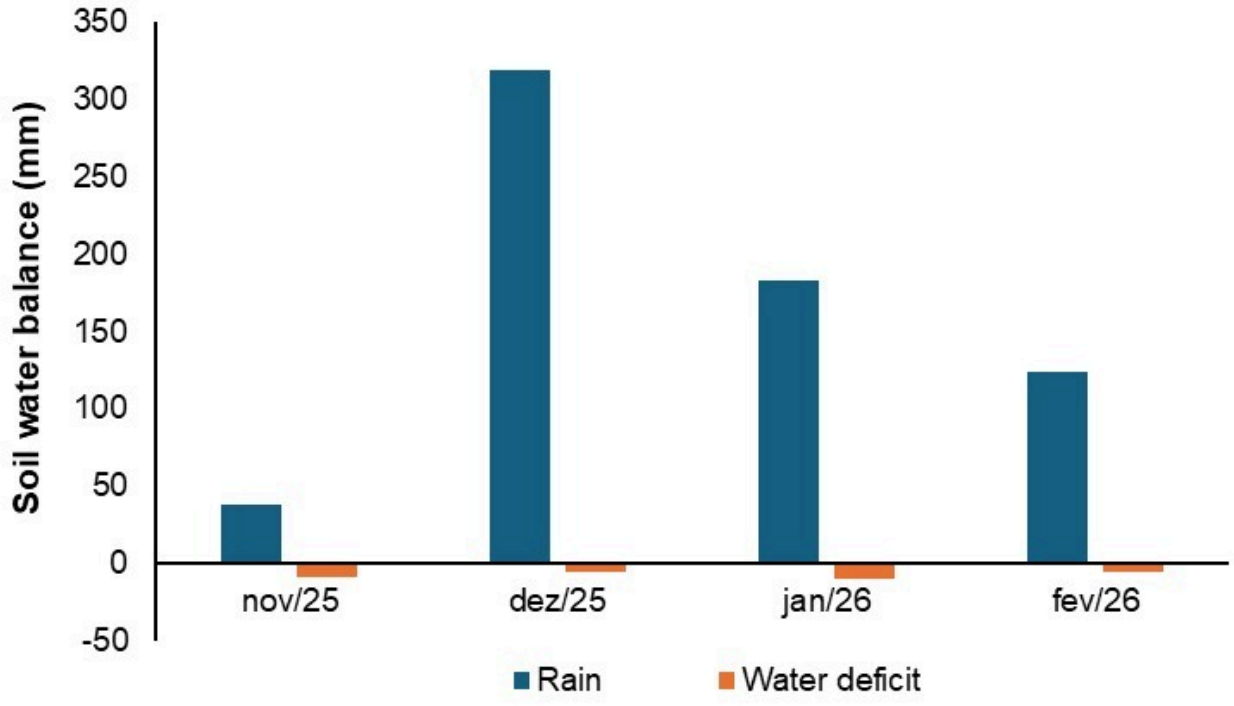


Figure 7

Water balance during the experimental period.