The Journal of Agricultural Sciences - Sri Lanka
Vol. 21 No 2, May 2026. pp 379-394
https://doi.org/10.4038/jas.v21i2.11527

RESEARCH

Effect of Water-soluble Zinc from Different Sources on Lettuce (*Lactuca sativa* L.) Seed Germination and Seedling Development

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Received: 28th February 2025 / Accepted: 14th February 2026

ABSTRACT

Purpose: *Zinc (Zn) is an essential micronutrient for crops and its availability in alkaline soils is very limited. In this study, the possible growth-promoting effects of soluble Zn present in an alkaline soil to which different Zn treatments have been applied are investigated.*

Research Method: *Four Zn chemicals (ZnSO4, ZnO-NP, Zn-EDTA or Zn-EDTA with humic and fulvic acids, Zn-EDTA+AC) at three different concentrations (2.5, 5 or 10, mg Zn kg¹) were applied to soil. The effect of soluble Zn solutions extracted from soil on the final germination percentage of lettuce (Lactuca sativa L.) seeds, radicle and shoot length and shoot dry weight in seedlings after eight days of incubation were evaluated.*

Findings and Values: *The results indicated that Zn EDTA and Zn EDTA+AC treatments showed a high effectiveness in providing soluble Zn in the soil solution (up to 17.8 times that of the control). Although no significant differences in germination percentage were observed between treatments, the highest radicle growth was observed with the Zn EDTA-2.5 treatment (1.3 times the control). The ZnSO treatments showed a dose-dependent promoter effect. Radicle growth under ZnO-NP and Zn EDTA+AC treatments did not show a consistent relationship with Zn concentrations, indicating a non-dose-dependent effect. The results of this study suggest that Zn EDTA extract alone is more effective in promoting seed growth than when combined with humic and fulvic acids.*

Keywords: *Lettuce, Nanoparticles, Radicle length, Seed germination, Zinc oxide.*

INTRODUCTION

Zinc (Zn) is an essential nutrient for agricultural crops and its deficiency causes negative effects on the plant. These effects include a reduction in leaf growth, appearance of chlorosis, and alteration of the ripening period of the plant resulting in a decrease in crop yield. Toxic concentrations of Zn in plants cause physiological disturbances and growth inhibition (Broadley *et al.* 2011).

Zinc availability in soils depends on various factors, including the role of microorganisms in enhancing its bioavailability and the influence of farming practices on soil Zn content and microbial activity (Helfenstein *et al.* 2016, Navarrete *et al.* 2017, Jalal *et al.* 2024, Singh *et al.* 2024).

The use of Zn fertilizers is of great importance especially in alkaline soils,

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where availability is very limited. The availability of this nutrient, when applied to the soil in the form of fertilizer, depends on the amount that remains available to the plant in the medium (Alloway 2013). In these alkaline soils, insoluble and non-bioavailable Zn compounds are formed.

The chemical characteristics of the product applied as a source of Zn have a great influence on the formation of these non-bioavailable compounds (Alloway 2008). Numerous studies have reported that soil chemical extractions can predict this amount of plant-available Zn (Almendros *et al.* 2020, Almendros *et al.* 2022). The most readily available fraction of Zn in soil is the water-soluble fraction. However, the use of other extractants, such as DTPA-TEA or the mixture of low molecular weight organic acids (LMWOAs) has been reported to adequately predict the amount of Zn taken up by the plant.

Applied sources that dissociate rapidly and readily release Zn2+ to the soil will be less effective in delivering the nutrient to the plant due to the formation of non-bioavailable compounds (Alloway 2013). However, chelated or complexed sources can protect the ion from the formation of insoluble and plant unavailable compounds. The difference between the two agents is that a complexing agent forms a planar or steric structure with a divalent or trivalent transition metal cation and the chelating agent has two or more sites that donate electron pairs to a central transition metal cation, as well as constituting a five- or six-ring cyclic structure (Freiser & Freiser 2017).

The need to implement strategies to reduce the environmental impact of fertilizer application on agricultural soils is currently becoming very important. In addition, it is necessary to ensure that soils are not excessively fertilized. These strategies include the use of plant biostimulants, defined as “a product that stimulates plant nutrition processes independently of the nutrient content of the product with the sole objective of improving nutrient use efficiency; tolerance to abiotic stress; plant quality traits or the availability of nutrients confined in the soil or rhizosphere” (EU 2019).

Different studies have reported the use of humic and fulvic acids as biostimulants, showing that they promote the growth and quality of different plants (Canellas *et al.* 2020, Nardi *et al.* 2021, Quijia-Pillajo *et al.* 2024). According to Canellas *et al.* (2015) biostimulant effects of humic substances of humic substances are characterized by both structural and physiological changes in roots and shoots related to nutrient uptake, assimilation and distribution (nutrient use efficiency traits).

In recent years, there has been growing interest in the possible use of ZnO nanoparticles as a possible source of Zn for crops. The effectiveness of these sources is strongly influenced by their physicochemical characteristics (De-Francisco *et al.* 2024a, De-Francisco *et al.* 2024b). One of the advantages of using ZnO nanoparticles in soil is their short- and medium-term dissolution kinetics, which allows for the gradual release of nutrients and high bioavailability. It also mitigates environmental problems such as deep water leaching observed with more traditional sources (De-Francisco *et al.* 2024c).

The effect of the different fertilizer products applied to a soil has a great influence on the early stages of seed germination. Since the first stage of seed germination consists of hydration, the effect of the added products is determined by the fraction of the nutrient, soluble in the soil solution. Zinc supplied to the seed is transported to the inner regions of the seed from the shell, increasing its availability to the region where it is most needed (Veena & Puthur 2022). The importance of an adequate Zn supply in the seed germination process lies in the participation of Zn in the activation of many enzymes necessary for key metabolism being crucial for protein production (Cakmak & Kutman 2018).

Previous studies have focused on the influence of the application of different Zn products in soils and their effect on the developed crop (Dapkekar *et al.* 2018, Ahmed *et al.* 2019, Mattiello *et al.* 2021, Meneghelli *et al.* 2021, Mirbolook *et al.* 2021, De-Francisco *et al.* 2024a). There are also numerous studies in which Zn products are applied in a prepared solution to evaluate seed germination (Lin & Xing 2007, Singh *et al.* 2016, Veena & Puthur 2022, De-Francisco *et al.* 2024b). However, to our knowledge, there are no studies evaluating the effect of soluble Zn contained in the soil on lettuce germination.

The aim of this study was to investigate how soil soluble Zn from different Zn sources influences different germination parameters of *Lactuca sativa* L. The hypothesis of this investigation is that soluble Zn in the soil could have different effects on seed germination and seed growth depending on the Zn treatment used to fertilize the soil.

MATERIALS AND METHODS

Pot experiment and Zn chemicals

For this experiment, an agricultural soil from Perales del Río, Spain (40°19’40.4” N, 3°40’3.1”W), was used. Soil samples were collected from the Ap horizon (0–20 cm) air-dried at room temperature for approximately 7 days until reaching constant weight, and then sieved to 2 mm. Soil characteristics were determined in accordance with the Spanish official methodology (MAPA 1994). The main characteristics of the soil were the following: pH, 7.8 slightly alkaline; sand, 656 g kg^{-1}; silt, 275 g kg^{-1}; clay, 69 g kg^{-1}; texture, sandy loam; electrical conductivity, 586 μS cm^{-1}; extractable P, 8.1 mg kg^{-1}; oxidizable OM, 4.7 g kg^{-1}; total N, 0.5 g kg^{-1}; C:N ratio, 5.8; exchange Na, 45 mg kg^{-1}; exchange K, 175.4 mg kg^{-1}; exchange Ca, 4382 mg kg^{-1}; and exchange Mg, 71.6 mg kg^{-1}. According to the concentration of available Zn (DTPA–triethanolamine (TEA)-extractable Zn) (0.63 mg kg^{-1}), the original soil was Zn deficient.

Pots with 0.35 kg soil dry weight were fertilized with Zn sources in different doses: 0, 2.5, 5 and 10 mg Zn kg^{-1} soil. The Zn application rates used in this experiment have been reported in other experimental studies to have a beneficial effect on the development of different crops (Almendros *et al.* 2015, Reddy Pullagurala *et al.* 2018, De-Francisco *et al.* 2024a). The Zn sources used in this study were as follows: i) $ZnSO_4 \cdot 7H_2O$ (22.7% w/w Zn, Merck) [$ZnSO_4$], ii) commercial Zn oxide nanoparticle (80.4% w/w Zn, Aldrich Chemistry) [ZnO-NP]. Briefly, the main characteristics of nanoparticles are as follows: Zeta potential value, -13.5 $\pm$ 1.0 mV indicates instability, so the solution in water will tend not to form stable suspensions and form aggregates; average particle size measured by TEM, 62 $\pm$ 21.28; specific surface area (SBET) values, 19.7 m^2 g^{-1}. These commercial nanoparticles have been characterized in detail by de-Francisco *et al.* (2024b) iii) Zn chelated with EDTA (105 g water-Soluble Zn L^{-1}) [Zn EDTA] and iv) Zn chelated with EDTA enriched with humic and fulvic acids (20 g water-Soluble Zn L^{-1}) [Zn EDTA + AC].

The solid products (ZnSO4·7H2O and ZnO-NP) were applied directly to 0.35 kg of air-dried soil to achieve the desired Zn application rates of 2.5, 5, and 10 mg Zn kg-1 soil. Accordingly, the amounts of ZnSO4·7H2O and ZnO-NP added were calculated based on their Zn content (22.7% and 80.4% w/w Zn, respectively). The solid materials were first weighed with analytical precision and then placed in a plastic container together with the dry soil. The mixture was homogenized by shaking on a rotary tumbler for approximately 10 minutes to ensure uniform particle distribution. For the ZnO-NP treatment, particular care was taken to disperse the nanoparticles evenly and minimize aggregation. The liquid products were added in an aqueous solution, as solutions of these products were previously prepared, with a concentration of 250 mg Zn L-1. Then, 3.5, 7 or 14 mL of these solutions were added to the soil samples, to reach the required study doses (2.5, 5 and 10 mg Zn kg-1 soil, respectively). Subsequently, the soil samples were homogenized. Each treatment was replicated three times, resulting in a total of 39 pots arranged in a randomized complete block design.

Status of Zn in soil

After 30 days of incubation with soil moisture at 60% of field capacity, the Zn status of the soil was determined for the different treatments. For this purpose, the soil was air-dried, homogenized and the following chemical extractions were carried out:

i) The concentration of soluble Zn in the soil was determined using 20 grams of soil shaken with double deionized water for 24 h (soil-water ratio of 1:10). After extraction, the soil suspension was centrifuged (4500 rpm, 15 min) (Almendros *et al.* 2020).

ii) The short-term available Zn concentration was assayed with the rhizosphere-based extraction method. For this, 2 g of soil were shaken for 16 hours with 20 mL of a mixture of low molecular weight organic acids (LMWOAs, 10-mM combination of acetic, lactic, citric, malic, and formic acids-in a molar ratio of 4:2:1:1:1:1:1) (Feng *et al.* 2005).

iii) The bioavailable Zn concentration was determined by shaking for 2 h, 10 grams of soil with 20 mL of DTPA-TEA (5×10^{-3} M diethylenetriaminepentaacetic acid, DTPA; 0.01 M $CaCl_2$; and 0.1 M triethanolamine, TEA), adjusted to pH 7.3 (Lindsay & Norvell 1978). The extracts were filtered, and Zn concentrations were determined by flame atomic absorption spectrophotometry (FAAS) (Perkin-Elmer model AAnalyst 900).

Germination experiment

Four different Zn chemicals were applied to soil: $ZnSO_4$ ($ZnSO_4 \cdot 7H_2O$), ZnO-NP (commercial Zn oxide nanoparticles), Zn EDTA (Zn chelated with EDTA), and Zn EDTA+AC (Zn chelated with EDTA enriched with humic and fulvic acids). Each chemical was tested at three concentrations: 2.5, 5, or 10 mg Zn kg^{-1} soil. The concentration of soluble Zn in the soil was determined using 20 g of soil shaken with 200 mL of double deionized water (1:10 soil-to-water ratio) for 24 hours. After extraction, the soil suspension was centrifuged at 4500 rpm for 15 minutes to separate the solid particles. Extracts from three replicates were combined, and their Zn concentration was determined using flame atomic absorption spectrophotometry (FAAS) (PerkinElmer, Analyst 900, Waltham, MA, USA).

To evaluate the influence of soluble Zn on seed germination, four replicates of 20 *Lactuca sativa* L. variety Romana seeds were used per treatment. Seeds were placed in 15 mm diameter Petri dishes containing two layers of filter paper and 12 mL of the different solutions of soil extracts containing soluble Zn, depending on the treatment under study. The dishes were sealed with Parafilm to prevent moisture loss during incubation. The plates were incubated vertically in a germination chamber to observe seedling growth. Incubation conditions were set at 20 °C with a photoperiod of 16 hours of light and 8 hours of darkness.

The germination rate was calculated using the T50 max parameter (Soltani *et al.* 2015). The time required for cumulative germination to reach 50% of the maximum final germination of the seed lot (T50 max) was determined. This parameter was calculated using the "drc" package in R (Ritz *et al.* 2015, Development Core Team 2022).

For the study of seedling development, images of the germination plates were captured after 8 days of incubation using a digital camera (Nikon Coolpix 4500) mounted on a support table (Kaiser 5410 RS2). Following image capture, the ImageJ software (version 1.43, Rasband, 1997–2015) (Rasband, no date) was used for image analysis to measure the length of the radicle and shoot. Biomass, measured as shoot dry weight of the seedlings, was measured after 8 days of incubation, analyzing each replicate separately. The samples were first weighed fresh, then dried for 15 hours at 65 °C to determine dry weight.

Statistical analysis

Statistical analysis was performed by using Statgraphics Centurion software (v.19, Manugistic Inc., Rockville, MD, USA). The main effects were differentiated using protected Fisher's LSD test at a probability level of $P \leq 5\%$. When the interaction between different factors was significant, we performed a new ANOVA to determine the main effects of the combined factor. Germination curves, expressed as germination percentage over incubation time, were modeled using the "drc" package available in R (Ritz *et al.* 2015, Development Core Team 2022). ANOVA was applied to compare germination curves using the F-test statistic. ANOVA was also used to compare the different germination rate parameters using the F-test statistic (Development Core Team 2022).

RESULTS AND DISCUSSION

Status of Zn in the soil

Table 1 presents the water-soluble and bio-available Zn concentrations in soil after the incubation period of the soil. As expected, the water-soluble and bio-available (DTPA-TEA) Zn concentration was higher for the Zn treatments compared to the control treatment. This effect was not observed in the case of bioavailable Zn extraction as the LMWOAs procedure, since the $ZnSO_4$ treatment at the lowest dose and all ZnO-NP application doses showed statistically similar concentrations to the untreated soil. On the other hand, ANOVA statistical analysis with Zn application rate effect shows a higher concentration of water-soluble and available Zn with increasing application rate. The concentration of water-soluble Zn when $ZnSO_4$ treatment was added to the soil showed the lowest values, followed by the concentrations obtained with the application of ZnO-NP. The Zn EDTA and Zn EDTA+AC treatments showed a high effectiveness in providing soluble Zn in the soil solution. These treatments reached the highest values of soluble Zn at all doses (Zn EDTA at 2.5 and 10 mg Zn kg^{-1} soil and Zn EDTA-AC at 5 mg Zn kg^{-1}). These results are consistent with previous studies showing that chelated Zn forms and humic-enriched amendments improve Zn solubility in soils, particularly in alkaline soils where Zn availability is limited (Alloway 2008, Cakmak 2008). For example, Zn EDTA has been reported to maintain higher soluble Zn concentrations over time compared to $ZnSO_4$ in calcareous soils (Almendros *et al.* 2012). Similarly, humic acid-enriched formulations have been shown to enhance micronutrient mobility and uptake in plants (Kaya *et al.* 2005).

The short-term available Zn concentration estimated with the LMWOAs method in this alkaline soil reached the lowest values compared to the other extractions used. No significant differences were obtained between any of the Zn doses applied with the $ZnSO_4$ and ZnO-NP sources and the untreated Zn treatment, which aligns with studies showing that Zn oxide nanoparticles have limited immediate solubility in neutral to alkaline soils (Dimkpa *et al.* 2017, De-Francisco *et al.* 2024a). In contrast, both Zn EDTA and Zn EDTA+AC treatments provided higher short-term available Zn, although no significant differences were found between them at each dose, confirming the effectiveness of chelation in maintaining Zn availability.

The bioavailable Zn concentrations estimated with the DTPA-TEA method reached the highest value of the different extractions used. The concentrations of Zn extracted with this method were higher when $ZnSO_4$ was applied than with the rest of the Zn sources. It is noteworthy that with the $ZnSO_4$ treatment the concentrations of Zn extracted exceeded the amount of Zn added, which indicates that this reagent extracts part of the Zn in the original soil. Similar observations have been reported in previous studies where strong chelating extractions can solubilize inherent Zn pools in soils (Lindsay & Norvell 1978). However, this behavior was not observed with the other Zn sources. The Zn concentrations extracted when ZnO-NP were applied ranged between 36 and 84% of the applied Zn (for doses 10 and 2.5 mg Zn kg^{-1}, respectively). These findings are consistent with reports of slow dissolution and gradual release of Zn from nanoparticles (Raliya *et al.* 2017). Again, no significant differences were observed between the bioavailable Zn concentrations estimated with DTPA-TEA between the Zn EDTA and Zn EDTA+AC treatments for each of the doses.

To study the differences between the Zn concentrations extracted by the different methods, linear correlations were performed. The short-term available Zn concentrations estimated with LMWOAs and soluble Zn-water reached the highest linear correlation ($r = 0.9077$ $P = 0.0000$, $n = 39$). Bioavailable Zn concentrations extracted with the DTPA-TEA method also showed significant correlations between the estimated concentrations with

Table 1. Water-soluble Zn concentration and bioavailable Zn in soil estimated with two different chemical extractions (rhizosphere-based extraction method -LMWOAs- and DTPA-TEA).

Zinc source[1]	Rate of application (mg Zn kg^{-1} soil)	Water-soluble Zn concentration	Short-term available Zn concentration	Bioavailable Zn concentration
Untreated soil	0	0.50 ± 0.03 a	0.28 ± 0.09 a	0.63 ± 0.02 a
$ZnSO_4$	2.5	0.71 ± 0.02 b	0.40 ± 0.06 a	3.79 ± 0.45 c
	5	0.81 ± 0.02 bc	0.88 ± 0.37 ab	5.09 ± 1.17 d
	10	0.92 ± 0.02 c	1.10 ± 0.31 ab	11.65 ± 0.91 f
ZnO-NP	2.5	0.82 ± 0.02 b	0.30 ± 0.03 a	2.10 ± 0.06 b
	5	1.18 ± 0.01 d	0.33 ± 0.11 a	2.37 ± 0.41 b
	10	1.75 ± 0.03 e	0.45 ± 0.09 a	3.60 ± 0.47 c
Zn EDTA	2.5	3.52 ± 0.11 g	1.95 ± 0.63 bc	1.91 ± 0.26 b
	5	4.17 ± 0.09 h	3.17 ± 0.50 d	4.50 ± 0.41 cd
	10	8.92 ± 0.06 k	5.84 ± 0.52 e	8.82 ± 1.09 e
Zn EDTA+AC	2.5	2.24 ± 0.07 f	1.65 ± 0.11 b	1.95 ± 0.03 b
	5	5.73 ± 0.09 i	2.99 ± 1.17 cd	4.91 ± 0.92 d
	10	6.07 ± 0.07 j	5.47 ± 1.99 e	7.77 ± 0.69 e
P-value		0.0000	0.0000	0.0000
Zn source[2]		Water-soluble Zn	Short-term available Zn	Bioavailable Zn
$ZnSO_4$		0.81 ± 0.09 a	0.79 ± 0.39 a	6.84 ± 3.73 b
ZnO-NP		1.25 ± 0.40 a	0.36 ± 0.10 a	2.69 ± 0.76 a
Zn EDTA		5.54 ± 2.55 b	3.65 ± 1.79 b	5.07 ± 3.09 ab
Zn EDTA+AC		4.68 ± 1.84 b	3.37 ± 2.04 b	4.88 ± 2.59 ab
P-value		0.0000	0.0000	< 0.05
Zn application rate[2]		Water-soluble Zn	Short-term available Zn	Bioavailable Zn
2.5		1.82 ± 1.33 a	1.08 ± 0.85 a	2.43 ± 0.91 a
5		2.97 ± 2.38 ab	1.84 ± 1.45 ab	4.22 ± 1.25 b
10		4.41 ± 3.76 b	3.21 ± 2.83 b	7.96 ± 3.34 c
P-value		< 0.05	< 0.05	0.0000

[1] $ZnSO_4$: $ZnSO_4 \cdot 7H_2O$; ZnO-NP: commercial Zn oxide nanoparticle; Zn EDTA: Zn chelated with EDTA; Zn EDTA+AC: Zn chelated with EDTA enriched with humic and fulvic acids.
[2] Mean Zn concentrations and ANOVA of the effects of Zn source or Zn application rate. Different lowercase letters in a column indicate significant differences between treatments. 3 replicates of each treatment.

short-term available and water-soluble Zn concentration (r = 0.4990, P=0.0012 and r = 0.4171, P = 0.0083, respectively n =39). These correlations are consistent with previous work demonstrating that soluble Zn is a key predictor of Zn availability to plants, whereas DTPA-extractable Zn reflects both added and native soil pools (Alloway 2008, Cakmak 2008).

Overall, these results suggest that chelated Zn forms and humic-enriched amendments are more effective at providing plant-available Zn in alkaline soils than conventional $ZnSO_4$ or ZnO nanoparticles. This finding aligns with earlier studies emphasizing the role of chelation and organic matter in improving micronutrient bioavailability and reducing Zn fixation in soils (Mackowiak *et al.* 2001, Noroozisharaf & Kaviani 2018, Zanin *et al.* 2019).

Effect of water-soluble Zn on germination parameters

Seed germination:

Final germination percentages remained unaffected by the different soil extracts, with all treatments showing consistently high germination rates. No significant differences were detected between seeds treated with various Zn-enriched soil extracts and those germinated in distilled water or untreated soil extract (Table 2). The germination dynamics of lettuce seeds were studied during the incubation period under various treatments. The results indicated minimal differences in germination dynamics across treatments with soil extracts enriched with various Zn sources (Table 2). The T50 max, which measures the time required for a seed lot to reach half of its maximum germination percentage, showed no significant differences between untreated seeds and those treated with most concentrations of Zn-enriched soil extracts. Similarly, seeds germinated in distilled water displayed comparable results to those under Zn treatments, further emphasizing the limited impact of these treatments on germination dynamics.

These findings are consistent with previous reports indicating that lettuce and other leafy vegetables are relatively tolerant to moderate Zn concentrations during germination (Broadley *et al.* 2011, Marschner 2023). For instance, Zn concentrations up to 10 mg Zn kg^{-1} in growth media are generally insufficient to induce toxicity symptoms or inhibit germination in lettuce seeds (Marschner 2023).

Seedling growth:

The radicle and shoot lengths of lettuce seedlings were evaluated after eight days of incubation with different soil extracts. Significant differences in radicle growth were observed between untreated seeds and those germinated in Zn-enriched soil extracts ($p < 0.001$). Most Zn treatments promoted greater growth (Fig. 1). Radicle lengths in the control and $ZnSO_4$-treated seeds at this concentration ranged from 22 to 30 mm, representing the lowest growth recorded. The greatest radicle growth, 41 mm, was observed with the Zn chelated with EDTA treatment at 2.5 mg Zn kg^{-1} soil (Zn EDTA-2.5) ($p < 0.0001$).Although radicle growth remained higher than the control at increased Zn concentrations, further significant growth was not observed (Fig. 1).

For example, while the lowest dose (2.5 mg Zn kg^{-1} soil) of $ZnSO_4$ had no significant impact compared to untreated seeds, higher concentrations (5 and 10 mg Zn kg^{-1} soil) significantly increased radicle length. Radicle growth under ZnO-NP and Zn EDTA+AC treatments also increased compared to the control but did not show a consistent relationship with Zn concentrations, indicating a non-dose-dependent effect.

The effect of treatments on shoot length was less pronounced, although significant differences were observed between untreated seeds and those germinated in Zn-enriched soil extracts ($p < 0.001$) (Fig. 2). The highest length, measuring 11 mm, was observed in the Zn oxide nanoparticles treatment at 10 mg Zn kg^{-1} soil (ZnNP-10). However, shoot growth under ZnSO, Zn EDTA, and Zn EDTA+AC treatments did not show a consistent relationship with Zn concentrations. Some doses even significantly reduce shoot length compared to the control, suggesting a non-dose-dependent effect.

Table 2. Seed final germination percentage and germination rate (T50 max) of *Lactuca sativa* L. seeds incubated in different soil extraction solutions.

Zinc source and rate of application[1] (mg Zn kg^{-1} soil)	Final germination (%)	Germination rate T50 max[2] (hours)
Distilled water	99.0 ± 1.0	38.1 ± 1.1 abcd
Untreated soil	91.3 ± 3.8	35.0 ± 1.1 ab
$ZnSO_4$ 2.5	100.0 ± 0.0	36.4 ± 1.1 abc
$ZnSO_4$ 5	96.3 ± 3.8	34.1 ± 1.2 a
$ZnSO_4$ 10	96.3 ± 1.3	40.0 ± 1.0 d
ZnO-NP 2.5	95.0 ± 2.0	37.7 ± 1.1 abcd
ZnO-NP 5	96.3 ± 1.3	36.4 ± 1.1 abcd
ZnO-NP 10	98.8 ± 1.3	37.8 ± 1.0 abcd
Zn EDTA 2.5	100.0 ± 0.0	37.3 ± 1.0 abcd
Zn EDTA 5	95.0 ± 3.5	37.7 ± 1.0 abcd
Zn EDTA 10	97.5 ± 1.4	38.2 ± 1.0 bcd
Zn EDTA+AC 2.5	97.5 ± 2.5	39.2 ± 1.0 bcd
Zn EDTA+AC 5	93.8 ± 4.7	39.1 ± 1.1 bcd
Zn EDTA+AC 10	96.3 ± 1.3	39.0 ± 1.0 bcd
P-value	NS	0.0000

[1] $ZnSO_4$: $ZnSO_4{\cdot}7H_2O$; ZnO-NP: commercial Zn oxide nanoparticle; Zn EDTA: Zn chelated with EDTA; Zn EDTA+AC: Zn chelated with EDTA enriched with humic and fulvic acids; at three concentrations (2.5, 5 or 10 mg Zn kg^{-1} soil) (four germination replicates).
[2] T50 max: time taken for the seeds to reach 50% of the final maximum germination of the seed lot. Data are expressed as mean values ± standard error. Different lowercase letters within a column indicate significant differences ($\alpha = 0.05$). NS: not significant.

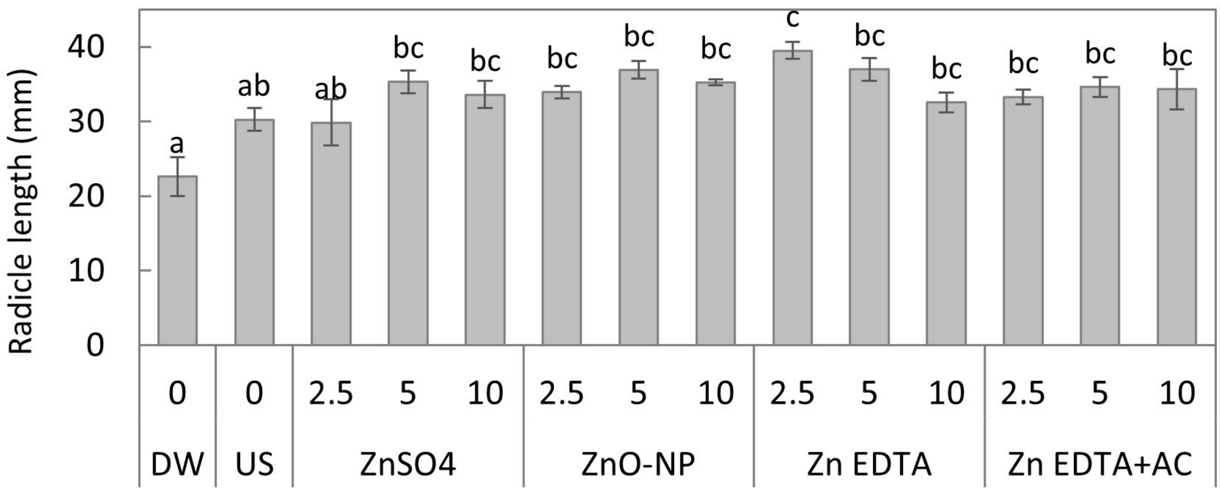

Figure 1. Radicle length of Lactuca sativa L. seedlings germinated after incubation in different soil extraction solutions. DW, distilled water; US, Untreated soil (no Zn application); ZnSO4: ZnSO4·7H2O; ZnO-NP, commercial Zn oxide nanoparticle; Zn EDTA, Zn chelated with EDTA; Zn EDTA+AC, Zn chelated with EDTA enriched with humic and fulvic acids. Rates Zn application: 0, 2.5, 5 and 10 mg Zn kg^{-1} soil. Significance: $p < 0.0001$. Different lowercase letters within a bar indicate significant differences (= 0.05) (four germination replicates).

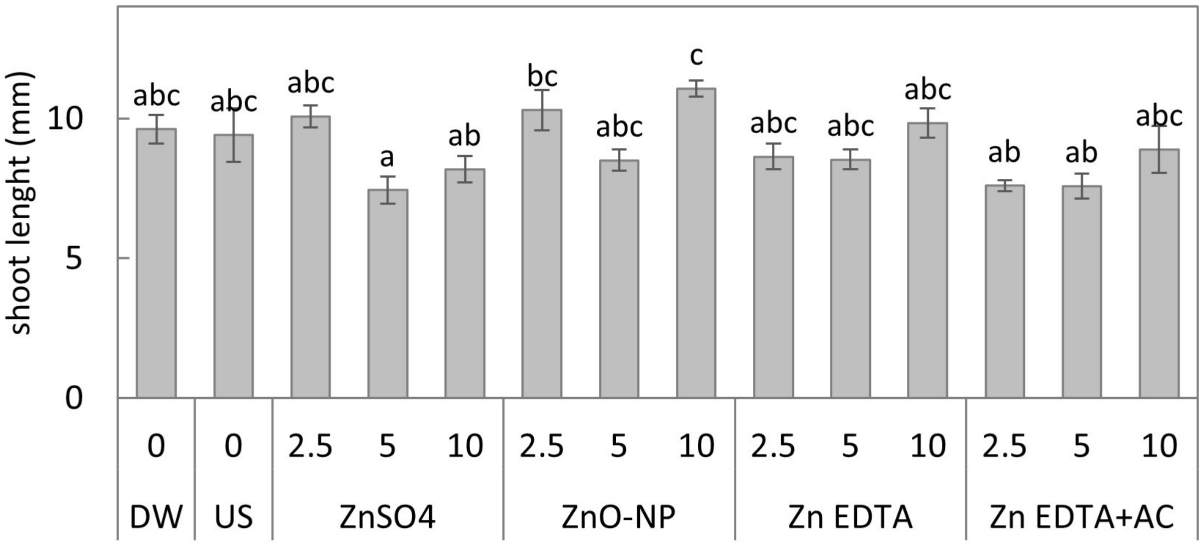

Figure 2. Shoot length of Lactuca sativa L. seedlings germinated after incubation in different soil extraction solutions. DW, distilled water; US, Untreated soil (no Zn application); ZnSO4: ZnSO4·7H2O; ZnO-NP, commercial Zn oxide nanoparticle; Zn EDTA, Zn chelated with EDTA; Zn EDTA+AC, Zn chelated with EDTA enriched with humic and fulvic acids. Rates Zn application: 0, 2.5, 5 and 10 mg Zn kg-1 soil. Significance: p < 0.0001. Different lowercase letters within a bar indicate significant differences (= 0.05).

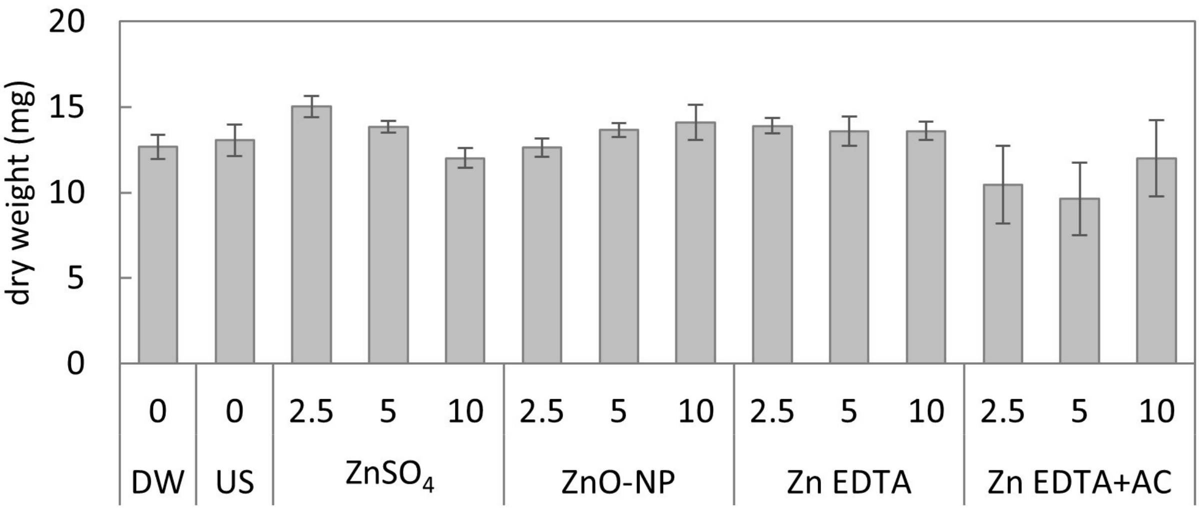

Figure 3. Shoot dry weight of Lactuca sativa L. seedlings germinated after incubation in different soil extraction solutions. DW, distilled water; US, Untreated soil (no Zn application); ZnSO4: ZnSO4·7H2O; ZnO-NP, commercial Zn oxide nanoparticle; Zn EDTA, Zn chelated with EDTA; Zn EDTA+AC, Zn chelated with EDTA enriched with humic and fulvic acids. Rates Zn application: 0, 2.5, 5 and 10 mg Zn kg-1 soil. No significance were detected.

The dry weight of lettuce shoots was assessed after eight days of incubation with different soil extracts as a way to evaluate biomass growth of cotyledon and true leaves. Biomass was similar across all treatments, as shown in Fig. 3. This observation aligns with the lack of significant differences in stem length among the treatments. The water-soluble Zn concentration obtained with the $ZnSO_4$ treatments in this alkaline soil reached very low values compared to the rest of the sources used. This effect could be due to a rapid release of Zn^{2+} and formation of non-water-soluble compounds in the incubation period of the soil (Alloway 2008, De-Francisco *et al*. 2024b). However, the extraction of bioavailable Zn with the regent DTPA-TEA extracted very high Zn concentrations compared to the other sources. Furthermore, the extracted Zn concentrations exceeded the amount of added Zn, indicating that this reagent extracts part of the Zn from the original soil. This effect could be due to the high extracting power of this complexing agent (DTPA), which is able to form very stable water-soluble complexes with Zn (Tewari *et al*. 2018). Chelating agents (such as DTPA) are strong extractants and can help to remove elements from adsorption sites to estimate potentially mobile Zn and thus potential bioavailability (Norvell 1991).

The soluble Zn concentration when ZnO-NP treatments were applied reached higher values than with the same doses of $ZnSO_4$ treatments. This indicated that the water solubility of these ZnO-NP in this soil and soil ageing conditions was higher than in the case of the Zn^{2+} form ($ZnSO_4$). However, the short-term available Zn was similar for both Zn sources ($ZnSO_4$ and ZnO-NP). Zinc oxides are relatively insoluble in water compared to $ZnSO_4$, depending on the physicochemical characteristics of the nanoparticles and the soil in which they are applied, should vary the rate and degree of dissolution in soils (Kochar *et al*. 2022).

The Zn EDTA and Zn EDTA+AC treatments showed a high efficiency in the supply of soluble Zn to the soil solution. Even the lowest soil application rate (2.5 mg Zn kg^{-1}) exceeded the water-soluble Zn obtained with $ZnSO_4$ or ZnO-NP sources applied at either rate. These chelated sources also both exhibited high concentrations of bioavailable Zn. The behavior of these chelated Zn sources can be attributed to the stability constants (K) of the chelates. The chelating agent EDTA has a high stability (log K Zn-EDTA = 17.5 at an ionic strength of 0.01 mol L^{-1}), maintaining a high Zn concentration in the soil solution. In contrast, non-chelated sources (such as $ZnSO_4$ and ZnO-NP) facilitate the retention of Zn by soil components. Chelating agents prevent the formation of insoluble compounds and enhance the desorption of metals from the soil, promoting metal uptake by plants from the reversible and irreversible phases (Norvell 1991, Tewari *et al*. 2018).

Germination was unaffected by the application of extracts from soil treated with Zn compounds, showing consistently high germination rates and no variations in germination speed. These findings align with previous studies on lettuce, suggesting that *Lactuca sativa* seeds exhibit tolerance to metals during the germination process. This tolerance is believed to result from the protective function of the seed coat, which likely acts as a barrier that blocks metals from reaching the developing embryo. This mechanism reduces stress and facilitates the cell elongation required for radicle emergence (Di Salvatore *et al*. 2008, Moreira *et al*. 2020).

In the context of nanoparticles, studies on Zn nanoparticles at low concentrations similarly reported no adverse effects on lettuce seed germination (Liu *et al*. 2016). While the impact of metal exposure on seed germination is highly species-dependent, comparable results have been observed in other plants. For instance, tomato seeds treated with Zn oxide nanoparticles (ZnO NPs) or fine particles at low doses showed no significant differences in germination rates compared to controls (Włodarczyk & Smolińska 2022, De-Francisco *et al*. 2024b). However, germination outcomes can vary based on the type and concentration of ZnO NPs. Sánchez-Pérez *et al*. (2023) reported that ZnO NPs synthesized via green methods improved germination rates, whereas chemically synthesized ZnO NPs at higher concentrations reduced germination percentages.

In contrast, radicle growth in lettuce seedlings was significantly influenced by Zn source and concentration, while shoot growth and biomass showed less pronounced effects and no consistent dose-dependent trends. Our result are in line with different work in Zn compounds application to lettuce germination suggesting that root elongation is a more sensitive measurement endpoint than germination rate, especially for lettuce, when Zn is crucial for enzyme activation and protein synthesis (You *et al*. 2019, Moreira *et al*. 2020). This positive effect depends on dose and the lettuce plant cultivar (Poštić *et al*. 2021) and it has been identified that low concentrations of Zn promoted the growth of green-leaf red-leaf lettuce (Moreira *et al*. 2020).

All Zn sources tested in this study promoted radicle length growth in lettuce seedlings: Zn sulphate ($ZnSO_4$), commercial Zn oxide nanoparticles (ZnO-NP), Zn chelated with EDTA (Zn EDTA), and Zn chelated with EDTA enriched with humic and fulvic acids (Zn EDTA+AC) (Fig. 1). The most effective treatment for promoting radicle growth was Zn EDTA, which resulted in a 30% increase at 2.5 mg Zn kg^{-1} soil. This was followed by a 22% increase observed with both Zn EDTA and ZnO-NP at 5 mg Zn kg^{-1} soil. The strong effect of Zn EDTA could be attributed to its high efficiency in supplying soluble Zn to the soil solution, as well as its ability to provide higher concentrations of bioavailable Zn compared to $ZnSO_4$ or ZnO-NP. It is known that properly applied chelated sources offer advantages such as root absorption, improved nutrient uptake, increased mobility and accessibility of nutrients (Huang *et al*., 1997). However, no significant positive effect was observed when increasing the dose of Zn-EDTA to 10 mg Zn kg^{-1} (Fig. 1). This effect could be due to the counterions present in the treatment solutions. For example, chelating agents such as EDTA could alter the availability of essential nutrients or create imbalances in the chemical environment, potentially contributing to the observed responses (Bloem *et al*. 2017). Further studies are needed to unravel these effects and to better monitor potential interactions between zinc and accompanying counterions.

Radicle growth under Zn EDTA+AC also increased compared to the control, but to a lesser extent (10–14% depending on the concentration), suggesting that Zn EDTA alone is more effective for promoting seed growth than when combined with humic and fulvic acids. Several studies have reported the positive effect of humic and fulvic acids on plant root development (Braziene *et al*. 2021, Zhang *et al*. 2021). Humic acids are considered compounds that increase the permeability of cell membranes in plants, improve the germination rate and the formation of roots (Kaya *et al*. 2005). However, in our research, no additional benefits have been found in the use of Zn-EDTA+AC with respect to the Zn-EDTA source. This may be due to the fact that water extraction limits the possible beneficial effect of these humic and fulvic acids, with respect to their use in the original soil (Cheshire *et al*. 1977).

The concentrations used in this study (2.5 to 10 mg Zn kg^{-1}) are within the range known to enhance plant growth and can be safely applied. This is supported by the lack of detrimental effects on shoot or root growth at higher Zn concentrations, indicating no toxic effects. Adequate Zn concentrations in the plant range between 20 and 100 mg Zn kg^{-1} dry matter (Khan *et al*.2022). Literature indicates that plants need moderate Zn levels for proper growth and development (Broadley *et al*. 2011). It is suggested that low metal concentrations can stimulate seedling growth by inducing oxidative stress, which activates reactive oxygen species signalling and triggers the plant's defense mechanisms (Bailly *et al*. 2008, Kranner & Colville 2011).

CONCLUSION

This study revealed that the chemical form of zinc, rather than its concentration, is the key factor controlling Zn solubility and its physiological effects on lettuce seedlings in alkaline soils. Among the evaluated sources, Zn–EDTA exhibited a markedly superior capacity to maintain soluble and bioavailable Zn and significantly enhanced radicle elongation, demonstrating a growth-promoting effect. In contrast, the incorporation of humic and fulvic acids into the chelated formulation (Zn–EDTA+AC) did not further improve Zn availability or seedling performance, suggesting that under alkaline conditions, the chelating strength of EDTA is sufficient to maximize Zn solubility and uptake potential. These results provide new evidence that soluble Zn extracted from chelated sources can stimulate root development without affecting germination, offering a practical approach for assessing micronutrient efficiency in soils. The applied Zn concentrations (2.5–10 mg Zn kg^{-1} soil) were confirmed to be safe and non-toxic for lettuce, indicating their suitability for targeted Zn fertilization strategies in Zn-deficient alkaline soils.

Future research should explore the behavior of different Zn sources across various soil types and plant species to assess the generalizability of the observed tolerance and growth responses. The potential interactions between Zn treatments and organic soil amendments, such as humic and fulvic acids, merit further examination to determine their influence on nutrient availability and plant performance. Additionally, expanding research to include long-term effects and different concentrations could provide deeper insights into the sustainability and safety of Zn applications in diverse agricultural contexts.

Acknowledgements

This study was funded by the Comunidad de Madrid through the call Research Grants for Young Investigators from Universidad Politécnica de Madrid (project: ECOnanoZn, reference APOYO-JOVENES-21-FUF0C0-61-VOXTPR).

REFERENCES

Ahmed N, Abid M, Ali MA, Masood S, Rashid A, Noreen S, Hussain S (2019) Zinc application enhances biological yield and alters nutrient uptake by Cotton (*Gossypium hirsutum* L.). *Communications in Soil Science and Plant Analysis*, 50, 265–274. http://doi.org/10.1080/00103624.2018.1559330.

Almendros P, Gonzalez D, Alvarez JM (2012) Evolution of residual-Zn available concentrations of Zn-EDTA chelate in two different soils. In: EGU General Assembly Conference Abstracts. 4270.

Almendros P, González D, Ibañez MA, Fernández MD, García-Gomez C, Smolders E, Obrador A (2020) Can diffusive gradients in thin films (DGT) technique and chemical extraction methods successfully predict both Zn bioaccumulation patterns in plant and leaching to groundwater in soils amended with engineered ZnO nanoparticles? *Journal of Soil Science and Plant Nutrition*, 20, 1714–1731. http://doi.org/10.1007/s42729-020-00241-x.

Almendros P, González D, Ibañez MA, Smolders E, Fernández MD, García-Gomez C, Obrador A (2022) Influence of ZnO particle size and soil characteristics on the estimation of long-term Zn bioavailability by chemical extraction methods and diffusive gradients in thin-films (DGT). *Journal of Soil Science and Plant Nutrition*, 22, 3901–3913. http://doi.org/10.1007/s42729-022-00938-1.

Almendros P, Obrador A, Gonzalez D, Alvarez JM (2015) Biofortification of zinc in onions (*Allium cepa* L.) and soil Zn status by the application of different organic Zn complexes. *Scientia Horticulturae*, 186, 254–265. http://doi.org/10.1016/j.scienta.2015.02.023.

Alloway B (2013) Bioavailability of elements in soil. In: O S (Ed.) Essentials of Medical Geology. Springer Netherlands, Dordrecht, 351–373.

Alloway BJ (2008) Zinc in soils and crop nutrition. 2nd Edition. International Zinc Association, Brussels, Belgium and Paris, France.

Bailly C, El-Maarouf-Bouteau H, Corbineau F (2008) From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. *Comptes Rendus Biologies*, 331, 806–814. http://doi.org/10.1016/j.crvi.2008.07.022.

Bloem E, Haneklaus S, Haensch R, Schnug E (2017) EDTA application on agricultural soils affects microelement uptake of plants. *Science of the Total Environment*, 577, 166–173. http://doi.org/10.1016/j.scitotenv.2016.10.153.

Braziene Z, Paltanavicius V, Avizienytė D (2021) The influence of fulvic acid on spring cereals and sugar beets seed germination and plant productivity. *Environmental Research*, 195, 110824. http://doi.org/10.1016/j.envres.2021.110824.

Broadley M, Brown P, Cakmak I, Rengel Z, Zhao F (2011) Function of Nutrients: Micronutrients. In: Marschner's Mineral Nutrition of Higher Plants: Third Edition. Elsevier Inc., pp 191–248.

Cakmak I (2008) Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant and Soil*, 302, 1–17. http://doi.org/10.1007/s11104-007-9466-3.

Cakmak I, Kutman UB (2018) Agronomic biofortification of cereals with zinc: a review. *European Journal of Soil Science*, 69, 172–180.

Canellas LP, Olivares FL, Aguiar NO, Jones DL, Nebbioso A, Mazzei P, Piccolo A (2015) Humic and fulvic acids as biostimulants in horticulture. *Scientia Horticulturae*, 196, 15–27. http://doi.org/10.1016/j.scienta.2015.09.013.

Canellas LP, Canellas NOA, Luiz Eduardo LES, Olivares FL, Piccolo A (2020) Plant chemical priming by humic acids. *Chemical and Biological Technologies in Agriculture*, 7, 1–17. http://doi.org/10.1186/s40538-020-00178-4.

Cheshire MV, Berrow ML, Goodman BA, Mundie CM (1977) Metal distribution and nature of some Cu, Mn and V complexes in humic and fulvic acid fractions of soil organic matter. *Geochimica et Cosmochimica Acta*, 41, 1131–1138. http://doi.org/10.1016/0016-7037(77)90108-9.

Dapkekar A, Deshpande P, Oak MD, Paknikar KM, Rajwade JM (2018) Zinc use efficiency is enhanced in wheat through nanofertilization. *Scientific Reports*, 8, 1–7. http://doi.org/10.1038/s41598-018-25247-5.

De-Francisco M, Fernandes-Silva P, Durães L, Romeiro A, Alvarez-Torrellas S, Almendros P (2024a) Influence of the application of different zinc oxide nanoparticles on a lettuce crop grown in an acidic Mediterranean soil. *Horticulturae*, 10, 681. http://doi.org/10.3390/horticulturae10070681.

De-Francisco M, Mira S, Durães L, Romeiro A, Alvarez-Torrellas S, Almendros P (2024b) Zinc oxide nanoparticles and fine particles: synthesis, characterization and evaluation of the toxic effect on germination and vigour of *Solanum lycopersicum* L. *Agronomy*, 14, 980. http://doi.org/10.3390/agronomy14050980.

De-Francisco M, Romeiro A, Durães L, Álvarez-Torrellas S, Ibañez M, Almendros P (2024c) Environmental behaviour of synthesized and commercial agricultural zinc products: leaching, migration, and availability in soils. *Journal of Soil Science and Plant Nutrition*, 24, 5293–5308. http://doi.org/10.1007/s42729-024-01908-5.

Development Core Team R (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org.

Di Salvatore M, Carafa AM, Carratù G (2008) Assessment of heavy metals phytotoxicity using seed germination and root elongation tests: A comparison of two growth substrates. Chemosphere, 73, 1461–1464. http://doi.org/10.1016/j.chemosphere.2008.07.061.

Dimkpa CO, White JC, Elmer WH, Gardea-Torresdey J (2017) Nanoparticle and ionic Zn promote nutrient loading of sorghum grain under low NPK fertilization. *Journal of Agricultural and Food Chemistry*, 65, 8552–8559. http://doi.org/10.1021/acs.jafc.7b02961.

EU (2019) Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019.

Feng MH, Shan X, Zhang S, Wen B (2005) A comparison of the rhizosphere-based method with DTPA, EDTA, CaCl2, and NaNO3 extraction methods for prediction of bioavailability of metals in soil to barley. *Environmental Pollution*, 137, 231–240. https://doi.org/10.1016/j.envpol.2005.02.003.

Freiser H, Freiser M (2017) Concepts and Calculations in Analytical Chemistry: A Spreadsheet Approach. CRC Press.

Helfenstein J, Müller I, Grüter R, Bhullar G, Mandloi L, Papritz A, Siegrist M, Schulin R, Frossard E (2016) Organic wheat farming improves grain zinc concentration. *PLoS One*, 11, e0160729. https://doi.org/10.1371/journal.pone.0160729.

Huang JW, Chen J, Berti WR, Cunningham SD (1997) Phytoremediadon of lead-contaminated soils: Role of synthetic chelates in lead phytoextraction. *Environmental Science and Technology*, 31, 800–805. https://doi.org/10.1021/es9604828.

Jalal A, Júnior EF, Teixeira Filho MCM (2024) Interaction of zinc mineral nutrition and plant growth-promoting bacteria in tropical agricultural systems: a review. *Plants*, 13, 571. https://doi.org/10.3390/plants13050571.

Kaya M, Atak M, Khawar M, Çiftçi CY, Özcan S (2005) Effect of pre-sowing seed treatment with zinc and foliar spray of humic acids on yield of Common Bean (Phaseolus vulgaris L.). *International Journal of Agriculture and Biology*, 7, 875–878.

Khan ST, Malik A, Alwarthan A, Shaik MR (2022) The enormity of the zinc deficiency problem and available solutions; an overview. *Arabian Journal of Chemistry*, 15, 103668. https://doi.org/10.1016/j.arabjc.2021.103668.

Kochar C, Taneja L, Kumar Yadav P, Swarupa Tripathy S (2022) Dissolution behaviour of nanoparticles and its usefulness in understanding the toxicity of nanoparticles- a review. *Materials Today: Proceedings*, 71, 254–258. https://doi.org/10.1016/j.matpr.2022.08.521.

Kranner I, Colville L (2011) Metals and seeds: Biochemical and molecular implications and their significance for seed germination. *Environmental and Experimental Botany*, 72, 93–105. https://doi.org/10.1016/j.envexpbot.2010.05.005.

Lin D, Xing B (2007) Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. *Environmental Pollution*, 150, 243–250. https://doi.org/10.1016/j.envpol.2007.01.016.

Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal*, 42, 421–428. https://doi.org/10.2136/sssaj1978.03615995004200030009x.

Liu R, Zhang H, Lal R (2016) Effects of Stabilized Nanoparticles of Copper, Zinc, Manganese, and Iron Oxides in Low Concentrations on Lettuce (Lactuca sativa) Seed Germination: Nanotoxicants or Nanonutrients? *Water, Air, and Soil Pollution*, 227, 1–12. https://doi.org/10.1007/s11270-015-2738-2.

Mackowiak CL, Grossl PR, Bugbee BG (2001) Beneficial effects of humic acid on micronutrient availability to wheat. *Soil Science Society of America Journal*, 65, 1744–1750. https://doi.org/10.2136/sssaj2001.1744.

MAPA (1994) Métodos oficiales de análisis. Tomo III. Ministerio de Agricultura, Pesca y Alimentación, Madrid, España. 768pp.

Marschner H (2023) Marschner's Mineral Nutrition of Plants, Fourth edition. Elsevier, London, United Kingdom. 1152pp.

Mattiello EM, Cancellier EL, Da Silva RC, Degryse F, Baird R, McLaughlin MJ (2021) Efficiency of soil-applied 67Zn-enriched fertiliser across three consecutive crops. *Pedosphere*, 31, 531–537. https://doi.org/10.1016/S1002-0160(20)60044-3.

Meneghelli CM, Fontes PCR, Milagres C do C, da Silva JM, Junior EG (2021) Zinc-biofortified lettuce in aeroponic system. *Journal of Plant Nutrition*, 44, 2146–2156. https://doi.org/10.1080/01904167.2021.1889587.

Mirbolook A, Rasouli M, Ebrahim S, Amir S, Mohammad L (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.

Moreira IN, Martins LL, Mourato M (2020) Effect of Cd, Cr, Cu, Mn, Ni, Pb and Zn on seed germination and seedling growth of two lettuce cultivars (*Lactuca sativa* L.). *Plant Physiology Reports*, 25, 347–358. https://doi.org/10.1007/s40502-020-00509-5.

Nardi S, Schiavon M, Francioso O (2021) Chemical structure and biological activity of humic substances define their role as plant growth promoters. *Molecules*, 26, 2256. https://doi.org/10.3390/molecules26082256.

Navarrete AA, Mellis EV, Escalas A, Lemos LN, Junior JL, Quaggio JA, Zhou J, Tsai SM (2017) Zinc concentration affects the functional groups of microbial communities in sugarcane-cultivated soil. Agriculture, Ecosystems Environment, 236, 187–197. https://doi.org/10.1016/j.agee.2016.12.009.

Noroozisharaf A, Kaviani M (2018) Effect of soil application of humic acid on nutrients uptake, essential oil and chemical compositions of garden thyme (Thymus vulgaris L.) under greenhouse conditions. *Physiology and Molecular Biology of Plants*, 24, 423–431. https://doi.org/10.1007/s12298-018-0510-y.

Norvell WA (1991) Reactions of metal chelates in soils. In: Mortvedt J (ed) Micronutrients in Agriculture. SSSA Book, Madison, Wisconsin, USA, pp 187–227.

Poštić D, Štrbanović R, Tabaković M, Popović T, Ćirić A, Banjac N, Trkulja NSR (2021) Germination and the initial seedling growth of lettuce, celeriac and wheat cultivars after micronutrient and a biological application pre-sowing seed treatment. *Plants*, 10, 1913. https://doi.org/10.3390/plants10091913.

Quijia-Pillajo J, Chapin LJ, Martins EM, Jones ML (2024) A Biostimulant Containing Humic and Fulvic Acids Promotes Growth and Health of Tomato 'Bush Beefsteak' Plants. *Horticulturae*, 10, 671. https://doi.org/10.3390/horticulturae10070671.

Raliya R, Saharan V, Dimkpa C, Biswas P (2017) Nanofertilizer for Precision and Sustainable Agriculture: Current State and Future Perspectives. *Journal of Agricultural and Food Chemistry*, 65, 9229–9239. https://doi.org/10.1021/acs.jafc.7b02178.

Rasband WS ImageJ, US National Institutes of Health, Bethesda, Maryland, USA.

Reddy Pullagurala VL, Adisa IO, Rawat S, Kim B, Barrios AC, Medina-Velo IA, Hernandez-Viezcas JA, Peralta-Videa JR, Gardea-Torresdey JL (2018) Finding the conditions for the beneficial use of ZnO nanoparticles towards plants-A review. *Environmental Pollution*, 241, 1175–1181. https://doi.org/10.1016/j.envpol.2018.06.036.

Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-response analysis using R. PLoS One, 10, 1–13. https://doi.org/10.1371/journal.pone.0146021.

Sánchez-Pérez DM, Márquez-Guerrero SY, Ramírez-Moreno A, Rodríguez-Sifuentes L, Galindo-Guzmán M, Flores-Loyola E, Marszalek JE (2023) Impact of biologically and chemically synthesized zinc oxide nanoparticles on seed germination and seedlings' growth. *Horticulturae*, 9, 1201. https://doi.org/10.3390/horticulturae9111201.

Singh A, Singh NB, Hussain I, Singh H, Yadav V, Singh SC (2016) Green synthesis of nano zinc oxide and evaluation of its impact on germination and metabolic activity of Solanum lycopersicum. *Journal of Biotechnology*, 233, 84–94. https://doi.org/10.1016/j.jbiotec.2016.07.010.

Singh P, Suyal DC, Kumar S, Singh DK, Goel R (2024) Long-term organic farming impact on soil nutrient status and grain yield at the foothill of Himalayas. *Frontiers in Environmental Science*, 12, 1–8. https://doi.org/10.3389/fenvs.2024.1378926.

Soltani E, Ghaderi-Far F, Baskin CC, Baskin JM (2015) Problems with using mean germination time to calculate rate of seed germination. *Australian Journal of Botany*, 63, 631–635.

Tewari G, Pareek N, Pachauri S, Pandey S (2018) Impact of chelation/complexation phenomenon on soil environment. *International Journal of Agricultural Science*, 9107, 7314–7316. https://www.bioinfopublic.

Veena M, Puthur JT (2022) Seed nutripriming with zinc is an apt tool to alleviate malnutrition. *Environmental Geochemistry and Health*, 44, 2355–2373. https://doi.org/10.1007/s10653-021-01054-2.

Włodarczyk K, Smolińska B (2022) The effect of nano-ZnO on seeds germination parameters of different tomatoes (*Solanum lycopersicum* L.) cultivars. Molecules, 27, 4963. https://doi.org/10.3390/molecules27154963.

You R, Domínguez C, Matamoros V, Bayona JM, Díez S (2019) Chemical characterization and phytotoxicity assessment of peri-urban soils using seed germination and root elongation tests. *Environmental Science and Pollution Research*, 26, 34401–34411. https://doi.org/10.1007/s11356-019-06574-0.

Zanin L, Tomasi N, Cesco S, Varanini Z, Pinton R (2019) Humic substances contribute to plant iron nutrition acting as chelators and biostimulants. *Frontiers in Plant Science*, 10, 1–10. https://doi.org/10.3389/fpls.2019.00675.

Zhang P, Zhang H, Wu G, Chen X, Gruda N, Li X, Dong J, Duan Z (2021) Dose-dependent application of straw-derived fulvic acid on yield and quality of tomato plants grown in a greenhouse. *Frontiers in Plant Science*, 12. https://doi.org/10.3389/fpls.2021.736613.