Agroind. sci. 15(1): 89-97 (2025)

Agroindustrial Science

Website: http://revistas.unitru.edu.pe/index.php/agroindscience

Escuela de Ingeniería Agroindustrial

Universidad Nacional de Trujillo

Aminochelated and microparticulated zinc applied to citrus grown in calcareous soil

Osbaldo Martínez-Ríos[1] *; José Isabel Cortés-Flores[1]; Alfredo López-Jiménez[2]; Jorge Dionisio Etchevers-Barra[1]; María Belia Contreras-Soto[3]

[1] Posgrado en Edafología. Colegio de Postgraduados, Campus Montecillo, Texcoco 56264, Estado de México, México.
[2] Posgrado en Recursos Genéticos y Productividad - Fruticultura. Colegio de Postgraduados, Campus Montecillo, Texcoco 56264, Estado de México, México.
[3] Laboratorio de Fitopatología, Centro de Investigación en Alimentación y Desarrollo. Campo el Diez, 80110, Culiacán, Sinaloa, México.

ORCID de los autores:
O. Martínez-Ríos https://orcid.org/0009-0009-1469-1571
A. López-Jiménez https://orcid.org/0000-0002-1515-1560
M. B. Contreras-Soto https://orci.org/0000-0002-2566-3711
J. I. Cortés-Flores https://orcid.org/0000-0002-0107-5069
J. D. Etchevers-Barra https://orcid.org/0000-0003-4663-6306

ABSTRACT

The objective of this study was to evaluate the effect of foliar fertilization with alternative zinc (Zn) sources on the nutritional status and growth of young ´Valencia´ orange (*Citrus sinensis* L. Osbeck) trees grown in calcareous soil (pH = 8.1). Nine treatments were tested: the commercial amino chelates Aton Zn (0.3% and 0.5%) and Kelatex Zn Forte (0.5% and 1.0%); the commercial Zn microparticles Basfoliar Zn 75 Flo (0.1% and 0.2%); $ZnSO_4H_2O$ (0.3% and 0.5%); and a control treatment with no Zn application. The Zn concentration in leaves increased with the application of Aton Zn (0.3%), Kelatex Zn Forte (0.5% and 1.0%), and $ZnSO_4H_2O$ (0.3% and 0.5%). The Zn concentration in roots increased only in trees sprayed with Kelatex Zn Forte (1.0%). The chlorophyll index (SPAD readings) decreased in most treatments, except in leaves sprayed with $ZnSO_4H_2O$ (0.3%) and Kelatex Zn Forte (1.0%). The N concentration in leaves increased with the application of $ZnSO_4H_2O$ (0.5%), while P levels did not increase in any treatment. The foliar K concentration increased in trees sprayed with Aton Zn (0.3% and 0.5%), Basfoliar Zn 75 Flo (0.1%) and $ZnSO_4H_2O$ (0.3%). The concentrations of Mn, Cu, and B in leaves remained unchanged across all treatments. Foliar Fe concentration increased in trees sprayed with Kelatex Zn Forte (0.5%) and $ZnSO_4H_2O$ (0.3%). Zn application had no significant effect on tree growth. The amino chelate Kelatex Zn Forte at a 1.0% dose shows promising potential by increasing Zn concentrations in leaves and roots while maintaining the chlorophyll index.

Keywords: young trees; *Citrus sinensis*; chlorophyll; Zn concentration; growth.

1. Introduction

´Valencia´ orange (*Citrus sinensis* L. Osbeck) is one of the main citrus species cultivated in northeastern Mexico, where calcareous soils predominate. Most crops established in these soils show zinc (Zn) deficiency (Bolan et al., 2023), which affects citrus production in soils with high calcium carbonate ($CaCO_3$) content and, occasionally, in acidic soils (Fu et al., 2016).

Zn is an essential element for plants, involved in the synthesis of chlorophyll, nucleic acids, and auxins. Additionally, it acts as a regulatory cofactor in various enzymes and participates in protein metabolism (Asadi et al., 2020). A deficient Zn concentration is critical in crops, as it leads to reduced tree vigor, lower photosynthetic activity, poor fruit setting, and smaller fruit size and quality (Fei et al., 2016).

Recibido 14 enero 2025
Aceptado 2 marzo 2025

* Autor correspondiente: martinez.osbaldo@colpos.mx (O. Martínez-Ríos)
DOI: http://doi.org/10.17268/agroind.sci.2025.01.10

The fertilizers commonly used to manage Zn deficiency are monohydrated zinc sulfate ($ZnSO_4H_2O$), heptahydrated zinc sulfate ($ZnSO_47H_2O$), and synthetic chelates such as EDTA-Zn and DTPA-Zn. In calcareous soils, these fertilizers exhibit low efficiency when applied directly to the soil or through fertigation, as Zn is rapidly retained by the $CaCO_3$ and $MgCO_3$ present in these soils (Srivastava & Singh, 2005).

Foliar fertilization is considered the most efficient method for correcting Zn deficiency in fruit crops grown in alkaline soils, compared to soil applications (Xie et al., 2020). However, the metal salts and synthetic chelates commonly used for this purpose exhibit low absorption by leaves (Souri & Hatamian, 2019). Their translocation to other plant organs such as the stem, roots, and fruit is limited, due to the low permeability of the cell membrane and the mobility of Zn within the plant (Gupta et al., 2016). For these reasons, Zn deficiency persists in citrus crops. An alternative approach to mitigate Zn deficiency is the application of alternative fertilizers to traditional ones, such as amino chelates and micro-fertilizers (flowable sources) (Macedo et al., 2021; Souri & Hatamian, 2019).

Amino chelates are newly formulated fertilizers that have been evaluated in various crops. They exhibit high efficiency of use and do not cause environmental pollution (Souri & Hatamian, 2019); additionally, they improve the translocation of micronutrients such as Zn within the plant (Ghasemi et al., 2013). Zinc oxide (ZnO) is poorly soluble in water, which limits Zn absorption by plant leaves when applied as a foliar fertilizer. However, this limitation can be overcome by reducing the particle size of the oxide to microparticle level (0.2-20 µm) (Du et al., 2015).

This research evaluated the effect of foliar application of amino-chelates and Zn microparticles on the nutritional status and growth of ´Valencia´ orange trees grown in calcareous soil, aiming to determine the most effective Zn source for correcting Zn deficiency.

2. Methodology

2.1 Growing conditions and experimental design

The experiment was conducted in a greenhouse at the Colegio de Postgraduados, Montecillo, Texcoco, State of Mexico, Mexico, located at latitude 19° 27' 30" N and longitude 98° 54' 14" W, at an altitude of 2,240 meters, with an average annual temperature of 15.9 °C and annual precipitation of 680 mm. Nine-month-old ´Valencia´ orange trees (*Citrus sinensis* L. Osbeck), grafted onto C35 rootstock (*Citrus sinensis* L. Osbeck x *Poncirus trifoliata* L. Raf.), were used. The average plant height at the time of transplanting was 75 cm. The trees were transplanted into 21 L capacity pots, which contained 18 kg of calcareous soil. The physical and chemical characteristics of the soil at the beginning of the experiment were as follows: pH = 8.1, clay texture, electrical conductivity (EC) of 0.36 dS m^{-1}, organic matter content of 3.2%, extractable Olsen phosphorus of 8.5 mg kg^{-1}, Kjeldahl nitrogen of 0.2%, and exchangeable potassium of 1.2 $cmol_{(+)}$ kg^{-1}. The concentrations of Zn, Cu, Mn, and Fe in the soil were 1.2, 0.5, 17, and 2.4 mg kg^{-1}, respectively, while the $CaCO_3$ concentration was 46.5%.

At the time of tree transplanting, the soil was fertilized with N, P, K, Mg, Fe, Cu, Mn, and B (30.0, 21.6, 30.0, 5.0, 1.4, 1.4, 1.4, and 0.1 g per tree, respectively), except for Zn, which was the element being evaluated. Urea and diammonium phosphate were used to supply N and P, while the respective sulfates provided K, Mg, Fe, Cu, and Mn, and B was supplied as borax. The doses of macro and micronutrients were determined according to the guidelines reported by Obreza et al. (2020). One-third of the nitrogen (N) was applied at transplanting, and the remaining amount was divided into equal parts and applied during the first ten irrigations, with an approximate interval of 5 days, without any loss of water or fertilizers. A topping pruning was performed 20 days (d) after transplanting, at 35 cm above the grafting point, to induce new vegetative growth and achieve greater tree uniformity. Irrigation was applied each time the Irrometer ISR-300® tensiometer (USA) recorded soil tension between 15 and 20 kPa. The average minimum and maximum temperatures inside the greenhouse were 9 °C and 37 °C, respectively, with an average relative humidity of 57%.

The treatments (T) consisted of the foliar application of four Zn sources, each at two doses: the amino chelates Aton Zn® at 0.3% and 0.5% (T1 and T2) and Kelatex Zn Forte® at 0.5% and 1.0% (T3 and T4); Zn microparticles Basfoliar Zn 75 Flo® at 0.1% and 0.2% (T5 and T6); $ZnSO_4H_2O$ at 0.3% and 0.5% (T7 and T8); and the control (T9), with no Zn application (Table 1).

The foliar application of treatments began on September 1, 2022, 50 days after transplanting the trees. Seven applications were made, two in September, October, and November 2022, with a final application in mid-March 2023. The amount

of solution used for spraying was proportional to the tree canopy to ensure good coverage. The pH of the solution for each treatment was adjusted to 5.5 with DAP-PLUS®, and INEX-A® was added as a surfactant at a dose of 0.1% (v/v).

Table 1
Treatments evaluated on young ´Valencia´ orange trees (*Citrus sinensis* L. Osbeck) grafted onto C35 rootstock (*Citrus sinensis* L. Osbeck x *Poncirus trifoliata* L. Raf) and transplanted into calcareous soil

Nº Treatment	Source of Zn	Dose (%)[a]
1	Aton Zn	0.3 (v/v)
2	Aton Zn	0.5 (v/v)
3	Kelatex Zn Forte	0.5 (w/v)
4	Kelatex Zn Forte	1.0 (w/v)
5	Basfoliar Zn 75 Flo	0.1 (v/v)
6	Basfoliar Zn 75 Flo	0.2 (v/v)
7	$ZnSO_4H_2O$	0.3 (w/v)
8	$ZnSO_4H_2O$	0.5 (w/v)
9	Control	-

[a](v/v) = volume/volume ratio; (w/v) = weight/volume ratio.

Tree growth was evaluated by stem diameter and tree height, number of sprouts, number of leaves per sprout, sprout length, leaf area, and aerial biomass dry weight. The nutritional status was analyzed based on the chlorophyll index (SPAD), concentrations of N, P, K, Zn, Fe, Mn, Cu, and B in leaves, and the Zn concentration in roots. All variables were determined in six trees per treatment, except for the Zn concentration in roots, which was evaluated at the end of the experiment in three trees per treatment.

2.2 Stem diameter and tree height

The stem diameter of the trees was measured using a Steren® HER-411 digital caliper (USA) at a height of 8 cm above the grafting point of the variety, and the tree height was measured with a tape measure from the soil level to the apex. Both variables were evaluated every 30 d throughout the experiment, with a total of eight measurements recorded.

2.3 Number of sprouts and leaves per sprout

The number of sprouts and leaves per sprout was counted, considering the spring, summer, and fall growth periods. Each sprout was identified and monitored. These determinations were made eight times, with a 30-day interval throughout the experiment.

2.4 Sprout length

The sprout length was measured using a tape measure, starting 80 d after the initiation of treatments. During the experimental period, five measurements were made, with a 40-day interval between each determination.

2.5 Leaf area

Leaf area was estimated on five occasions. In the first four, it was obtained using a non-destructive method, measuring the length and width of the leaf with a 30 cm ruler. In the fifth and final measurement, a leaf area integrator (LI-COR-3000A; Lincoln, NE, USA) was used. This variable was assessed at 48-day intervals during the experimental period.

2.6 Aerial biomass dry weight

The dry weight of aerial biomass was determined at the end of the experiment, 240 d after the initiation of treatments. The stem and leaves of each tree were dried in a forced-air oven (Riossa, HCF-125, Mexico) at 70 °C until reaching a constant weight (approximately 72 h), then weighed using an analytical balance (Adventurer Ohaus Pro, AV213C; Parsippany, NJ, USA). The rootstock stem of the trees was not included.

2.7 Chlorophyll index

The chlorophyll index was determined based on SPAD readings, which provide a relative index closely correlated with chlorophyll content in leaves. Eight evaluations were made throughout the experimental period using a SPAD Plus Minolta 502 (USA), which measures light transmission at wavelengths between 650 and 940 nm over an area of 0.06 cm² (Kulig et al., 2024). The readings were taken at three points on a fully developed leaf, between the middle portion and the apex of three buds of the same age on each tree, and the average was calculated (Intrigliolo et al., 2000). The leaves were marked, and measurements were taken every 30 d during the experimental period.

2.8 Concentration of macro and micronutrients in leaves

Leaf sampling for macro- and micronutrient analysis was conducted twice: at the beginning of the treatments and at the end of the experiment, that is, 240 d after transplanting. One day before the first application of the treatments, the initial leaf sampling was performed on 20 trees out of the 54 that made up the experiment, considering that uniform management conditions were applied to all plants at the start. Six leaves were collected per tree from spring 2022 shoots that were four to six months old. The leaves were successively washed in running water, a 0.1M HCl acid solution, and

distilled water in the laboratory. They were then left at room temperature on kraft paper for 30 min and subsequently dried in a forced air oven (Riossa, HCF-125, Mexico) at 70 °C until they reached a constant weight (approximately 72 h). The samples were ground using a Thomas-Wiley stainless steel mill (NJ, USA). For the analysis of P, K, Ca, Mg, Zn, Fe, Mn, and Cu, 0.5 g of leaf tissue was weighed and then digested with a mixture of $HNO_3/HClO_4$ (v/v, 2:1). The concentration of K, Ca, Mg, Zn, Fe, Mn, and Cu in the extracts was measured using an atomic absorption spectrometer (Varian SPECTRAA 220 FS, Australia). The concentration of P was determined using a HACH UV/VIS DR 5000 (Iowa, USA) by visible spectrophotometry. The determination of N was performed using the modified semi-micro Kjeldahl method (Bremner, 1965). For digestion, 0.1 g of the sample was weighed, and a mixture of $H_2SO_4/C_7H_6O_3$ was added. The concentration of B was determined by the azomethine-H method (Gaines & Mitchell, 1979). A second sampling and analysis were performed at the end of the experiment. Twenty leaves, four to six months old, were collected from the autumn 2022 flush, in each of the six trees per treatment. The processing of the foliar tissue samples and their analysis were conducted in the same manner as the first, as described above. The same nutrients determined at the start of the treatments were analyzed. The nutrient concentrations were expressed relative to dry matter (DM).

2.9 Concentration of Zn in the root

Root sampling for the analysis of Zn was conducted at the end of the experiment, that is, 240 d after the application of treatments. To obtain the roots, each tree was removed from the pot. The aerial part was separated from the root ball, which was treated with successive volumes of running water to remove the adhered soil. The cleaning, drying, and grinding of the samples were performed in the same way as the foliar tissue samples described earlier. Half a gram of the dry, ground root was digested with the $HNO_3/HClO_4$ (v/v, 2:1) mixture, and the extract was then analyzed using an atomic absorption spectrophotometer (Varian SPECTRAA 220 FS, Australia).

2.10 Statistical analysis

The effect of treatments on the response variables: stem diameter and tree height, number of shoots and leaves per shoot, shoot length, leaf area, dry biomass of the aerial part, chlorophyll index, concentration of macro and micronutrients in leaves, and concentration of Zn in the root, was evaluated by performing an ANOVA (Analysis of Variance). The Shapiro-Wilk (q-q plot) and Bartlett tests were used to verify the assumptions of normality and homogeneity of variances, respectively. The means were compared using the Tukey test at a probability level of 0.05. The statistical program R-studio version 4.1.1 was used.

3. Results and discussion

3.1 Tree Growth

No significant effect was observed from the foliar Zn applications on tree growth at any evaluation date (Table 2). Zn deficiency affects plant metabolism and consequently growth, as it is involved in the regulation and activation of genes, protein synthesis, and carbohydrate metabolism (Clemens et al., 2022; Hamzah et al., 2022). Moreover, Zn is involved in the regulation of hormones and participates in the synthesis of tryptophan (precursor of indoleacetic acid, the predominant auxin in plants) (Sourati et al., 2022).

Table 2
Effect of foliar applications with different sources and doses of Zn on the growth of young ´Valencia´ orange trees transplanted in calcareous soil

Source of Zn (%)	SD (mm) †	TH (cm)†	NS†	NLS†	SL (cm) †	LA (cm^2) †	DM (g) †
AZn (0.3)	7.5 a	105.9 a	11.6 a	6.2 a	19.6 a	2165.4 a	23.0 a
AZn (0.5)	7.5 a	102.6 a	7.5 a	5.3 a	18.7 a	1965.0 a	21.6 a
KZn (0.5)	7.7 a	104.0 a	12.8 a	4.2 a	16.0 a	1968.6 a	21.2 a
KZn (1.0)	7.3 a	99.7 a	17.3 a	5.1 a	17.1 a	1898.5 a	24.5 a
BZn (0.1)	6.8 a	93.9 a	9.6 a	5.4 a	19.9 a	1733.8 a	21.0 a
BZn (0.2)	7.3 a	103.6 a	11.1 a	4.8 a	20.2 a	2204.8 a	23.6 a
$ZnSO_4H_2O$ (0.3)	7.4 a	100.8 a	11.5 a	5.1 a	18.5 a	1492.0 a	22.8 a
$ZnSO_4H_2O$ (0.5)	7.1 a	97.9 a	14.0 a	5.5 a	14.9 a	1851.8 a	20.7 a
Control	7.2 a	104.4 a	16.1 a	5.6 a	16.0 a	2082.9 a	27.3 a
MSD	1.32	17.85	15.89	4.09	5.74	1716.3	14.49

AZn = Aton Zn; KZn = Kelatex Zn Forte; BZn = Basfoliar Zn 75 Flo; $ZnSO_4H_2O$ = monohydrated zinc sulfate; MSD = minimum significant difference; SD = stem diameter; TH = tree height; NS = number of shoots; NLS = number of leaves per shoot; SL = shoot length; LA = leaf area; DM = dry biomass. † Mean values ± SE with different letters in the column indicate significant differences (Tukey, $p < 0.05$).

Auxins are involved in apical dominance, phototropism, senescence, response to pathogens, response to abiotic stress and fruit formation, thus regulating plant growth and development (Gao et al., 2024). In young ´Washington navel´ orange trees grown in alkaline soil, foliar application of solutions containing 0.2, 0.4, 0.6, 0.8, and 1.0% $ZnSO_4$ increased diameter and height after two years of evaluation (Dawood et al., 2001). Similarly, foliar applications of Zn increased the leaf area in pecan trees (*Carya illinoinensis*) established in soil with 30% $CaCO_3$ (Ojeda et al., 2014). The authors report positive effects on plant growth after two years of evaluation in soils with a 30% concentration of $CaCO_3$. However, in this experiment, the $CaCO_3$ concentration of the soil was 46.5%, and the study period was eight months, conditions that may have influenced the lack of response.

3.2 Chlorophyll Index

No statistical differences were found in the chlorophyll index in response to the application of the different Zn sources, at any evaluation date, compared to the control. However, the results of the last measurement show that SPAD values tended to decrease in most treatments, except for trees treated with $ZnSO_4H_2O$ at a low dose, which showed a slight increase of 1.7 SPADs (Table 3). Trees sprayed with the aminoquelate Kelatex Zn Forte at a high dose showed a minimal decrease of 1.0 SPADs (Table 3). Applications of Zn increase the chlorophyll content in plants (Umair et al., 2020), because Zn regulates the amount of iron (Fe) and magnesium (Mg) in the cytoplasm, which are essential nutrients in chlorophyll molecule synthesis (Makhasha et al., 2024). In ´Fengwan´ navel orange trees (*Citrus sinensis* L. Osbeck), chlorophyll index in leaves increased when sprayed with $ZnSO_4$, $ZnCl_2$, and $Zn(NO_3)_2$ (Fu et al., 2016).

3.3 Concentration of macronutrients in leaves

The macronutrient concentrations at the beginning of the treatments were low: N (22.0 g kg^{-1}), P (1.1 g kg^{-1}), and K (11.3 g kg^{-1}), compared to the concentrations indicated by Obreza et al. (2020). The concentration of N in the leaves sprayed with $ZnSO_4H_2O$ at 0.5% increased significantly compared to the control. At the end of the experiment, this concentration was 43.0 g kg^{-1} (Figure 1A).

Similar results were obtained by Nasir et al. (2016) when they applied moringa extract, K, and Zn to the foliage of ´Kinnow´ mandarin, increasing the foliar N concentration. $ZnSO_4H_2O$ applied at the high dose (0.5%) also induced a higher level of foliar Zn (Table 4), demonstrating the synergism between Zn and N (Prasad et al., 2016). In all treatments, the N concentration in leaves was excessive at the end of the evaluation (>30.0 g kg^{-1}), but no toxicity symptoms due to this element were observed.

The concentration of P in leaves did not show a significant increase compared to the control (Figure 1B). Although the P level was high in all treatments (0.17 – 0.30 g kg^{-1}), it did not affect the Zn concentration in some treatments, where an optimal Zn status was observed in leaves (25 – 100 mg kg^{-1} Zn) (Obreza et al., 2020). An excess of P causes a Zn deficiency in plants (Bouain et al., 2014). However, in the present experiment, the trees sprayed with $ZnSO_4H_2O$ at the high dose (0.5%) showed the highest concentration of both Zn and P in the leaves.

Significant differences were observed in the leaf K concentration (Figure 1C). At the end of the experiment, Aton Zn increased the K concentration to 27.9 and 30.7 g kg^{-1} for trees sprayed with 0.3% and 0.5%, respectively. Similarly, Basfoliar Zn 75 Flo (0.1%) and $ZnSO_4H_2O$ (0.3%) increased the K concentration in leaves to 30.0 and 29.0 g kg^{-1}, respectively.

Table 3
Chlorophyll Index (SPAD) in ´Valencia´ orange leaves sprayed with different Zn sources at 0 and 240 days

Source of Zn (%)	Chlorophyll Index (SPAD) †		Difference in Chlorophyll Index ($SPAD_{240D}$ - $SPAD_{0D}$)
	Start of Evaluation (0D)	End of evaluation (240D)	
AZn (0.3)	44.9 ± 3.8 a	30.7 ± 5.5 b	- 14.2
AZn (0.5)	47.9 ± 2.3 a	37.5 ± 6.6 ab	- 10.4
KZn (0.5)	52.8 ± 12.0 a	44.4 ± 7.1 a	- 8.4
KZn (1.0)	45.5 ± 4.0 a	44.4 ± 2.8 a	- 1.0
BZn (0.1)	51.8 ± 14.4 a	42.4 ± 4.3 a	- 9.3
BZn (0.2)	50.2 ± 2.8 a	45.4 ± 3.0 a	- 4.8
$ZnSO_4H_2O$ (0.3)	46.0 ± 5.9 a	47.8 ± 6.1 a	1.7
$ZnSO_4H_2O$ (0.5)	50.3 ± 5.8 a	44.0 ± 3.4 a	- 6.2
Control	52.8 ± 7.6 a	46.7 ± 6.9 a	- 6.1

AZn = Aton Zn; KZn = Kelatex Zn Forte; BZn = Basfoliar Zn 75 Flo; $ZnSO_4H_2O$ = monohydrated zinc sulfate; D = days. † Average values ± SE with different letters in the column indicate significant differences (Tukey, $p < 0.05$).

Mosa et al. (2021) published similar results concerning aminochelates, *i.e.*, the application of tryptophan and glycine to the leaves caused an increase in the leaf content of N, P, K, and Ca in ´Anna´ apple trees (*Malus domestica* L. Borkh). At the end of the experiment, the K concentration in leaves was high (18.0 – 24.0 g kg^{-1}) in the control trees and those sprayed with Kelatex Zn Forte (0.5%), while the rest of the treatments showed excess levels (>24.0 g kg^{-1}) (Obreza et al., 2020). The trees sprayed with Kelatex Zn Forte (0.5%) had the lowest concentrations of N, P, and K in the leaves at the end of the experiment; however, these levels remained above the optimal level for ´Valencia´ orange trees (Figure 1A, 1B y 1C). The low concentrations of N, P, and K in the same treatment may be due to interactions between these nutrients. A low K level leads to reduced N absorption by plants because K is essential for the absorption and transport of nitrate (NO_3^-) (Fageria, 2001). Synergism between N and P in plants has been documented, with N promoting the absorption of P (Krouk & Kiba, 2020). It is believed that the low K concentration in the leaves led to a lower N level, which resulted in a similar P content due to the synergism between these nutrients.

The soil fertilization carried out at the time of transplanting adequately provided the required amounts of N, P, and K in all treatments, including the control. The leaf concentrations of these elements at the beginning of the treatments were low, and by the end of the experiment, they were high and/or excessive (Obreza et al., 2020).

3.4 Micronutrient Concentration in Leaves

Regarding the micronutrient concentration in leaves, at the beginning of the treatments, Zn (19.3 mg kg^{-1}) and Fe (45.9 mg kg^{-1}) were at low levels, Mn (7.1 mg kg^{-1}) was deficient, while Cu (4.8 mg kg^{-1}) and B (83.8 mg kg^{-1}) were at optimal levels (Obreza et al., 2020).

Foliar applications of Zn had a significant effect on Zn concentration in the leaves. Trees sprayed with Aton Zn (0.3%), Kelatex Zn Forte (0.5% and 1.0%), and $ZnSO_4H_2O$ (0.3% and 0.5%) showed a significant increase in foliar Zn concentration by the end of the experiment, placing them within the optimal range for ´Valencia´ orange trees (25–100 mg kg^{-1} Zn) Obreza et al., 2020) (Table 4).

Aton Zn (0.3%) increased the Zn concentration 2.35-fold compared to the control. Similarly, Kelatex Zn Forte enhanced the Zn concentration by 2.05 and 3.77 times relative to the control for doses of 0.5% and 1.0%, respectively (Table 4).

A study on the effect of foliar applications of Zn complexed with the amino acids methionine and lysine, and $ZnSO_4$ on pistachio (*Pistacia vera* L.) ´Akbari´ reported a higher Zn concentration in leaves sprayed with $ZnSO_4$ (58 mg kg^{-1} Zn), followed by the treatment with Zn complexed with methionine (45 mg kg^{-1} Zn) (Najizadeh & Khoshgoftarmanesh, 2019).

$ZnSO_4H_2O$ applied at doses of 0.3% and 0.5% increased the foliar Zn concentration by 3.57 and 5.43 times, respectively, compared to the control (Table 4). Boaretto et al. (2023) reported an increase in foliar Zn concentration up to 70 mg kg^{-1} in leaves of ´Pera´ orange trees (*Citrus sinensis* L. Osbeck) after foliar sprays with $ZnSO_47H_2O$ at a dose of 2 kg ha^{-1}, while the control showed concentrations ranging from 10 to 27 mg kg^{-1} Zn. Frequent foliar sprays with $ZnSO_4$ can correct Zn deficiency in leaves; however, its translocation to other plant organs is limited (Zekri & Obreza, 2003).

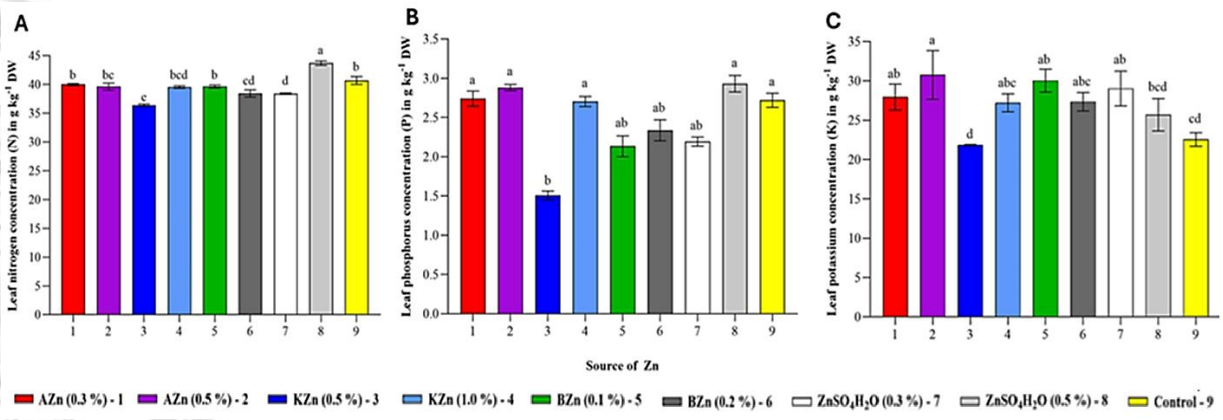

Figure 1. Foliar concentration of nitrogen (A), phosphorus (B), and potassium (C) in one year and five-month-old ´Valencia´ orange trees, transplanted into calcareous soil and sprayed with different Zn sources. AZn = Aton Zn; KZn = Kelatex Zn Forte; BZn = Basfoliar Zn 75 Flo; $ZnSO_4H_2O$ = monohydrate zinc sulfate. Different letters indicate significant differences (Tukey, $p < 0.05$).

Table 4
Increase in Zn concentration in ´Valencia´ orange leaves (number of times higher than the control value) following foliar application of different Zn sources

Source of Zn (%)	Zn Concentration (mg kg^{-1}) †	Number of times (Zn treatment/Control treatment) - 1
Aton Zn (0.3)	32.14 c	2.35
Aton Zn (0.5)	14.48 e	0.50
Kelatex Zn Forte (0.5)	29.29 d	2.05
Kelatex Zn Forte (1.0)	45.78 b	3.77
Basfoliar Zn 75 Flo (0.1)	10.86 f	0.13
Basfoliar Zn 75 Flo (0.2)	11.95 ef	0.24
$ZnSO_4H_2O$ (0.3)	43.86 b	3.57
$ZnSO_4H_2O$ (0.5)	61.73 a	5.43
Control	9.59 f	-

† Average values with different letters in the column indicate significant differences (Tukey, $p < 0.05$).

Zinc microparticles (Basfoliar Zn 75 Flo) did not significantly increase Zn concentration in leaves, resulting in deficient levels for optimal requirements (25–100 mg kg^{-1} Zn) (Obreza et al., 2020) (Table 4). In contrast, an increase in foliar Zn concentration was observed in ´Pera´ orange trees following foliar sprays with ZnO microparticles (Oliveira et al., 2020).

The foliar concentration of Fe at the end of the experiment showed a significant increase only in trees sprayed with Kelatex Zn Forte (0.5%) and $ZnSO_4H_2O$ (0.3%), being 21.71% and 15.89% higher than the control, respectively (Figure 2A).

No significant differences were observed in the foliar concentration of Mn, Cu, and B compared to the control (Figure 2B, 2C y 2D). Najizadeh & Khoshgoftarmanesh (2019) found no effect on the concentrations of Mn and Cu in leaves when performing foliar sprays with Zn amino chelates in pistachio trees; they only observed a significant increase in the foliar content of Fe. At the conclusion of this experiment, in all treatments, the Mn leaf status was optimal (25 – 100 mg kg^{-1}), B was high (101 – 200 mg kg^{-1}), and Fe (>200 mg kg-1) and Cu (>20 mg kg^{-1}) were in excess, with reference to Obreza et al. (2020).

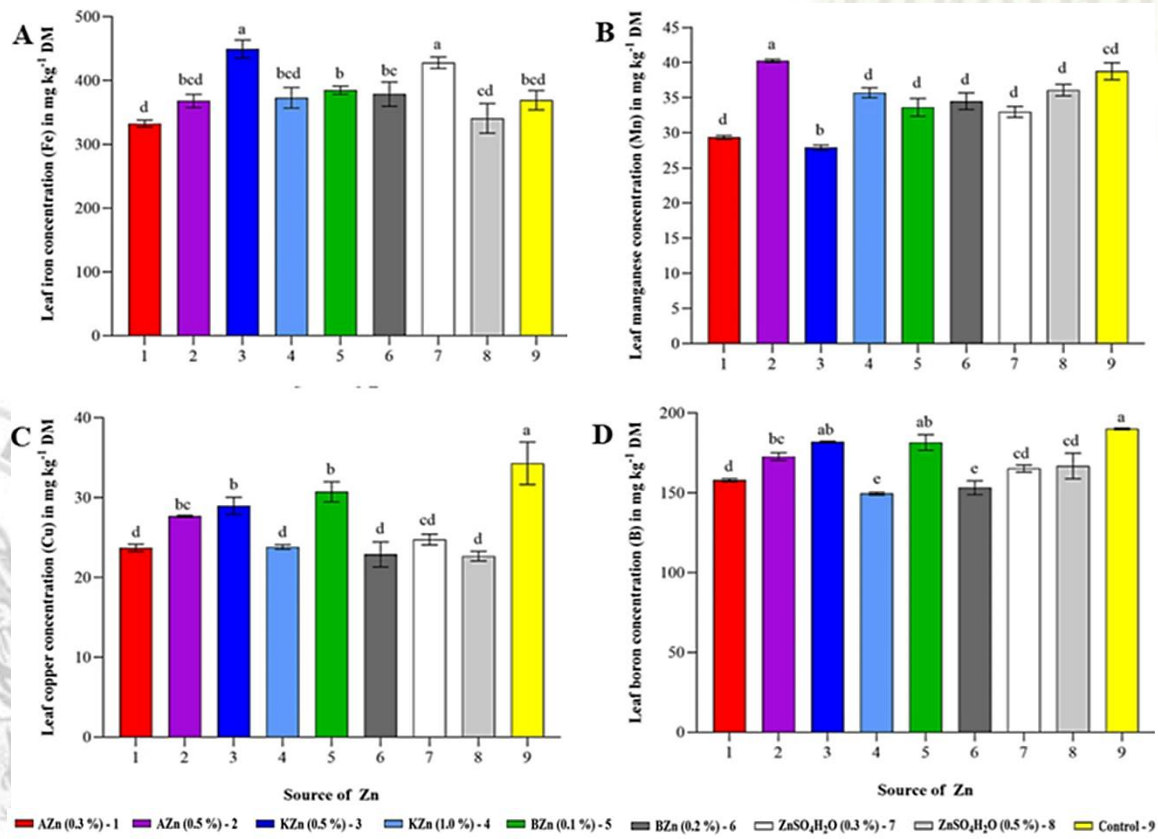

Figure 2. Leaf iron (A), manganese (B), copper (C), and boron (D) concentrations in one-year-and-five-month-old 'Valencia' orange trees transplanted into calcareous soil and sprayed with different sources of Zn. Different letters indicate significant differences between them (Tukey, $p < 0.05$).

3.5 Zinc concentration in the root

The zinc concentration in the roots of trees treated with the amino chelate Aton Zn (0.5 %) increased to 23.59 mg kg^{-1}, and this increase was significant. Trees that received this treatment also had an optimal foliar zinc concentration (45.7 mg kg^{-1}) (Obreza et al., 2020) (Table 4).

No significant differences were observed in the zinc concentration in the roots for the other treatments. Mirboloock et al. (2021) reported that foliar applications of zinc complexed with glycine, phenylalanine, tryptophan, and alanine increased the zinc concentration in the roots and stems of bean plants (*Phaseolus vulgaris* L). Similarly, Rafie et al. (2023) reported that foliar applications of zinc chelated with lysine in onions (*Allium cepa* L.) increased the zinc level in the bulbs compared to $ZnSO_4$ and the control (no zinc application). These results are consistent whit those obtained in the present study. However, limited information is available on the translocation of zinc after applying amino chelates in fruit trees.

Amino chelates improve the translocation of elements such as zinc within the plant (Souri & Hatamian, 2019). Therefore, when the leaves are sprayed with this form of zinc, it can increase the concentration of the nutrient in the fruit, stem, or roots. This gives an advantage to these fertilizers compared to metal salts (sulfates).

4. Conclusions

The foliar application of different Zn sources did not cause, over the eight-month evaluation period, a significant effect on the growth of young ´Valencia´ orange trees established on calcareous soil. Regarding nutritional status, only the amino-chelate Kelatex Zn Forte at a 1.0% dose proved to be a promising Zn source, as it increased the Zn concentration in roots and leaves and caused a minimal decrease in the chlorophyll index. The foliar application of this Zn source in citrus trees grown on calcareous soil shows potential to mitigate Zn deficiency; however, its evaluation over a longer period is warranted.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

O. Martínez-Ríos: Methodology, Research, Writing–draft, Resources. J. I. Cortés-Flores: Methodology, Conceptualization, Writing–draft, Resources. A. López-Jiménez: Writing–review, Editing, Validation, Resources. J. D. Etchevers-Barra: Methodology, Writing–review, Formal analysis, Editing. M.B. Contreras-Soto: Writing–review, Editing, Software, Conceptualization.

References

Asadi, K. A., & Akhlaghi, A. N. (2020). Evaluation of growth rate and vegetative and physiological characteristics of Satsuma mandarin on C-35 rootstock in some calcareous soils. Iranian *Journal of Soil Research, 34*(2), 215–233. https://doi.org/10.22092/IJSR.2020.122521

Boaretto, R. M., Hippler, F. W. R., Teixeira L. A. J., Fornari, R. C., Quaggio, J. A., & Mattos, D. (2023) Zinc fertilizers for Citrus production: assessing nutrient supply via fertigation or foliar application. *Plant and Soil, 496*, 1–14. https://doi.org/10.1007/s11104-023-05969-w

Bolan, N., Srivastava, P., Rao, C. S., Satyanaraya, P. V., Anderson, G. C., Bolan, S., et al. (2023). Distribution, characteristics and management of calcareous soils. *Advances in agronomy, 182*, 81–130. https://doi.org/10.1016/bs.agron.2023.06.002

Bouain, N., Shahzad, Z., Rouached, A., Khan, G. A., Berthomieu, P., Abdelly, C., & Rouached, H. (2014). Phosphate and zinc transport and signalling in plants: toward a better understanding of their homeostasis interaction. *Journal of Experimental Botany, 65*(20), 5725–5741. https://doi.org/10.1093/jxb/eru314

Bremner, J. M. (1965). Total nitrogen. In: Methods of soil analysis. Part 2. Chemical and Microbiological Properties. A. G. Norman (ed.). American Society of Agronomy. Madison, WI, USA. pp: 1149–1178. https://doi.org/10.2134/agronmonogr9.2.c32

Clemens, S. (2022). The cell biology of zinc. *Journal of Experimental Botany, 73*(6), 1688–1698. https://doi.org/10.1093/jxb/erab481

Dawood, S. A., Meligy, M. S., & El-Hamady, M. M. (2001). Influence of Zn sulfate application on tree leaf and fruit characters of three young citrus varieties grown on slightly alkaline soil. *Annals of Agriculture Science Moshtohor, 39*(1), 433–447. https://doi.org/10.1080/01904167.2013.785567

Du, Y., Li, P., Nguyen, A. V., Xu, Z. P., Mulligan, D., & Huang, L. (2015). Zinc uptake and distribution in tomato plants in response to foliar supply of Zn hydroxide-nitrate nanocrystal suspension with controlled Zn solubility. *Journal of Plant Nutrition and Soil Science, 178*(5), 722–731. https://doi.org/10.1002/jpln.201400213

Fageria, V. D. (2001). Nutrient interactions in crop plants. *Journal of plant nutrition, 24*(8), 1269–1290. https://doi.org/10.1081/PLN-100106981

Fei, X. I. N. G., Fu, X. Z., Wang, N. Q., Xi J. L., Huang, Y., Wei, Z. H. O. U., & Peng, L. Z. (2016). Physiological changes and expression characteristics of ZIP family genes under zinc deficiency in navel orange (*Citrus sinensis*). *Journal of integrative agriculture, 15*(4), 803–811. https://doi.org/10.1016/S2095-3119(15)61276-X

Fu, X. Z., Xing, F., Chao, L., Chun, C. P., Ling, L.L., Jiang, C. L., & Peng, L. Z. (2016). Effects of foliar application of various zinc fertilizers with organosilicone on correcting citrus zinc deficiency. *HortScience, 51*(4), 422–426. https://doi.org/10.21273/HORTSCI.51.4.422

Gaines, T. P., & Mitchell, G. A. (1979). Boron determination in plant tissue by the azomethine H method. *Communications in Soil Science and Plant Analysis, 10*(8), 1099–1108. https://doi.org/10.1080/00103627909366965

Gao, J., Zhuang, S., & Zhang, W. (2024). Advances in plant auxin biology: synthesis, metabolism, signaling, interaction with other hormones, and roles under abiotic stress. *Plants, 13*(17), 2523. https://doi.org/10.3390/plants13172523

Ghasemi, S., Khoshgoftarmanesh, A. H., Afyuni, M., & Hadadzadeh, H. (2013). The effectiveness of foliar applications of synthesized zinc-amino acid chelates in comparison with zinc sulfate to increase yield and grain nutritional quality of wheat. *European Journal of Agronomy, 45*, 68–74. https://doi.org/10.1016/j.eja.2012.10.012

Gupta, N., Ram, H., & Kumar, B. (2016). Mechanism of Zinc absorption in plants: uptake, transport, translocation and accumulation. *Reviews in Environmental Science and Bio/Technology, 15*, 89–109. https://doi.org/10.1007/s11157-016-9390-1

Hamzah, S. M., Usman, K., Rizwan, M., Al Jabri, H., & Alsafran, M. (2022). Functions and strategies for enhancing zinc availability in plants for sustainable agriculture. *Frontiers in Plant Science, 13*, 1033092. https://doi.org/10.3389/fpls.2022.1033092

Intrigliolo, F., Giuffrida, A., Rapisarda, P., Calabretta, M., & Roccuzzo, G. (2000). SPAD as an indicator of nitrogen status in Citrus. In: Proceeding of the IXth international citrus congress, Orlando, FL, USA, pp: 665–667.

Krouk, G., & Kiba, T. (2020). Nitrogen and phosphorus interactions in plants: from agronomic to physiological and molecular insights. *Current Opinion in Plant Biology, 57*, 104–109, https://doi.org/10.1016/j.pbi.2020.07.002

Kulig, B., Klimek-Kopyra, A., Ślizowska, A., Oleksy, A., Skowera, B., Lepiarczyk, A., & Grygierzec, W. (2024). A comparison of the methods used to assess the nutritional status of selected crop species. *Journal of Water and Land Development,* 1–10. https://doi.org/10.24425/jwld.2024.151784

Macedo, L. O., Mattos, D., Jacobassi, R. C., Petená, G., Quaggio, J. A., & Boaretto, R. M. (2021). Characterization and use efficiency of sparingly soluble fertilizer of boron and zinc for foliar application in coffee plants. *Bragantia, 80*, e3421. https://doi.org/10.1590/1678-4499.20200329

Makhasha, E., Al-Obeed, R. S., & Abdel-Sattar, M. (2024). Responses of Nutritional Status and Productivity of Timor Mango Trees to Foliar Spray of Conventional and/or Nano Zinc. *Sustainability, 16*(14), 6060. https://doi.org/10.3390/su16146060

Mirboloock, A., Rasouli, S. M., Sepehr, E., Lakzian, A., & Hakimi, M. (2021). Synthesized Zn (II)-amino acid and chitosan chelates to increase Zn uptake by bean (*Phaseolus vulgaris*) plants. *Journal of Plant Growth Regulation, 40*, 831–847. https://doi.org/10.1007/s00344-020-10151-y

Mosa, W. F., Ali, H. M., & Abdelsalam, N. R. (2021). The utilization of tryptophan and glycine amino acids as safe alternatives to chemical fertilizers in apple orchards. *Environmental Science and Pollution Research, 28*, 1983–1991. https://doi.org/10.1007/s11356-020-10658-7

Najizadeh, A., & Khoshgoftarmanesh, A. H. (2019). Effects of foliar applied zinc in the form of ZnSO4 and Zn-amino acid complexes on pistachio nut yield and quality. *Journal of Plant Nutrition, 42*(18), 2299–2309. https://doi.org/10.1080/01904167.2019.1655043

Nasir, M., Khan, A. S., Basra, S. A., & Malik, A. U. (2016). Foliar application of moringa leaf extract, potassium and zinc influence yield and fruit quality of 'Kinnow'mandarin. *Scientia Horticulturae, 210*, 227–235. https://doi.org/10.1016/j.scienta.2016.07.032

Obreza, T. A., Zekri, M., & Hanlon, E. A. (2020). Soil and leaf tissue testing. In: Morgan K. T., D and M. Kadyampakeni (eds.), Nutrition of Florida citrus trees (3rd ed.) SL458/SS671. EDIS: Florida, USA, pp: 23–32.

Ojeda, B. D. L., Perea, P. E., Hernández, R. O. A., Ávila, Q. G., Abadía, J., & Lombardini, L. (2014). Foliar fertilization with zinc in pecan trees. *HortScience, 49*(5), 562–566. https://doi.org/10.21273/HORTSCI.49.5.562

Oliveira, M. L., Mattos, J. D., Jacobassi, R., Rieger, H. F. W., Quaggio, J. A., & Boraetto, R. M. (2020). Efficiency of foliar application of sparingly soluble sources of boron and zinc in citrus. *Scientia Agricola, 78*(1), e20180387. https://doi.org/10.1590/1678-992X-2018-0387

Prasad, R., Shivay, Y. S., & Kumar, D. (2016). Interactions of zinc with other nutrients in soils and plants-A Review. *Indian Journal of Fertilizers, 12*, 16–26.

Rafie, M. R., Khoshgoftarmanesh, A. H., Shariatmadari, H., & Darabi, A. (2023). Apoplastic and symplastic zinc concentration of intact leaves of field onion (Allium cepa) as affected by foliar application of ZnSO4 and Zn-amino chelates. *Journal of Plant Nutrition, 46*(5), 731–742. https://doi.org/10.1080/01904167.2022.2044046

Sourati, R., Sharifi, P., Poorghasemi, M., Alves Vieira, E., Seidavi, A., Anjum, N. A., et al. (2022). Effects of naphthaleneacetic acid, indole-3-butyric acid and zinc sulfate on the rooting and growth of mulberry cuttings. *International Journal of Plant Biology, 13*(3), 245–256. https://doi.org/10.3390/ijpb13030021

Souri, K. M., & Hatamian, M. (2019). Aminochelates in plant nutrition: a review. *Journal of Plant Nutrition, 42*(1), 67–78. https://doi.org/10.1080/01904167.2018.1549671

Srivastava, A. K., & Singh, S. (2005). Zinc nutrition, a global concern for sustainable citrus production. *Journal of Sustainable Agriculture, 25*(3), 5–42. https://doi.org/10.1300/J064v25n03_03

Umair, H., Aamer, M., Umer, C. M., Haiying, M., Shahzad, T., Barbanti, B., et al. (2020). The critical role of zinc in plants facing the drought stress. *Agriculture, 10*(9), 396. https://doi.org/10.3390/agriculture10090396

Xie, R., Zhao, J., Lu, L., Brown, P., Guo, J., & Tian, S. (2020). Penetration of foliar-applied Zn and its impact on apple plant nutrition status: in vivo evaluation by synchrotron-based X-ray fluorescence microscopy. *Horticulture research, 7*, 147. https://doi.org/10.1038/s41438-020-00369-y

Zekri, M., & Obreza, T. (2003). Micronutrient deficiencies in citrus: iron, zinc and manganese. EDIS. SL 204. EDIS. (2). https://doi.org/10.32473/edis-ss423-2003