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Foliar Application and Translocation of Radiolabeled Zinc Oxide Suspension vs. Zinc Sulfate Solution by Soybean Plants

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Abstract: The present study employed a ^{65}Zn radioactive isotope as a tracer to investigate the foliar uptake and distribution patterns of ZnO concentrated suspension- and $ZnSO_4$ solution-sprayed on soybean plant leaves. The radiolabeled foliar treatments were sprayed on the leaves at V4 and V8 phenological stages. The radioactivity of ^{65}Zn in the leaves, roots, stems, and pods was determined using γ-ray spectrometry. After the first foliar spray, V4, the partition of radiolabeled Zn in plants treated with ZnO and $ZnSO_4$ was 99.22% and 98.12% in treated leaves, 0.15% and 0.39% in stems, 0.16% and 0.29% in roots, and 0.47% and 1.19% in newly expanded non-treated leaves, respectively. After two sprayings, V4 and V8, the partition of radiolabeled Zn in plants treated with ZnO and $ZnSO_4$ was 92.56% and 92.18% in treated leaves, 0.92% and 0.70% in stems, 0.52% and 0.39% in roots, 5.60% and 6.15% in newly expanded non-treated leaves, and 0.43% and 0.61% in grains, respectively. The total fraction translocated from the application tissue was 0.79% and 1.91% for ZnO and $ZnSO_4$, respectively, after 12 days and 8.03% and 8.48% for ZnO and $ZnSO_4$, respectively, after 72 days. An anatomical analysis revealed that plants cultivated in a nutrition solution with 10% ionic strength had 63% fewer stomata, and the xylem vessels were 63% smaller compared to plants grown in a solution with 100% Zn ionic. One can conclude that after a short period, 12 days, the absorption and translocation of $ZnSO_4$ was higher and faster than ZnO, and after the long period, 72 days, their performance was similar.

Keywords: ^{65}Zn; $ZnSO_4$; ZnO; foliar spray; leaf anatomy

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1. Introduction

The foliar spraying of nutrients is a consolidated practice aiming at complementing soil nutrient supply. It can be deployed under several conditions, such as (i) chronically low soil nutrient levels, such as those observed for transition metal micronutrients under natural alkaline conditions or at the surface layer of limed soils in a no-till system [1]; (ii) temporary soil deficiency, such as that observed for Mn under flooded oxisol conditions due to the competition with excess Fe^{2+} [2]; (iii) for the agronomic biofortification of grains and fruits [3]; and (iv) to deliver nutrients in specific moments of high demand, guaranteeing that they will not limit the plant yield potential [4].

Zn is directly related to the synthesis of growth hormones and thus directly linked to leaf area and the number of leaf organs, as shown by [5]. Zn is predominantly transported as complexed Zn^{2+} ions through the phloem from the treated leaf to other parts of the plant [6]; the excess of Zn is also responsible for opening the stomata and determines the rate of K^+ influx in these cells, and its excess can cause a reduction in the number of guard cells and the size of the stomata [7]. It is estimated that around 9% of eukaryote proteome contains zinc (Zn) [8]. This trace element is involved in cell division and elongation, which are critical processes in plant growth and development. It plays a role in the regulation of auxins, which are plant hormones that promote cell elongation and act in the maintenance of activity of the cambium cells responsible for producing secondary xylem and phloem [1,9,10].

In soybean cultivation, plant tissues are considered deficient in zinc (Zn) when concentrations fall below 20 mg/kg, as noted by [11]. Furthermore, a critical threshold for soil Zn concentration is typically below 1 mg/dm^3. In soybean grains, Zn concentrations range between 30 and 50 mg/kg, with the peak accumulation occurring between reproductive stages R5 and R6 [12].

Soybean zinc extraction is highly dependent on yield, but it falls around 80–100 g/ha of Zn over their growth cycle, of which 30–50 g/ha is exported through the harvested grains. Zinc is classified as a low-mobility micronutrient within the plant. Once absorbed by the roots and translocated to various tissues, Zn becomes strongly bound to proteins and enzymes [12]. This strong binding hinders its redistribution to new, actively growing parts of the plant. Therefore, symptoms are observed in newer leaves.

Several zinc sources are available in the fertilizer market; they can be loosely grouped into two large groups: solutions and suspensions. As a representative of solutions, one can highlight salts, such as $ZnSO_4$, chelates, such as Zn-EDTA, and complexes combining Zn^{2+} with amino acids or sulfonated lignocellulosic products. Suspensions are usually obtained by grinding and suspending ZnO or $ZnCO_3$ down to micrometer to submicron size range. Alternatively, nanoparticles can also be employed to form colloidal dispersions. Some of the critical differences between solutions and suspensions involve the concentration of dissolved Zn; in contrast to solutions, in suspensions, only a small fraction of Zn is dissolved. This fraction can be calculated from the Ksp of the solid, and it also depends on the pH. Usually, the concentration available for immediate absorption is smaller than 1% of the total Zn. The concentration of Zn in a solution, among many other parameters, impacts the rate of Zn absorption by plant leaves. Such a fact raises concerns about the extent to which plants can absorb Zn coming from suspensions.

Thus, understanding absorption and transport processes is of utmost importance for product development and adequate application for end users. Radioisotopes can be employed to investigate the absorption and movement of certain elements in plants. For example, Zn can be traced by monitoring ^{65}Zn, a 244-day half-living radioactive isotope that emits gamma radiation at 1.11 MeV.

Using autoradiography and γ-ray spectrometry, the behavior of ^{65}Zn applied to wheat leaves in various forms ($ZnCl_2$, ZnEDTA, ZnO nanoparticles, and ZnO microparticles) was analyzed, with each treatment having an activity of 131 MBq. A 5 µL droplet was applied to the youngest fully expanded leaf, and samples were collected after 1 and 5 days. Leaves treated with $^{65}ZnCl_2$ showed the highest absorption but had the lowest translocation from the treated leaf after 15 days. ^{65}ZnEDTA displayed intermediate absorption and translocation, while most ^{65}Zn from all treatments remained concentrated in the treated leaf [13].

A study focused on assessing how different cultivars of rye, bread wheat, and durum wheat responded to environments with limited zinc availability was employed using ^{65}Zn

isotope to compare the zinc root absorption capacities under zinc-deficient conditions. Plants were grown under 0.1 μM (Zn-deficient plants) and 10 μM (Zn-sufficient plants). For the uptake experiment, past 9, 11, and 13, the plants were exposed to a nutrient solution containing 8 μM $^{65}ZnSO_4$ + 18.2 μM ^{65}Zn-HEDTA, labeled with 37 kBq of ^{65}Zn, lasting 8 h. When Zn was adequately supplied, there were no noticeable differences in the uptake or the root-to-shoot translocation of ^{65}Zn among the cultivars. However, under Zn-deficient conditions, significant variations were observed. Rye displayed the highest Zn uptake, while durum wheat had the lowest. Among the bread wheat cultivars, the ^{65}Zn uptake rates were similar and not correlated with their Zn efficiency, which refers to their ability to maintain growth and yield under Zn deficiency. In conditions lacking Zn, rye had the fastest root-to-shoot translocation of ^{65}Zn, whereas bread and durum wheat cultivars exhibited comparable rates of ^{65}Zn movement from roots to shoots [14].

Likewise, in another study aimed to evaluate the transport pathways of zinc applied to wheat leaves in experiments designed to track the movement of ^{65}Zn, plants were grown in a nutrient solution without zinc. A stock solution of 0.71 mM ^{65}Zn with a specific activity of 50 MBq/mmol was used to prepare a testing solution at 5 mM ^{65}Zn. The application of ^{65}Zn was carried out in three ways: by immersing the cut leaf tip into the labeled solution, by applying a droplet of the ^{65}Zn-containing solution to the upper surface of the leaf, or by adding ^{65}Zn to one side of split-root pots. The leaf surface application of ^{65}Zn showed that the addition of a surfactant improved Zn uptake by approximately 15%. Therefore, subsequent surface application experiments used the surfactant-mixed solution. The majority of surface-applied ^{65}Zn, around 85% of Zn, moved toward the tip of the treated leaf, while the remaining portion was distributed among the stem, leaves, roots, and to a lesser extent, older leaves [15].

Also, another study examined the effects of foliar applications of $ZnSO_4$ and zinc oxide nanoparticles (ZnO NPs) on green bean plants, specifically assessing Zn uptake. The treatments involved applying either an aqueous $ZnSO_4$ solution or a suspension of ZnO NPs, both at a concentration of 150 mg·L^{-1}. The results showed that ZnO NP applications notably enhanced Zn^{2+} concentrations in leaflets, roots, stems, and pods [16].

The effects of ZnO nanocolloid, ZnO nanoparticles, and micrometric ZnO particles on corn growth were evaluated by [17]. A concentration of 2 ppm of each ZnO particle type was applied through irrigation water. The findings indicate that all three forms of ZnO enhanced shoot dry matter and leaf area index. The most significant improvements were observed with the ZnO nanoparticle treatment, which increased the shoot dry matter and leaf area index by 63.8% and 69.7%, respectively, on average. These results suggest that zinc nanoparticles have the potential to improve corn growth and yield, particularly in soils with low mineral availability.

These studies help us understand how Zn can move in the plant through absorption by the leaves, and the radiation levels allowed us to evaluate how much Zn resulting from the treatments is partitioned in the tissues. However, we detected a knowledge gap regarding the capacity of soybeans to absorb and translocate Zn upon foliar spraying. Given the yield gain reported by farmers, we hypothesize that the absorption of Zn from ZnO may happen through the leaves, but this is somehow contradictory with the low concentration of dissolved Zn in the suspension. Hence, the present study established two goals: Firstly, this study sought to determine the fraction translocated from leaves sprayed with Zn to non-treated other plant tissues. Secondly, it aimed to evaluate how Zn deficiency affects plant anatomy.

2. Materials and Methods

2.1. Experimental Design and Foliar Application

The experiment was carried out in a greenhouse located at the Center for Nuclear Energy in Agriculture, Piracicaba-SP, Brazil (22.70748 °S, 47.64531 °W), from July to October 2022. To complement the natural light irradiation, we employed 800 μmol of photon/m^2 from LED lights.

Soybean seeds of the NS6700 IPRO variety were germinated in paper towels moistened with $CaSO_4$ solution at 2 g L^{-1} for 7 days to avoid disease contamination, and then they were transplanted to 3 L pots containing growth solution. The solution, whose chemical composition is disclosed below, was replaced every 5 days. The water level of the pots was replenished every 2 days. The composition of the solution at 100% ionic strength was an adaptation of [18], as follows: 1 M of KNO_3; 1 M of $Ca(NO_3).4H_2O$; 1 M of $NH_4H_2PO_4$; 1 M of $MgSO_4{\cdot}7H_2O$; 25 mM of KCl, 13 μM of H_3BO_3; 1 μM of $MnSO_4{\cdot}7H_2O$; 1 μM of $ZnSO_4{\cdot}7H_2O$; 0.25 μM of $CuSO_4.5H_2O$; 0.34 μM of $(NH_4)_6Mo_7O_{24}$; and 50 μM of NaFeEDTA. The pots were permanently aerated.

The plants that received the foliar treatments, $ZnSO_4{\cdot}7H_2O$ solution or ZnO suspension, were grown in a solution bearing 10% of the ionic strength stated above. Additionally, we grew two controls: (i) complete nutrient solution, in which plants received water foliar spraying instead of Zn treatments, and (ii) nutrient solution with 10% of Zn recommendation, in which plants received water foliar spraying instead of Zn treatments. The experiment was carried out with five biological replications in a completely randomized block design (CRB), summing up 40 pots, half of which were harvested in V6–V8 (12 days after 1st treatment application), while the other half was harvested in R5.3–R5.5 (60 days after the 2nd treatment application).

Foliar applications of formulated ZnO suspension and $ZnSO_4$ solution, both radiolabeled, occurred in two moments. The surfaces of pots were properly covered to avoid any contamination with radiolabeled Zn. Half of the plants received a single spray at V4, and the other half of the plants received two sprays at stages V4 and V6–V8, each plant received 1.3 mL of treatment, resulting in a concentration of 0.65 mg Zn per plant. The treatments were applied to the 3 oldest trefoils, and monitoring the fraction of the applied zinc that was translocated to other parts of the plant was carried out at stages V6–V8 and R5.3–R5.5.

The first harvest occurred 12 days after the first foliar application, stages V6–V8, and the harvests were divided into (i) treated leaves, which received foliar treatment and (ii) non-treated leaves, which did not receive foliar, root, or stem applications. Once harvested, the samples were dried for 72 h at 65 °C and ground. At this point, half of the experiment was finished, leaving only 20 pots that received the 2nd foliar application.

The second application was carried out in the new leaves at the V6–V8 stage, and the sampling was performed at stages R5.3–R5.5.

For the sake of clarity, the experimental procedure is summarized and illustrated in Figure 1.

The ground plant material was weighed and transferred to plastic vials. The determination of ^{65}Zn was carried out using γ-ray spectrometry with a well-type HPGe detector, Ortec (Wokingham, UK) model GWL22015. All activities were corrected for decay, assuming 26 August 2022 as the reference date.

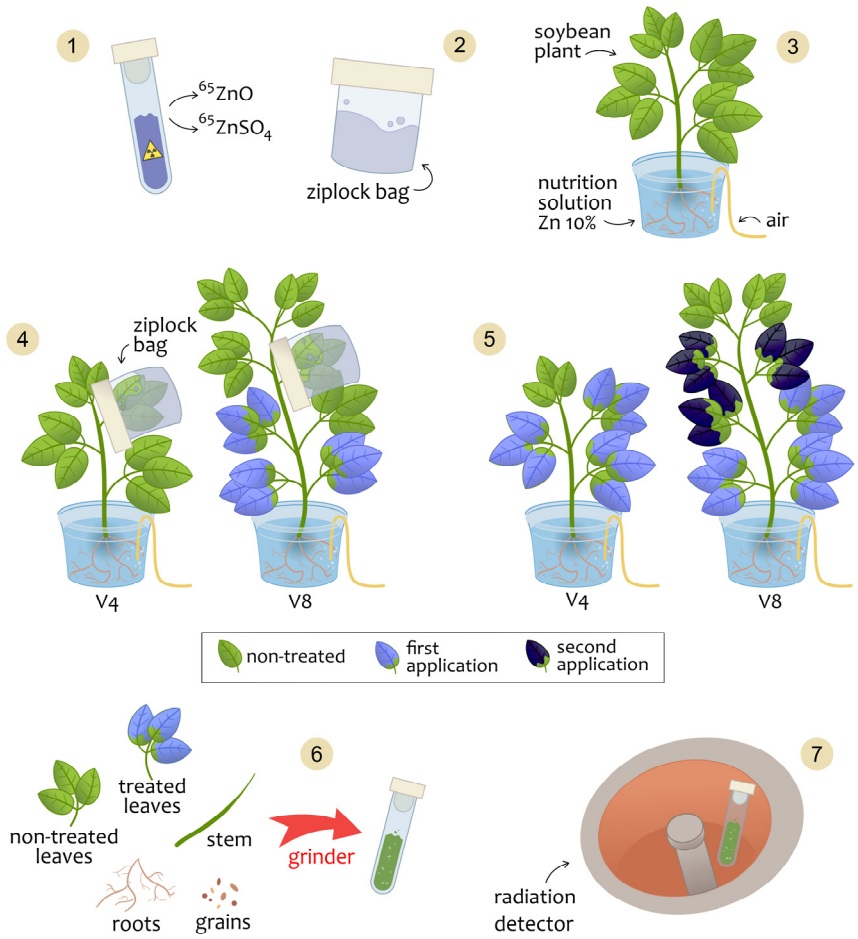

Figure 1. Process of application of ^{65}Zn. This figure represents the entire process of ^{65}Zn spaying in different V stages and how the plants were harvested.

2.2. Preparation of Radiolabeled Sources

The radiolabeled zinc materials were obtained through the activation of ZnO supplied by Yara (Paulínia, Brazil). Yara International ASA is a leading global agricultural company headquartered in Oslo, Norway. The same ZnO used in the production of the commercial product Zintrac is utilized in this context, and $ZnSO_4 \cdot 7H_2O$ at a thermal neutron flux of 8×10^{12} cm^{-2} s^{-1} for 4 h in the nuclear research reactor IEA-R1 of the Nuclear and Energy Research Institute (IPEN) was supplied by Merck (Darmstadt, Germany). Two polyethylene capsules with 16 mg of ZnO and two capsules with 57 mg of $ZnSO_4$ were irradiated, yielding individual activities of 162 kBq and 164 kBq of ^{65}Zn, respectively.

The powdered ^{65}ZnO was mixed with two liquid proprietary surfactants supplied by Yara company. The $^{65}ZnSO_4$ was dissolved in water. The concentration of Zn in both treatments was 0.5 mg/mL.

2.3. Stomatal Density, Trichome, and Stem Anatomy Analyses

For this experiment, the plants were cultivated as described above. Twenty pots were distributed in a completely randomized block (CRD) with 10 repetitions and 2 treatments, in which 10 pots contained soybeans grown in nutrition solution with an ionic strength of 100% Zn (control), resulting in 100% of the Zn recommendation, and the other 10 pots cultivated soybeans in a solution with an ionic strength of 10% Zn (treatment), resulting in 10% of the Zn recommendation. At the phenological stage, V4, the third mature trefoils were collected and analyzed under an ultraviolet light microscope, as described by [19].

The trichomes and stomata density of the abaxial and adaxial surfaces were counted using ImageJ 1.54g software.

Stem segments (5 cm from the crown) from the same plants in the experiment mentioned above were harvested and processed for anatomical analysis, as described by [20]. For instance, the sample was fixed in Karnovsky solution and submitted to a vacuum pump to remove the air contained in the intercellular spaces; then, the samples were dehydrated in ethanol and embedded in glycol methacrylate Technovit histories. The samples were then cut into a microtome with a 7 µm-thick slice to be analyzed in a light microscope. The slides were stained with toluidine blue and analyzed under a light microscope, as shown in Figure 2.

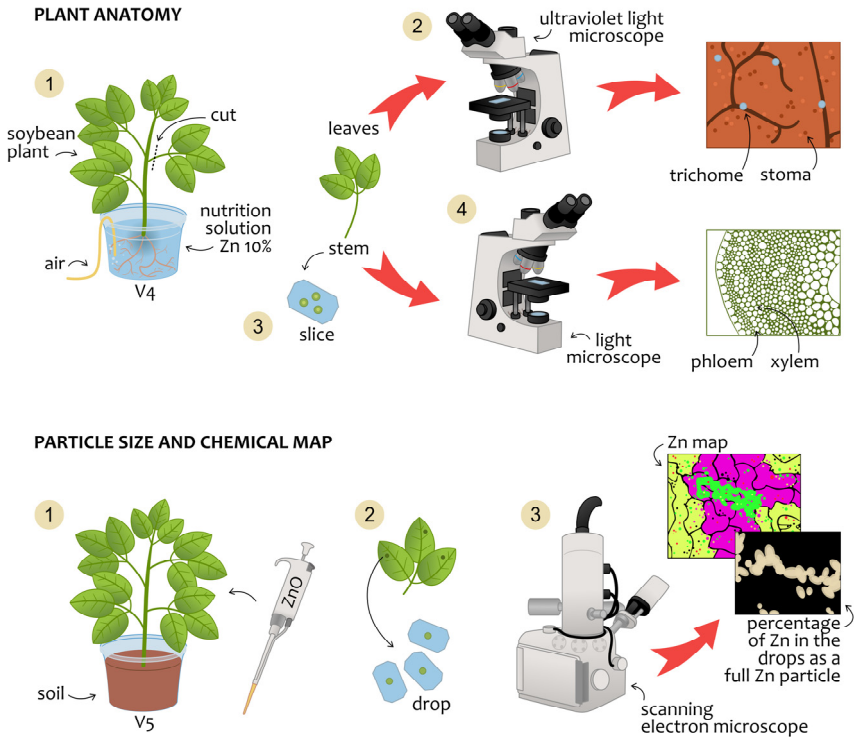

Figure 2. Steps of anatomical analyses and particle distribution on the leaf surface. Plant anatomy represents the steps to analyze the number of trichomes and stomata abaxial and adaxial and the sizes of the vessel, xylem, and phloem. Particle size and chemical map represent the steps to measure the particles of ZnO and the concentration of Zn in the drops.

2.4. Monitoring of Particle Size and Chemical Map

To monitor the shape and size distribution of the ZnO particles deposited on the leaf surface, a Zintrac-Yara^{TM} suspension was prepared to yield a suspension containing 245 mg/L. Twelve soybean plants were cultivated in a greenhouse, which received a single treatment, three droplets of 1 µL deposited with a micropipette on the adaxial surface of soybean leaves in the V5 phenological stage. The plants were cultivated under greenhouse conditions in sandy loam soil containing 0.4 mg Zn/kg of soil extracted using DTPA [21]. The thermal and air relative humidity amplitude during the experiment were 37.5 °C maximum and 14.4 °C minimum and 89.2% maximum and 29% minimum, respectively.

Four regions of leaves containing the dried fertilizer droplets were sampled from distinct plants, each representing a biological replicate; they were collected at 0.04 days (1 h), 1 day, 14 days, and 21 days. No previous treatment (fixation or dehydration) was conducted to avoid washing the Zn fertilizer on the soybean leaf surface. These samples of plant leaves bearing the dried Zn droplets were placed on an aluminum sample holder covered with double-sided carbon adhesive tape. In the next step, the samples were first coated with

flash carbon under vacuum using a Balzers MED 010 evaporator (Technotrade International, Manchester, NH, USA) and then transferred to a desiccator for the dehydration process. Then, they were analyzed using the scanning electron microscope JEOL IT 300 with 20 kV voltage. In addition, SEM EDX analyses are performed with an Oxford X-ray detector and using AzTech 3.0 software. The EDX chemical images of three spots per drop under 300× magnification were used as a method to reveal the concentration of Zn on the droplet region, and the SEM images were employed to determine the dimensions of the ZnO particles that remained on the surface; for this last step, at least 500 particles were assessed per sample. Figure 2 summarizes and illustrates the procedure.

2.5. Statistical Analysis

Statistical analyses were performed using JMP SAS 16.1 software. An analysis of variance (ANOVA) was performed, and when $p < 0.10$, the test of means was LSD 10%. The choice of a p-value threshold of $p < 0.1$ was established prior to the analysis based on the practical objectives of the research and the agronomic context, ensuring that the decision was not influenced by the results obtained and balancing scientific rigor with practical applicability. While $p < 0.05$ is a general convention, $p < 0.1$ was adopted to ensure that the relevant effects for agronomic management were not overlooked due to a more restrictive approach.

3. Results

3.1. Radiolabeled Sources

Figure 3A shows the ^{65}Zn activity in plant parts at the V6–V8 phenological stages that received the foliar spraying at the V4 stage. It reveals that past 12 days, 92.56% and 92.18% of the Zn applied as ZnO and $ZnSO_4$, respectively, remained in the leaves that received the treatment. It also demonstrates that Zn, from both $ZnSO_4$ and ZnO, translocated to non-treated leaves that expanded after the spraying; likewise, ^{65}Zn counts above the controls were detected in roots and stems. We also noticed that the counts of ^{65}Zn were higher in the non-treated tissues of plants sprayed with $ZnSO_4$ than in plants sprayed with ZnO.

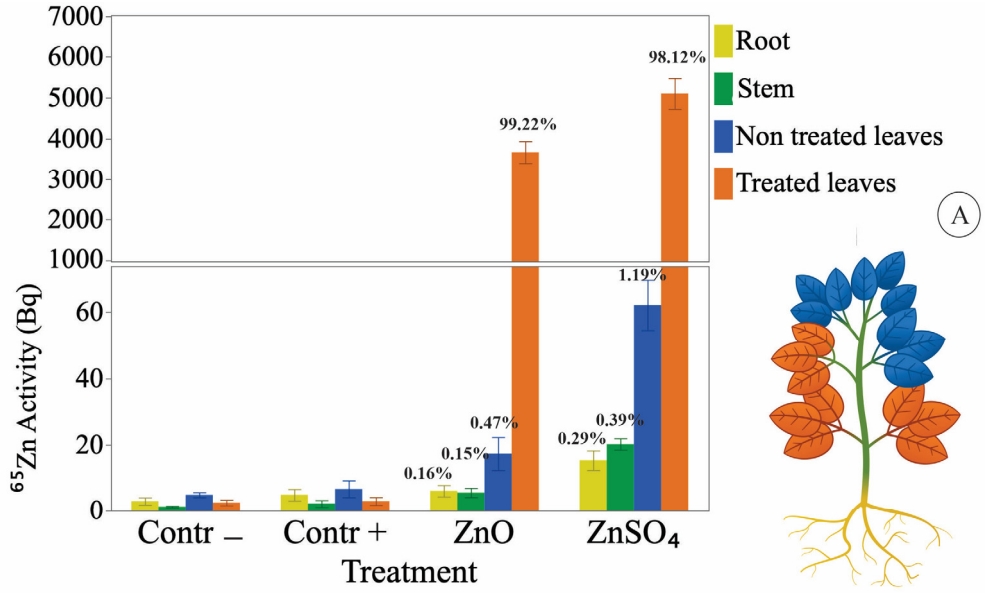

Figure 3. *Cont.*

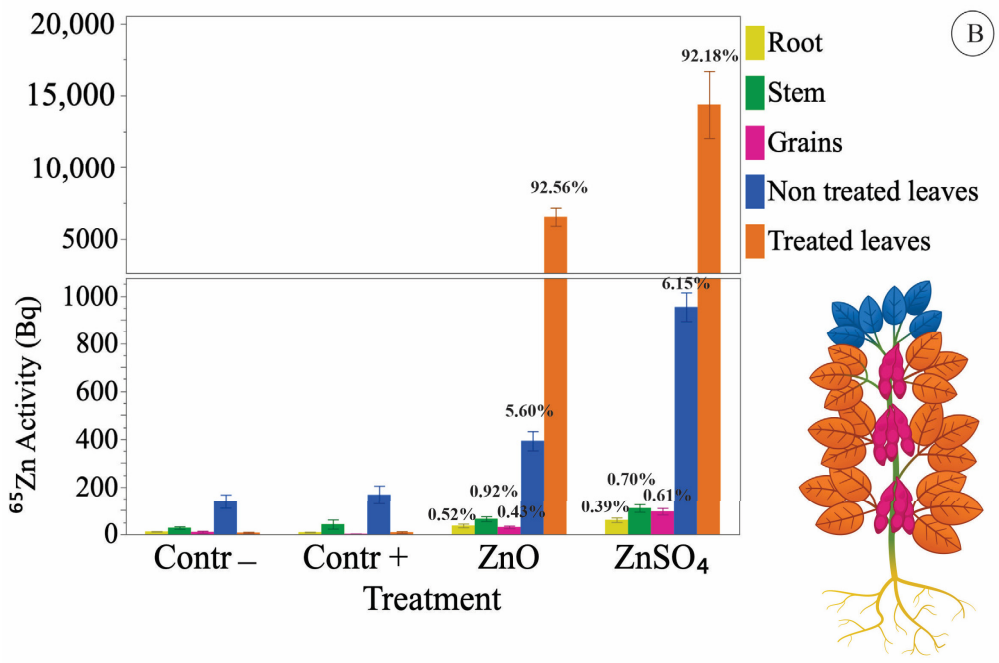

Figure 3. (**A**) 65Zn activity in plant parts from the harvest after the first harvest (treatment in V4 and harvest V6–V8). (**B**) 65Zn activity in plant parts from the harvest second harvest (treatment in V6–V8 and harvest R5.3–R5.4). Contr− is the plants cultivated in solution with 10% of Zn recommendation and no leaf application, and Contr+ is plants cultivated in solution with 100% of Zn recommendation and no leaf application. The bars indicate the standard error of the mean. The coefficient of variation is represented as CV%. Zn applied on the plant leaves was translocated to other plant parts. Values in % represent the activity of Zn in each part of the plant relative to the total activity across all parts of the plant.

Figure 3B shows the ^{65}Zn activity for plant parts at R5.3–R5.5; these plants were treated twice at the V4 and V6–V8 stages. During the 60 days between the second treatment and tissue sampling, the plants had developed new leaves and grains. The activity of ^{65}Zn in the non-treated leaves, grains, roots, and stems shows that the Zn coming from both sources was absorbed and translocated within the plant.

Table 1 shows that the translocation of both sources represents less than 2% of the total Zn deposited on the leaves by the spray in the past 12 days. More than 92% of the Zn remained in the treated leaves. Unfortunately, the experimental approach employed by the present study did not allow us to determine the fraction of Zn that was absorbed and how much remained attached to the outer part of the leaf. The choice of experimental approach was made to minimize the amount of radioactive waste generated by the experiment. It is worth mentioning that upon the first harvest, 12 days after the application, the translocation of ZnO represented only 41% of that of $ZnSO_4$, and after 72 days, the translocation of ZnO represented 94% of that of $ZnSO_4$.

Table 1. γ-ray spectrometry data showing the proportion of total applied ^{65}Zn detected in each of the harvested soybeans.

Exposure Time	Treatment	Treated Leaf (Bq)	New Leaf (Bq)	Steam (Bq)	Root (Bq)	Grain (Bq)	Translocated Relatively to the Total Applied on Leaves (%)
1st harvest (12 days after application)	ZnO	3668.00	17.29	5.58	6.10	-	0.79
	$ZnSO_4$	5103.00	62.14	20.15	15.31	-	1.91
2nd harvest (72 days after application)	ZnO	6524.20	392.80	64.62	36.48	30.27	8.03
	$ZnSO_4$	14,387.30	954.30	109.68	60.10	95.86	8.48

3.2. Effect of Zn on Plant Anatomy

The leaf is amphistomatic, with an abaxial face that has a greater density of trichomes and stomata than the abaxial face at 32.2% and 13.6%, respectively, in 100% Zn ionic-strength solution (Figure 4). Also, the abaxial face of the plants carried out in 10% Zn ionic-strength solution showed 27% more trichomes and 10.7% more stomata than the adaxial face. Here, we present the interference of Zn in forming these leaf structures. The stem cross-sections indicate that the Zn-deficient soybean stems presented decreased cambium activity and reduced thickness of the secondary xylem (Figure 5). Interestingly, no differences were observed in the phloem. Also, no statistical differences were verified in the diameter of the vessel elements (Ve—arrows shown in Figure 5).

Also, plants grown under Zn deficiency conditions had 43% fewer abaxial stomata and 42.14% fewer adaxial stomata than plants cultivated with 100% of the recommended Zn levels. Although studies have shown that trichomes play an important role in nutrient absorption, no difference in trichome density was observed when plants were grown with low Zn levels. In this study, plants subjected to Zn deficiency had xylem vessels that were 37% smaller and had 63% fewer stomata than those in plants with sufficient Zn.

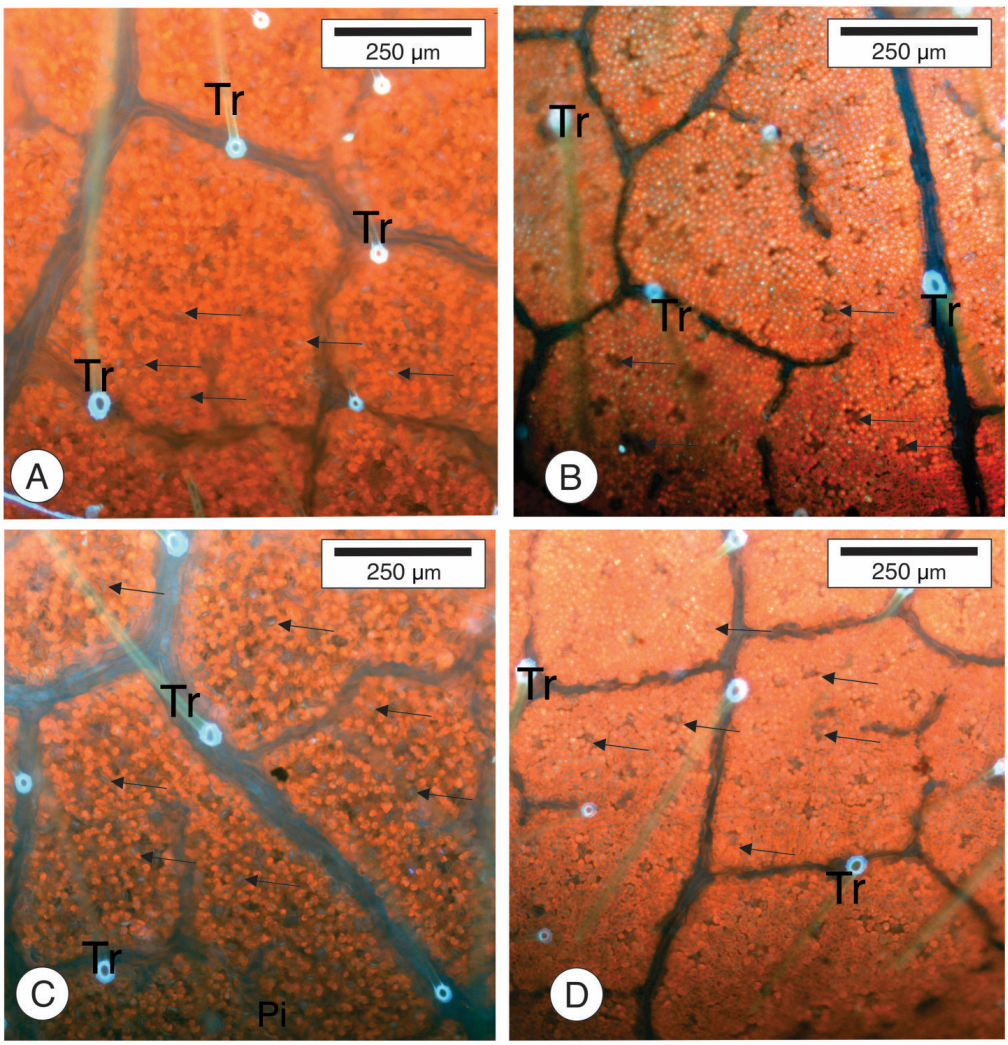

Figure 4. *Cont.*

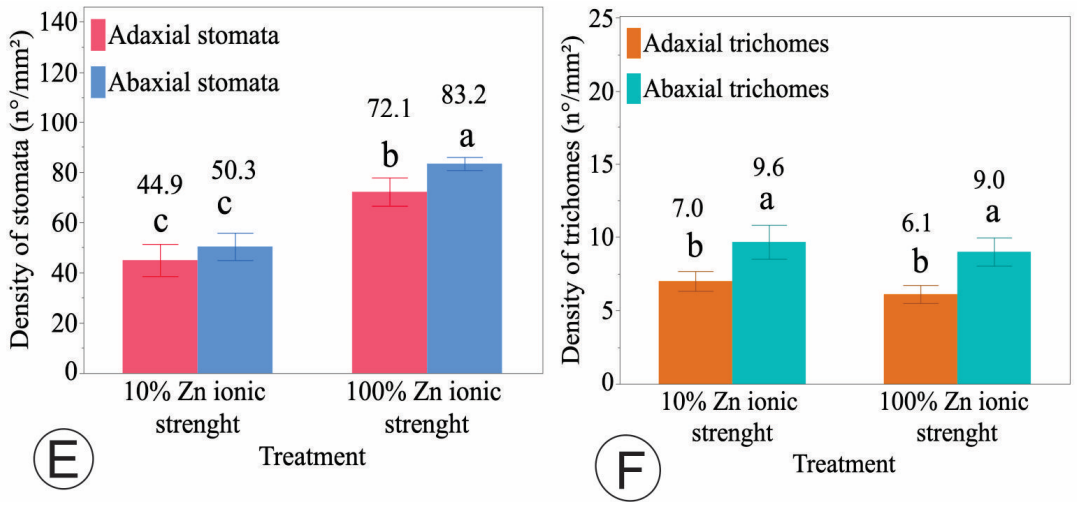

Figure 4. Analysis of the adaxial (**A**,**C**) and abaxial (**B**,**D**) of the soybean leaf surface under UV light and analyzed using epifluorescence microscopy with Imagens. It is possible to verify the autofluorescence within the guard cells—stomata (arrows) and the trichome base (Tr). (**E**,**F**) Density of stomata and trichomes on soybean leaves. Means followed by equal letters do not differ statistically from each other on an LSD ($p < 0.10$) probability test, and the bars indicate the standard error of the mean.

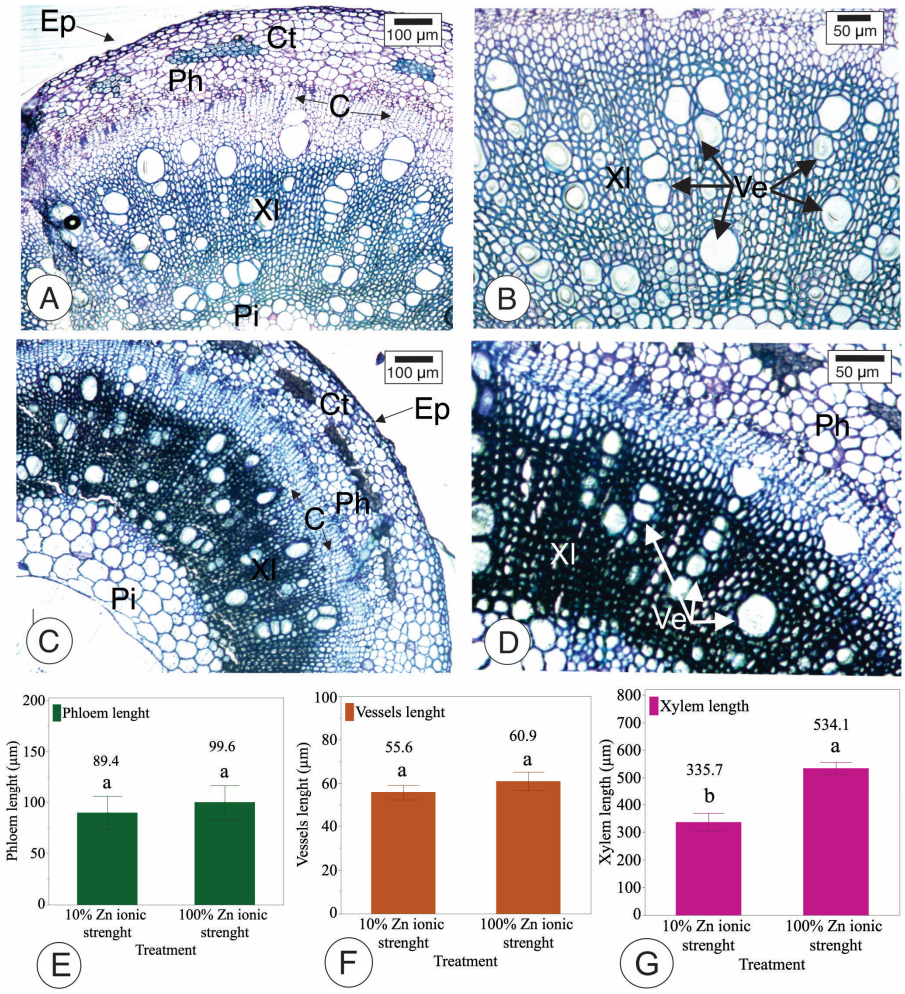

Figure 5. Cross-section of the soybean stem (**A**–**D**) of the control (**A**,**B**) and treatment (**C**,**D**) plants and biometric data of the phloem, vessel element diameter, and xylem (**E**–**G**). The soybean stems

with the recommended dose of Zn present more cells of secondary xylem compared to the 10% Zn. Size of xylem and phloem in soybean stems. It is possible to verify the autofluorescence within the Epidemis (Ep—arrows), Cortex (Ct), Phloem (Ph), Cambium (C—arrows), Vessel element (Ve—arrows), Pith (Pi) and Xylem (Xl). Means followed by equal letters do not differ statistically from each other on an LSD ($p < 0.10$) probability test, and the bars indicate the standard error of the mean.

3.3. Shape, Size, and Concentration of ZnO on the Leaf Surface as a Function of Time

Figure 6 presents the size distribution of the ZnO particles on the top of the leaves as a function of time; Figure 6A shows the length along the major axis, and Figure 6B shows the length along the minor axis of the particles. The particles deposited on the surface of the leaves present a normal distribution; as time elapses we observe that the standard error changes, which shows that the size distribution of the particles is changing. This supports the hypothesis that the particles are dissolving on the left surface, and the ionic Zn is being absorbed. The fact that the mean particle size decreases and then increases may indicate that the particles can agglomerate once they are resuspended whenever dew or water films are formed on the leaf surface.

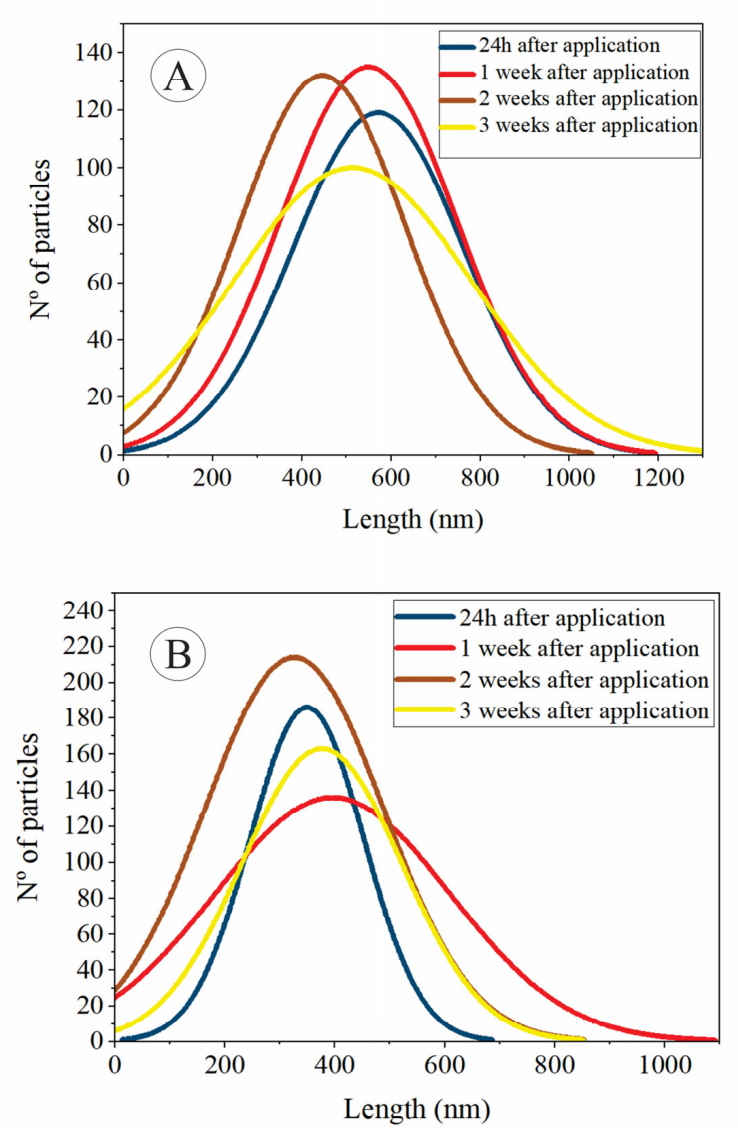

Figure 6. Histogram of the minor axis length (**A**). Histogram of the major axis length of the Zn nanoparticles. (**B**).

Figure 7 presents the spatial distribution of Zn in the ZnO within the droplet. It can be seen that Zn disappears from the surface since the leaves have not been washed; the conclusion is that Zn is being absorbed. By integrating the Zn X-ray fluorescence Zn count rate, one can determine the concentration of Zn on the surface of the leaf.

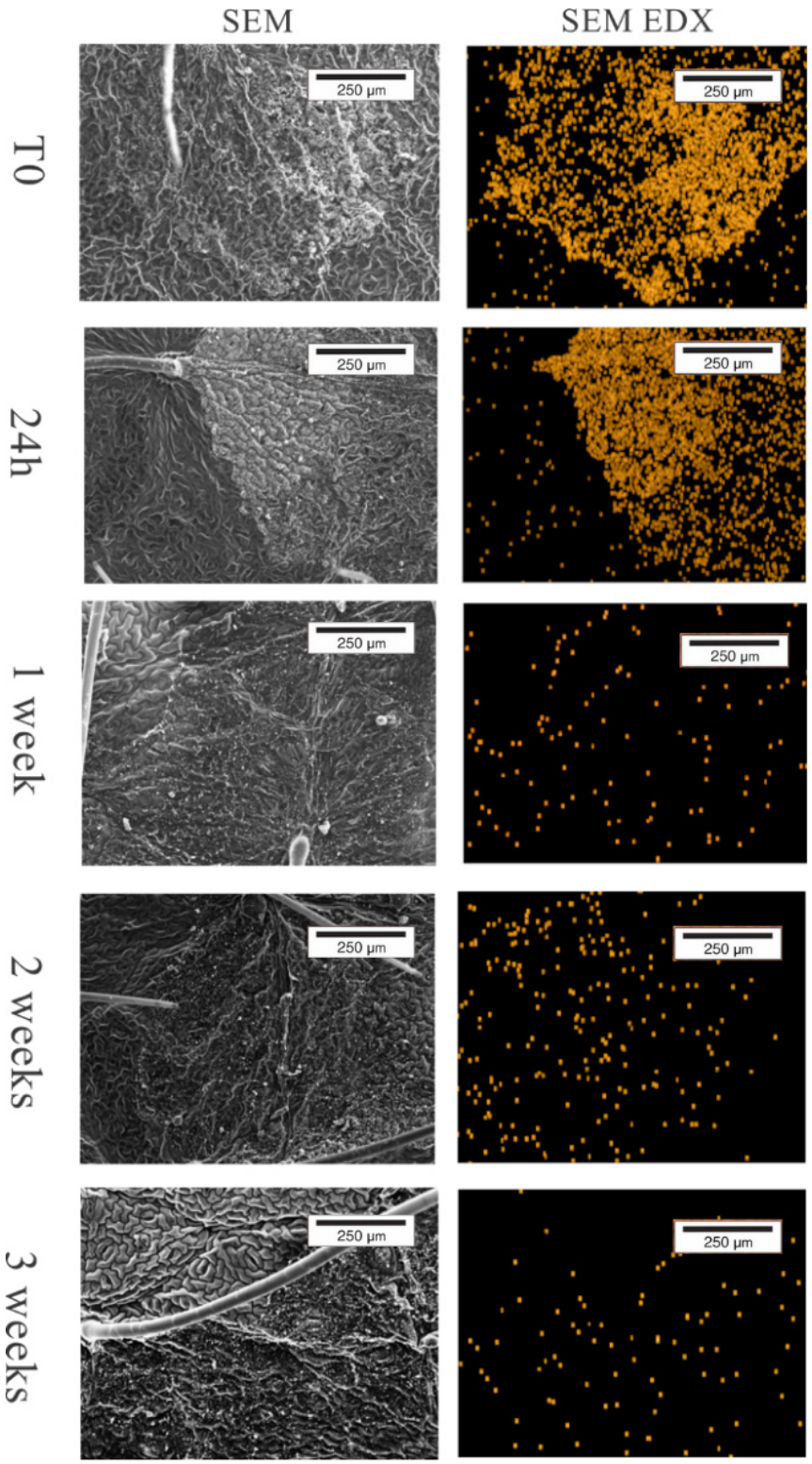

Figure 7. Scanning electron microscopy of soybean leaf adaxial surface revealing the leaf surface morphology and the spatial distribution of Zn on the surface leaf as a function of time.

Figure 8 shows the concentration of Zn in the droplets on the surface of the leaves as a function of time. The depth probed by the electron probe was approximately 4 µm, which ensures that this Zn quantification represents the Zn lying on the leaf surface, including within the cuticle layer, which is around 1 ± 10 µm thick. The Zn concentration on the analyzed spots decreases as a function of time; it takes 20 days for the concentration to reduce to 50% of the initial concentration.

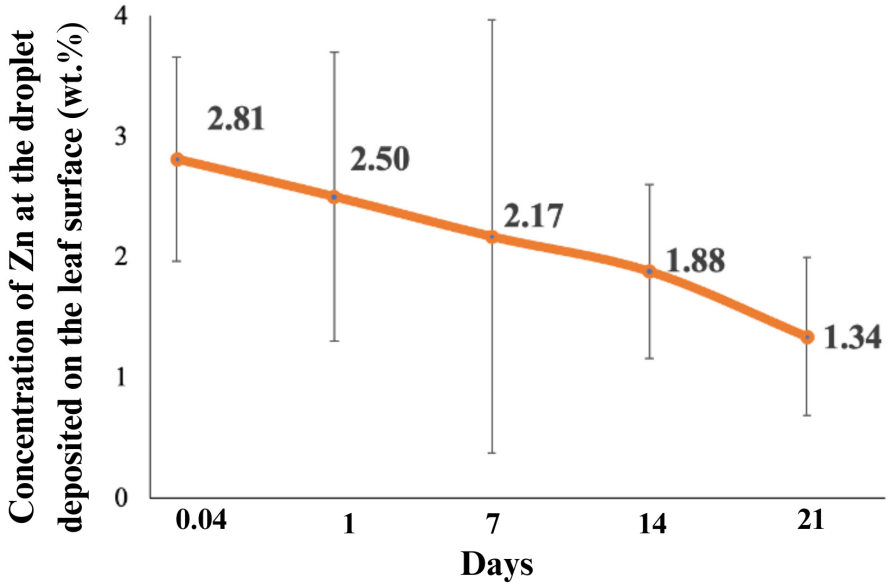

Figure 8. Concentration of Zn in the ZnO droplet deposited on the surface of the leaf as a function of days. Bars represent standard deviation.

4. Discussion

Like the present study, previous research has investigated the foliar uptake and transport of $ZnSO_4$ and ZnO by soybeans one week after spraying and found that in a short time range, the absorption of Zn from $ZnSO_4$ is faster than that of Zn from ZnO [22]. Similarly, in wheat leaves, the foliar application of Zn from both $ZnSO_4$ and ZnEDTA traveled less than 25 mm from the application site within 24 h. Most of the movement occurred between 3 h and 12 h post-application, with less movement observed between 12 and 24 h [23]. The present study, however, shows that in the long term, i.e., a timespan of a few weeks, the foliar sprayed nutrients can translocate much longer distances.

In durum and bread wheat, leaves treated with $^{65}ZnSO_4$ exhibited higher translocation in plants grown in a Zn-deficient nutrient solution. Approximately 40% of the total absorbed ^{65}Zn was translocated from the treated leaf to the roots and other shoot parts within 8 days. In contrast, in Zn-sufficient plants, the proportion of translocated ^{65}Zn was around 26% of the total absorbed. After 6 days of growth in nutrient solutions with Zn supplies, the oldest leaf tip sections were immersed in a ^{65}Zn solution for 10 s. The application solution contained 0.1 mM of $^{65}ZnSO_4$ (111 KBq ^{65}Zn). Plants were harvested 4 and 8 days after application. The treated leaf tips were cut and washed for approximately 10 min in a 10 mM $ZnSO_4$ solution to remove any ^{65}Zn adsorbed on the leaf surface and within the leaf apoplastic spaces [24].

Another study with a ^{68}Zn-stable isotope was conducted to demonstrate that the mature leaves of pistachio and walnut absorbed 12% and 8%, respectively, of the total applied zinc. Of this retained zinc, approximately 6.5% in pistachio and 3.5% in walnut was absorbed into the leaves and subsequently translocated beyond the treated area. These

findings suggest that the ability of leaves to absorb and redistribute Zn varies between crop species. Zinc absorption was measured at intervals from 10 min to 24 h post-application [25].

After 72 days and taking into account that the treatments were not rinsed by rain, it can be considered that the absorption was 100%, or close to it, for both sources. In this hypothetical scenario, the fraction of the translocated zinc relative to the absorbed zinc would be 7.8% for $ZnSO_4$ and 7.4% for ZnO; the values are similar since the translocation and remobilization are related to the Zn chemical species inside the plant, which is putatively the same for both Zn sources. However, we should consider that the rate of translocation is certainly dependent on the rate of absorption, which in turn depends on the source of the nutrient, the concentration of the spray, adjuvants, the air relative humidity, and the points of deliquesce and efflorescence of the formulations, which can be modified by adjuvants, as well. Therefore, using independent studies published in the literature to carry out a quantitative comparison of the translocation rates from one species to another might be challenging because the experimental design is not uniform. Some articles have reviewed the effects of nutrient deficiency on plant absorption and metabolism, highlighting that under deficient conditions, plants can exhibit adaptive mechanisms that lead to a reduction in nutrient uptake. This occurs as a response of the plant to conserve energy and allocate it to vital processes during nutrient scarcity.

Likewise, Zn deficiency resulted in a significant decrease in foliar Zn absorption from $ZnSO_4$ in sunflowers, ranging from 50% to 66%, compared to zinc-sufficient plants [26]. The same study showed that the primary factors contributing to the reduced Zn absorption under zinc deficiency conditions were the decrease in leaf trichome density and potential alterations in the composition and structure of the adaxial leaf surface compared to those that we could study in this experiment. When the plants were conducted in 10% lower Zn, the number of trichomes and stomas decreased, explaining why the absorption of label sources was low. Thus, in the present study, the relatively low translocation of Zn shown in Table 1 might also have contributed to the low Zn content in the growth solution that contained only 10% of the regular Zn ionic strength.

In Bromeliaceae, only the negative control (no Zn and no Se) produced a lower stomatal density [27]. The experiment was carried out in vitro for 75 days in Zn concentrations (0, 30, and 300 μM) combined with two Se concentrations (0 and 4 μM). The plants were treated 21 days before harvest with Hoagland nutrient solution [28]. These results were observed in a study on safflower crops, which were grown in a nutrient solution containing 0, 10, and 20 mg dm^{-3} of Zn ($ZnSO_4$) for three weeks. Plants receiving 20 mg dm^{-3} of Zn exhibited a greater number of xylem vessels with larger diameters compared to those treated with lower Zn concentrations [29].

The cuticle composition in the basal cells of non-glandular trichomes in sunflowers differs from that of regular leaf epidermal cells, which may facilitate Zn absorption [30]. Both the basal cells and trichomes possess pectin-rich, hydrophilic cell walls that could enhance nutrient uptake [31]. Differences in stomatal nutrient absorption may be influenced by external factors affecting the wettability of guard cells, potentially activating stomata for solute transport [32]. For instance, Zn phosphite particles can agglomerate in stomata and release ions in humid conditions, thus increasing Zn uptake through this pathway [33], which is a response also noted in rice treated with ZnO nanoparticles [34]. Acidic Zn treatments reduce cation fixation on leaf cuticles, aiding Zn entry [6]. Furthermore, Zn was primarily detected in the xylem and parenchyma cell walls of treated plants, but not in the controls, potentially explaining the xylem size differences between the control and 10% Zn treatments [35].

5. Conclusions

The present study concludes that both ZnO and $ZnSO_4$ sources can supply Zn to soybean plants through foliar application. The radiolabeled Zn deposited on the leaves was detected in roots, stems, new leaves, and even grains.

Although it was not possible to determine the rates of absorption, the rate of transport of Zn from $ZnSO_4$ was higher than that of ZnO at the first harvest, 12 days after spray application. Based on previously published results and a parallel ongoing investigation by our group, we believe that this is explained by the higher absorption rate of Zn from $ZnSO_4$ compared to ZnO. However, after a longer period, specifically, 72 days past the first spraying, the translocation rates of both ZnO and $ZnSO_4$ were similar. This happens because under such a long period, the time is not a limiting factor for ZnO absorption. We can also conclude that ZnO acts as a slow-release source of Zn; it requires around 20 days for 50% of the Zn to be absorbed by the soybean leaves.

The fraction of Zn transported to plant parts that did not receive the spray was below 2% of the total Zn applied to the plants after 12 days. This emphasized the importance of splitting the applications since most of the Zn will remain in the treated organs. After 72 days, the fraction of translocated Zn was 8.03% for ZnO and 8.48% for $ZnSO_4$, relative to the total Zn deposited on the plant leaves.

Zinc deficiency significantly affects the number of stomata and the size of the xylem, suggesting that Zn plays a role in modulating the xylem development or expansion but does not have a significant influence on trichomes.

Also, the results from the chemical map demonstrate that when ZnO droplets were applied to the leaves, a noticeable reduction in droplet size was observed. This result reinforces that leaves can absorb ZnO fertilizer, causing the droplets to disappear in size as the nutrients were taken up by the plant.

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