

Efficiency of a Zinc Lignosulfonate as Zn Source for Wheat (*Triticum aestivum* L.) and Corn (*Zea mays* L.) under Hydroponic Culture Conditions

DIEGO MARTÍN-ORTIZ, LOURDES HERNÁNDEZ-APAOLAZA,* AND AGUSTIN GÁRATE

Departamento de Química Agrícola, Facultad de Ciencias, Universidad Autónoma de Madrid, Francisco Tomás y Valiente 7, 28049 Madrid, Spain

The objective of this study was to evaluate the efficiency of a zinc lignosulfonate (ZnLS) as Zn source for wheat and corn plants under hydroponic conditions. The Zn-complexing capacity of three commercial lignosulfonates (byproducts of the paper and pulp industry) was tested, and a LS-NH₄, from spruce wood, was selected. Its efficacy as Zn fertilizer for wheat and corn plants was assessed at different pH values (7.0 and 8.0) in comparison with a chelate (ZnEDTA) and an inorganic salt (ZnSO₄). For wheat at pH 7.0, it was concluded that the efficacy of the Zn fertilizers followed the sequence Zn-EDTA > Zn-LS ≈ ZnSO₄ > zero-Zn; and for wheat and corn at pH 8.0, similar results were obtained: Zn-LS > ZnSO₄ ≈ 0 Zn. These data give evidence that ZnLS could be used as Zn source to the roots of wheat and corn and seems to be more efficient than ZnSO₄ to correct Zn deficiency in both plants.

KEYWORDS: Zn-complexing capacity; lignosulfonate; Zn fertilizer; wheat; corn; hydroponic conditions

INTRODUCTION

Globally, 30% of cultivated soils are deficient in Zn (1). This has implications for plant production and for the nutritive value of crops. Zinc is an essential component of over 300 enzymes (2). It plays catalytic, cocatalytic, or structural roles in many plant processes (3). The relatively recent discovery of widespread Zn deficiency problems in rice, wheat, and corn is linked to the intensification of farming in many developed countries (4). About 50% of the soils used for cereal production in the world contain low levels of plant-available Zn, which reduces not only grain yield but also the nutritional quality of grain (5). Continuous use of phosphoric fertilizers and intensive cultivation of high-yield crop varieties have resulted in a widespread deficiency of Zn that has produced a decrease in the growth of many field crops in almost all soil types (6).

This deficiency is traditionally corrected by application to the soil of inorganic Zn salts such as ZnSO₄ (7). When Zn is added to calcareous soils in the form of soluble inorganic salts, it is fixed to a large extent (8). The use of synthetic chelates is the most common and efficient agricultural practice to treat metal deficiencies, but it is an expensive practice, used only in cash crops (9). Moreover, when stable chelates, such as ZnEDTA, are used on alkaline soils, they can migrate through the soil profile and leach (10, 11).

Zinc lignosulfonate (ZnLS) is a complexed organic fertilizer that is formed by reacting ZnSO₄ with sulfonated lignin wastes produced by the paper and pulp industry. Although the exact

structure of lignosulfonates has not been elucidated, it is generally accepted that a wide variety of functional groups are present in the lignosulfonate molecules such as aliphatic, aromatic, sulfonic, carboxylic, and hydroxyl groups. These products are commercially available as calcium, sodium, potassium, or ammonium salts (12).

In 2006, the number of Zn complexes available at the Spanish fertilizer market were 216. Within the complexes, the amount of ZnLS increased significantly from 2000 (71 products) to 2006 (113 products) (52% of the total). Nowadays, lignosulfonates are the most used agents to complex Zn at the Spanish market (13).

Many claims are made regarding the relative efficiency of organic versus inorganic Zn sources. Producers of organic sources generally claim a 10:1 advantage of organic source versus inorganic source (i.e., ZnSO₄) to satisfy the agronomic demand. However, most research has found that there is approximately a 3:1 to 5:1 advantage for ZnEDTA in comparison to ZnSO₄ (14, 15).

Complexes, such as LS, provide several advantages in comparison to other Zn fertilizers. Lignosulfonates have less risk of contamination in some environmental compartments (16) due to their lower stability than chelates. With regard to this, Álvarez et al. (11) studied the relative mobility of ZnEDTA and ZnLS added to calcareous soil columns, concluding that ZnLS remained mostly in the upper zone of the leaching columns and ZnEDTA could migrate to lower zones of the soil. Moreover, complexes are cheaper than synthetic chelates, so they can be used in a large number of crops even if they had lower efficacy than chelates (17).

* Corresponding author (telephone 00-34-91-4976859; fax 00-34-91-4973826; e-mail lourdes.hernandez@uam.es).

High pH and CaCO₃ concentration of soils are usually referred to as the reasons for the low availability of Zn (18, 19). Moreover, Rico et al. (20) tested the efficacy of some Zn fertilizers to provide this element to corn plants grown in calcareous soil and showed that both sources gave rise to a good response by the maize. However, the increments of yield and concentration of Zn in the plants were higher when ZnEDTA was added. On the other hand, another set of experiments was conducted under neutral pH (21–23), which supported the best effectiveness of the ZnEDTA as Zn source compared to ZnLS and ZnSO₄.

Despite there being some studies that tested the ZnLS as a Zn source for plants roots (7, 11, 20–24), Spanish legislation allows the ZnLS only in foliar or fertirrigation applications (25). Nowadays, soil ZnLS applications are not legally accepted in Europe, partly due to the lack of an analytical method to quantify these types of products and partly because of the little evidence of their efficacy available in the literature. One approach to find an analytical method was done by Villén et al. (17). This method is established only on the basis of the compounds metal-complexing capacity and avoided their quantification. Further research is needed at this point. On the other hand, biological data about the efficacy of metal-LS as soil fertilizers for different crops are necessary. For this purpose, hydroponical assays are required to establish the efficiency of these products to provide Zn to roots.

The aim of this work was divided in two studies. In the first, a ZnLS product was prepared from commercial LS salts and selected in order to its better characteristics to provide Zn to roots of crops. In the second one, the efficacy of ZnLS to provide Zn to wheat and corn was evaluated. For this, first an experiment with wheat at pH 7.0 was designed to test the ability of the ZnLS to supply Zn to the root system in comparison with other sources under neutral conditions. After that, another experiment was prepared under simulated calcareous conditions both with wheat and with corn.

MATERIALS AND METHODS

Reagents. All reagents used to form the Zn complexes (ZnSO₄·7H₂O, Panreac), to apply the AOAC modified method (17) [H₂O₂ (33%) and NaOH, Panreac], and in the nutrient solutions of biological experiments [Ca(NO₃)₂, KNO₃, KH₂PO₄, MgSO₄, NaCl, CuSO₄, MnSO₄, MoO₂(NH₄)₆, H₃BO₃, Panreac, FeEDDHA (as Torneo, Syngenta), H₅DTPA (as Tritiplex V, Merck) and Na₂EDTA (as Tritiplex III, Merck)] were of recognized analytical grade. The water used for the preparation of reagents or standards conforms to EN ISO 3696 (26), grade I, free of organic contaminants.

ZnLS-Complexing Capacity. The Zn-complexing capacity of three commercial lignosulfonates has been determined by using the method described by Villén et al. (17). The method seeks the amount of Zn that remains in solution after pH increase; this Zn concentration was the so-called complexed Zn.

Ten grams of lignosulfonates was dissolved in type I water. The volume was made up to 200 mL. Twenty milliliters of this solution was added to 60 mL polyethylene vessels. Increasing volumes of ZnSO₄ (100 g L⁻¹) and 2 drops of H₂O₂ (33%) were added to each vessel (1.0, 1.5, 3.5, 5.0, 7.0, 12.0, 13.0, 15.0, 17.0, and 20.0 mL, respectively). After the additions, the pH was raised to 9.0 with 0.5 M NaOH. After 30 min, pH values were readjusted to 9.0 and the polyethylene vessels were stoppered. Samples were left to stand overnight in the dark. After that, the pH was readjusted to 9.0, if necessary. Solutions were transferred to a 100 mL volumetric flask and made up to volume with type I water. If a precipitate appeared, samples were centrifuged for 10 min at 7500 min⁻¹ before being filtered through a 0.45 μm Millipore filter. Removal of the organic compounds was made in accordance with method 9.3 (EC 2003/2003 Regulation). Twenty-five milliliters of the solutions was added to a 100 mL vessel, and 5 mL of H₂O₂ (33% w/v)

Table 1. Physicochemical Characteristics of the Lignosulfonates Tested

	lignosulfonate products		
	spruce NH ₄	spruce Na	eucalyptus Na
% LS	87.7	54.8	87.4
pH	4.4	6.0	9.5
total S (%)	6.2	5.3	8.0
phenolic groups (%)	1.9	2.0	1.3
carboxylic groups (%)	2.6	6.6	5.5

and 5 mL of 0.5 M HCl were added. Solutions were left to stand during 1 h and then heated for 30 min at 90 °C for the digestion of the samples. If it was required, an extra 5 mL of H₂O₂ could be added, with additional heating. Finally, solutions were made up to 50 mL volume. The Zn remaining in solution (complexed Zn) of each replicate was determined by atomic absorption spectroscopy (AAs) (AAnalyst 800, Perkin-Elmer). Three replicates per addition of Zn were done.

Preparation of Zn Lignosulfonates. Three lignosulfonates, kindly provided by Lignotech Ibérica S.A., were tested in this study; two of them were from spruce wood and the other was from eucalyptus wood with different accompanying cations: spruce NH₄-LS, spruce Na-LS, and eucalyptus Na-LS. The physicochemical characteristics of the products provided by the company are described in **Table 1**.

On the basis of their maximum complexing capacity, 3 μM Zn as ZnLS stock solutions was prepared by introducing into a 100 mL vessel the suitable amount of LS [taking into account the percentage of LS of each formulation (**Table 1**)] and the corresponding volume, to attain this maximum, of a 100 g L⁻¹ of ZnSO₄. After agitation of the mixture during one night in the dark, solutions were made up to volume. The final Zn concentration was assessed by AAs.

Biological Experiments. Wheat Experiment at pH 7.0. Wheat seeds (*Triticum aestivum* L. cv. Perico) were germinated in the dark on moist filter paper at 30 °C. After germination, seedlings were transferred to a 12 L container with a complete nutrient solution without Zn, in a greenhouse at the Autónoma University of Madrid, where climatic conditions were (day/night) temperature, 25/18 °C, and relative humidity, 50/80%. Humidity and temperature were maintained by using a fog cooling system. The composition of nutrient solution was 3.0 mM Ca(NO₃)₂, 4.0 mM KNO₃, 2.0 mM KH₂PO₄, 1.0 mM MgSO₄, and 0.5 mM NaCl with microelements 50 μM FeEDDHA (as Sequestrene), 0.5 μM CuSO₄, 5.0 μM MnSO₄, 0.5 μM MoO₂(NH₄)₆, and 25 μM H₃BO₃. This nutrient solution was buffered by a DTPA-excess solution (27), and the pH was kept at 7.0 ± 0.3. After 7 days, 10 seedlings were placed in 3 L pots (covered with aluminum foil), and treatments were applied: a control without adding Zn (0 Zn), 3.0 μM ZnSO₄ (sulfate), 3.0 μM ZnLS (lignosulfonate), and 3.0 μM ZnEDTA (EDTA). The nutrient solution when treatments were applied was not DTPA-excess buffered, in order to avoid competition between LS and the chelate for the nutrients. Solutions were continuously aerated with a pump and renewed weekly. Three replicates by treatment were done. After 14 days, shoots and roots were separated.

Wheat Experiment at pH 8.0. Wheat seeds were germinated and grown under Zn deficiency as described in the previous experiment. After 7 days, 10 seedlings were placed in 3 L pots (covered with aluminum foil), and treatments were applied (four replicates each one): a control without added Zn (0 Zn), a treatment with 3.0 μM ZnSO₄, and one treatment with 3.0 μM ZnLS. The pH of the nutrient solution was kept at 8.0 ± 0.3, and 0.1 g L⁻¹ of solid CaCO₃ was added to simulate calcareous conditions. The nutrient solutions were aerated with a pump and renewed weekly. After 14 days, shoots and roots were separated. The experiment were done in a growth chamber, with a photoperiod of 16 h of light and 8 h of darkness with corresponding temperatures of 25 and 18 °C and relative humidities of 50 and 80%. The radiation flux was 500 μmol m⁻² s⁻¹.

Corn Experiment at pH 8.0. An assay similar to the previous pH 8.0 wheat experiment was done with corn seeds (*Zea mays* L. cv. PR5500). The experiment included only one variation: the number of seedlings transferred to experimental pots (after Zn deficiency) was 7 instead of 10.

All of the experiments were set up in a completely randomized block.

Analytical Procedures. Shoots and roots were weighed and thoroughly washed with a Tween–HCl mixture [Tween 0.1% (v/v) and 0.1 M HCl] (28) and deionized water. After that, plant material was dried at 70 °C for at least 2 days to determine dry weight.

Concentrations of Zn, Mn, Cu, and Fe in dried plant material samples were measured by AAs after mineralization of the samples at 480 °C for 4 h and dissolving ashes in 2% HCl at 80 °C for 30 min (29). Phosphorus was measured by a colorimetric UV–visible determination (UV-160A, Shimadzu) (30).

Concentrations of Zn, Mn, Cu, and Fe in dried wheat and corn seeds were measured by AAs after mineralization of the samples (29) in order to determine the initial concentration of micronutrients in these seeds (five replicates were done).

Statistical Analyses. Differences among Zn treatments were analyzed by one-way ANOVA, followed by a post hoc multiple comparison of means using the Duncan test ($\alpha = 0.05$). The statistical analysis was performed with the statistical program SPSS 14.0.

RESULTS AND DISCUSSION

ZnLS-Complexing Capacity. Figure 1 shows the Zn-complexing capacity of the three lignosulfonates tested. Zinc that remains in solution is represented versus the Zn added. In all cases, the graphs present a rising segment, which would correspond to the Zn-complexing process by lignosulfonates. This segment was followed by another decreasing segment, probably due to the coagulation of the material by an excess of metal (17). The intersection of the two segments represents the highest complexing capacity point; lines were obtained from the linear regression for the rising and decreasing segments (Figure 1). Table 2 shows the amount of Zn complexed and the LS/Zn ratio at the highest complexing capacity point of the three products tested. An optimum Zn product for crops must have a high Zn-complexing capacity but a low LS/Zn ratio to support as much Zn as possible and reduce the production costs. In our experiment ammonium spruce LS had the highest Zn-complexing capacity and the minor LS/Zn ratio. The spruce sodium LS had a Zn-complexing capacity similar to the ammonium form, but its LS/Zn ratio was higher. Besides, of the two spruce LS, the sodium form was more difficult to handle than the ammonium form. It was sticky and remained in the laboratory material, introducing errors to the process. Moreover, industrial management of this product would not be possible, so this product were ruled out. Finally, eucalyptus sodium LS presented the smallest Zn-complexing capacity and the highest LS/Zn ratio. These different complexing capacities could be associated with the kind and percentage of the functional groups presented in the LS (31). Table 1 shows the percentage of total sulfur, phenolic, and carboxylic groups of the commercial LS tested. Spruce ammonium LS had the highest Zn-complexing capacity (Table 2); it could be attributed both to its phenolic content, which is higher than in eucalyptus sodium product, and its total sulfur content (related to sulfonic groups), which is higher than for the sodium spruce LS. Pang et al. (31) studied the calcium-complexing capacity of a LS based on the phenolic, carboxylic, and sulfonic groups content on its structure. These authors indicated that the concentration of complexing cation in the LS increased as the phenolic group concentration increased, whereas the amount of sulfonic groups changed little its complexing capacity. On the other hand, Pang et al. (31) observed that as the carboxylic groups increased, the amount of calcium complexed decreased. These data agree with our results, as the sodium eucalyptus product tested in our experiment had the highest content of carboxylic groups and the lowest amount of complexed Zn.

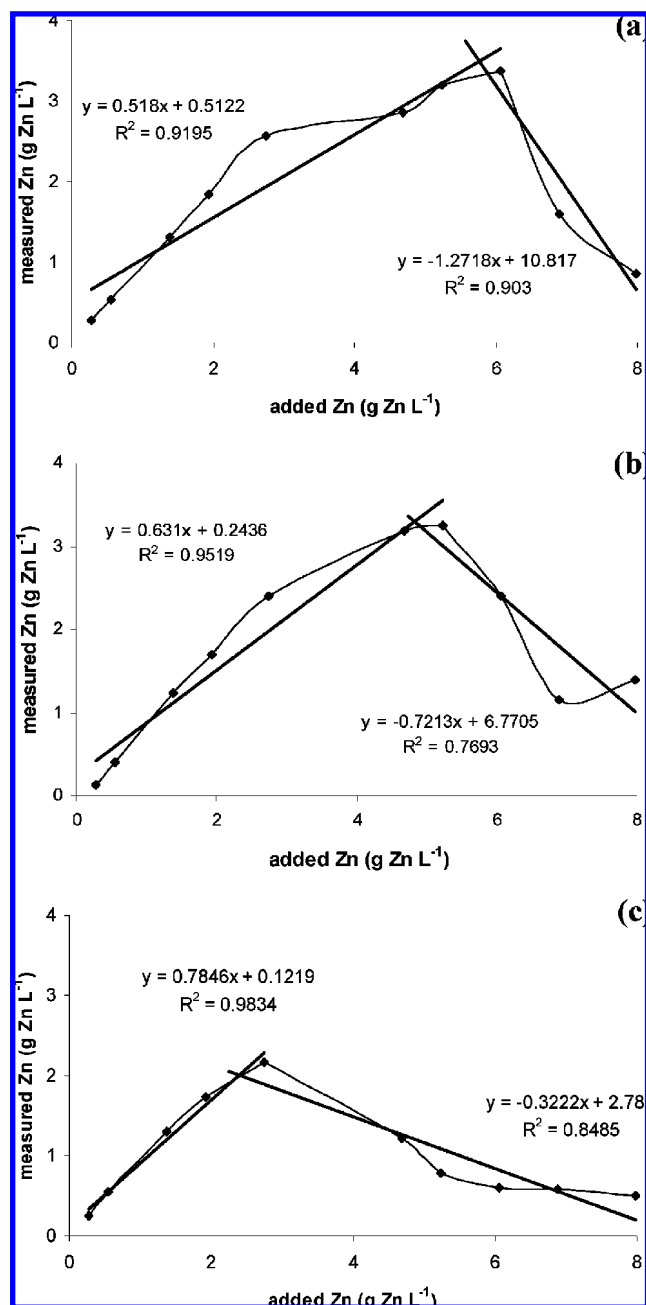


Figure 1. Complexing capacity of lignosulfonates (LS) with Zn: (a) spruce NH_4 ; (b) spruce Na; (c) eucalyptus Na.

Table 2. Zinc-Complexing Capacity and LS/Zn Ratio of the Three Commercial Lignosulfonates Tested

	lignosulfonate products		
	spruce NH_4	spruce Na	eucalyptus Na
Zn-complexing capacity (g of Zn L^{-1})	3.5	3.3	2.0
LS/Zn ratio	1.7	2.1	5.0

Pang et al. (31) found that when the LS pH increased, the Ca-complexing ability increased. On the contrary, our results showed that when the commercial LS pH increased (Table 1), the Zn-complexing capacity decreased (Table 2).

Finally, the ammonium spruce lignosulfonate was selected for testing the efficiency of this product as Zn fertilizer.

Biological Experiments. Initial concentrations of Zn, Cu, Mn, and Fe in seeds were determined prior to the hydroponic experiments. Values obtained in wheat seeds were 23, 7, 26,

Table 3. Dry Weight and Zn Concentration in Wheat Shoots and Roots^a

treatment	shoots				roots				
	dry wt (g)	Zn ($\mu\text{g g}^{-1}$ of DW)	P (mg g^{-1} of DW)	Zn \times 1000/P	Fe ($\mu\text{g g}^{-1}$ of DW)	Mn ($\mu\text{g g}^{-1}$ of DW)	Cu ($\mu\text{g g}^{-1}$ of DW)	dry wt (g)	Zn ($\mu\text{g g}^{-1}$ of DW)
0 Zn	1.7 \pm 0.1 a	25 \pm 4 c	1.6 \pm 0.1 a	15	40 \pm 6 c	84 \pm 8 a	8 \pm 2 ab	0.7 \pm 0.0 a	23.1 \pm 1.1 c
sulfate	1.8 \pm 0.1 a	104 \pm 10b	1.4 \pm 0.1 b	77	84 \pm 6 b	48 \pm 5 b	10 \pm 2 ab	0.6 \pm 0.1 a	136 \pm 12 b
lignosulfonate	1.7 \pm 0.1 a	108 \pm 7 b	0.6 \pm 0.1 c	180	103 \pm 15 a	42 \pm 8 bc	7 \pm 1 b	0.7 \pm 0.1 a	123 \pm 9 b
EDTA	1.7 \pm 0.1 a	132 \pm 6 a	0.3 \pm 0.1 d	471	97 \pm 7 a	37 \pm 4 c	12 \pm 2 a	0.7 \pm 0.1 a	155 \pm 9 a

^a Phosphorus concentration (mg g^{-1} of DW \pm SE), Zn \times 1000/P ratio, and Fe, Mn, and Cu concentrations ($\mu\text{g g}^{-1}$ of DW \pm SE) in wheat shoots in experiment at pH 7.0. For each column, different letters denote significant differences among the treatments according to Duncan's multiple-range test ($\alpha = 0.05$).

Table 4. Dry Weight and Zn Concentration in Wheat and Corn Shoots and Roots^a

treatment	shoots				roots				
	dry wt (g)	Zn ($\mu\text{g g}^{-1}$ of DW)	P (mg g^{-1} of DW)	Zn \times 1000/P	Fe ($\mu\text{g g}^{-1}$ of DW)	Mn ($\mu\text{g g}^{-1}$ of DW)	Cu ($\mu\text{g g}^{-1}$ of DW)	dry wt (g)	Zn ($\mu\text{g g}^{-1}$ of DW)
Wheat Plants									
0 Zn	0.9 \pm 0.2 b	33 \pm 2 c	0.9 \pm 0.1 a	36	70 \pm 2 c	43 \pm 4 a	18 \pm 5 a	0.7 \pm 0.2 b	27 \pm 4 b
sulfate	0.8 \pm 0.0 b	75 \pm 19 b	0.9 \pm 0.1 a	87	88 \pm 10 b	37 \pm 4 a	9 \pm 4 ab	0.6 \pm 0.1 b	40 \pm 1 b
lignosulfonate	1.6 \pm 0.4 a	105 \pm 11 a	0.5 \pm 0.2 b	233	116 \pm 9 a	37 \pm 5 a	7 \pm 2 b	1.3 \pm 0.3 a	110 \pm 12 a
Corn Plants									
0 Zn	1.5 \pm 0.1 b	23 \pm 4 c	7.3 \pm 0.3 a	4	62 \pm 2 a	63 \pm 6 a	14 \pm 5 a	1.6 \pm 0.2 a	28 \pm 2 c
sulfate	1.5 \pm 0.1 b	52 \pm 4 b	4.0 \pm 0.6 b	28	60 \pm 5 a	44 \pm 3 b	5 \pm 1 b	1.5 \pm 0.1 a	92 \pm 15 b
lignosulfonate	2.0 \pm 0.2 a	152 \pm 16 a	2.1 \pm 0.2 b	149	61 \pm 6 a	42 \pm 5 b	6 \pm 1 b	1.2 \pm 0.2 a	189 \pm 10 a

^a Phosphorus concentration (mg g^{-1} of DW \pm SE), Zn \times 1000/P ratio, and Fe, Mn, and Cu concentrations ($\mu\text{g g}^{-1}$ of DW \pm SE) in wheat and corn shoots in experiments at pH 8.0. For each column, different letters denote significant differences among the treatments according to Duncan's multiple-range test ($\alpha = 0.05$).

and 39 $\mu\text{g g}^{-1}$ of dry weight (DW), respectively. These data agree with Zn values reported by other authors in modern varieties of wheat (32) and barley (33). This relatively high Zn concentration in wheat seeds could be an initial source of this element when seeds are grown under Zn-deficient conditions. On the other hand, the concentrations of Zn, Cu, Mn, and Fe measured in corn seeds were 12, 21, 8, and 18 $\mu\text{g g}^{-1}$ of DW, respectively. The Zn concentration in corn seeds was lower than Zn measured in wheat grains.

Wheat Experiment at pH 7.0. Shoot and root dry weights were not significantly affected by the treatments applied (**Table 3**). Similar data were obtained by Álvarez and Gonzalez (22) when ZnEDTA and ZnLS (among other sources) were compared as fertilizers for corn plants in neutral soil. On the other hand, wheat plants presented a shoot growth that was higher than root growth in all treatments. Zhang et al. (34) showed that under Zn deficiency shoot growth is usually more inhibited than root growth in nutrient solutions by wheat plants. In addition, Cumbus (35) observed that root growth might even be enhanced at the expense of the shoot in wheat plants. However, in contrast, Alam and Shereen (6) showed that the dry weight of wheat shoots was usually higher than that of roots harvested in a study with different concentrations of Zn (0.0–20.0 mg kg^{-1}) added as ZnSO₄ salt at neutral pH. These authors (6) found that shoot growth was higher than root growth in Zn-sufficient and -deficient conditions.

Zinc concentration in shoots and roots presented significant differences among treatments (**Table 3**). Plants with no added Zn showed the lowest Zn concentration, in both shoots and roots. The highest Zn concentration was achieved when Zn EDTA was added to the nutrient solution. Inorganic (ZnSO₄) and organic (ZnLS) sources did not present significant differences between them. Similar data were obtained by Gangloff et al. (21) when Zn EDTA, ZnSO₄, and ZnLS were compared for corn plants in a limed soil at pH 7.2.

Some authors (3, 4) remarked on the importance of the interaction between Zn and P in plant nutrition, despite the

mechanisms responsible being still not completely understood. **Table 3** shows that plants treated with ZnEDTA presented the lowest concentration of P in shoots. Plants that were treated with ZnLS presented lower P concentrations in comparison with plants that had grown under 0 Zn and ZnSO₄ nutrient solutions. These data agree with Loneragan et al. (24). These authors found in a hydroponic experiment for ochra (*Abelmoschus esculentum* L.) plants that when Zn concentration increased in shoot, a decrease in P concentration was observed. Cakmak and Marschner (36) proposed that the main reason for the high phosphorus content in aerial tissues is that Zn deficiency enhances both the uptake rate of phosphorus by the roots and its translocation to the shoots. Moreover, the authors explained that this enhancement effect is specific for Zn deficiency and is not observed when other micronutrients are deficient.

The Zn \times 1000/P ratio (**Table 3**) relates the antagonism between Zn and P in shoots. This ratio was higher in plants treated with ZnLS in comparison with plants treated with ZnSO₄ and lower when it was compared to ZnEDTA plants. As in our experiment the P source did not change in the nutrient solutions, one could think that the ratio Zn \times 1000/P was determined by the availability of Zn in the nutrient solution.

Wheat Experiment at pH 8.0. Results obtained in the experiment at pH 8.0 with wheat plants are shown in **Table 4**. In simulated calcareous conditions, plants grown under ZnLS treatment presented a significantly higher shoot dry weight than the plants treated with ZnSO₄ and, as expected, than the plants grown in solution without any added Zn. Similar results were found in roots. As in the pH 7.0 experiment, wheat plants presented a shoot growth that was higher than root growth in all treatments. The results agree with the trend obtained by Alam and Shereen (6).

The highest Zn concentration in both shoots and roots has been measured in plants grown under ZnLS treatment. On the other hand, plants with ZnSO₄ presented greater Zn concentration in shoots than 0 Zn plants. Similar differences between ZnLS and 0 Zn treatments were found by Rico et al. (20) when

they applied ZnLS to maize plants in calcareous soil. The Zn concentration measured in plants treated with ZnSO₄ was lower than the same plants of the experiment at pH 7.0. This could be explained on the basis of the fact that when an inorganic salt (such as ZnSO₄) is added, in alkaline conditions, the Zn²⁺ activity decreases. Some soluble forms of Zn can precipitate at basic pH. These precipitate forms (such as zinc hydroxides) did not form an available source of Zn by the crops in the nutrient solution (37, 38).

In our experiment, P shoot concentrations measured in plants grown under 0 Zn and ZnSO₄ treatments did not present significant differences between them (Table 4). Plants with ZnLS in their nutrient solution had a significant decrease in P measured in comparison with other treatments. These results agree with Cakmak and Marschner (36) and with Loneragan et al. (24), as explained in the previous experiment. A different explanation of our results could be the interaction between P and Zn mentioned by other authors in calcareous conditions. May and Pritts (39) showed that Zn interacted with soil P by formation of insoluble phosphates of Zn for strawberry crops in a calcareous soil.

On the other hand, for all treatments, the concentration of P measured in the aerial part of the plants was lower than in the pH 7.0 experiment. There could be a chemical reason for this. In general, the uptake of phosphorus in plants is mainly as H₂PO₄⁻ form (3). The formation constant (log K° = 7.20), which relates H₂PO₄⁻/HPO₄²⁻, is numerically equal to the pH at which the reacting species have equal activities. An increase in pH of 1 unit decreases the ratio H₂PO₄⁻/HPO₄²⁻ by 10-fold (19). The Zn × 1000/P ratio keeps the trend shown in the pH 7.0 experiment but with higher amounts of these ratios in all treatments. This could be attributed to the decrease in the availability of P at pH 8.0.

Corn Experiment at pH 8.0. Results obtained for corn plants in the pH 8.0 experiment are shown in Table 4. In the same way as in the experiment with wheat at pH 8.0, corn plants grown with ZnLS supply presented a significantly higher shoot dry weight than the plants of the other treatments. This was confirmed by other authors including Goos et al. (40) for maize plants grown in soil under greenhouse conditions. Root dry weights were not significantly affected by the treatments applied. The highest Zn concentration in shoots and roots was found in plants grown under ZnLS nutrient solution, as in the wheat pH 8.0 experiment. The ZnSO₄ treatment was less effective, probably due to the precipitation of the Zn in the nutrient solution at pH 8.0 (37, 38), which was much lower for the ZnLS.

Phosphorus concentration in corn shoots presents significant differences among treatments. The largest P level was found in control plants followed by plants treated with ZnSO₄. The lowest P concentration was measured in plants grown in ZnLS nutrient solution. The same trend was observed in wheat plants but with much lower P values than in the corn plants. The results agree with those of Loneragan et al. (24). Those authors explained that in the absence of Zn, or with low external Zn concentrations, the P level in shoots is higher when the level of Zn supply is normal.

The Zn × 1000/P ratio in corn shoots presented the same trend shown in wheat; the ZnLS treatment presented higher values of this index than the other treatments. Differences found in corn were higher than in wheat, which could be related to the higher sensitivity of the maize crop in comparison with wheat plants to the Zn deficiency (41). The Zn × 1000/P ratio seems to be a sensitive index of Zn nutrition as it shows important differences among Zn treatments.

Lignin represents a basic substance that can exhibit various kinds and percentages of functional groups depending on the vegetal species from which it is derived, in contrast to other biopolymers that have a regular structure (42). Moreover, the structural changes suffered by lignin (therefore, in lignosulfonates, too) during pulping and sulfonation processes can exert a great influence on its characteristics (such as complexing capacity) (28). The AOAC modified method combined with the addition of metal solutions may provide a tool for the determination of complexing capacity in lignosulfonates (17).

Some authors have proved the highest efficiency of ZnEDTA as Zn fertilizer in calcareous and neutral soils (22), but the high cost of the products (9) forced farmers to apply inorganic salts as sulfate in extensively grown crops such as cereals (43). This fact and some environmental problems (16) limit the utilization of organic chelates to cash crops. Our results give evidence that ZnLS is a useful Zn source for wheat and corn and seems to be more efficient than ZnSO₄ to correct Zn deficiency in both plants under hydroponic calcareous conditions. Further experiments are needed to evaluate the efficiency of ZnLS as Zn source of extensive crops under field conditions.

ABBREVIATIONS USED

AOAC, Association of Official Analytical Chemists; LS, lignosulfonate; EDTA, ethylenediaminetetraacetic acid; ED-DHA, ethylenediamine-di-*o*-hydroxyphenylacetic acid; DTPA, diethylenetriaminepentaacetic acid; AAs, flame atomic absorption spectrometry; UV, ultraviolet; ANOVA, analysis of variance.

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