Wheat (*Triticum aestivum* L.) Response to a Zinc Fertilizer Applied as Zinc Lignosulfonate Adhered to a NPK Fertilizer

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The efficacy as Zn fertilizers for wheat of zinc lignosulfonate (ZnLS) products adhered to NPK was evaluated by three plant experimental designs. In the first and second assays, wheat plants were grown under controlled conditions with perlite and a calcareous soil as substrate, respectively. Shoot dry matter and Zn concentration showed that NPK + ZnLS was a better Zn source for wheat than NPK + ZnSO$_4$ under our experimental conditions. A third experiment was conducted under field conditions on a calcareous soil with a low Zn level. Wheat samples were taken at five growth stages of the crop. Although at early stages NPK + ZnLS was the most efficient source of Zn, at harvest no significant differences among treatments were found. Despite that, NPK + ZnLS showed evidence of being a useful Zn source for wheat crop under calcareous conditions.

INTRODUCTION

Zinc deficiency is by far the most ubiquitous micronutrient deficiency problem in the world as a whole (*1*). A high percentage of the soils used for cereal production in the world contain low levels of plant-available Zn, especially in soils with high pH and high CaCO$_3$ content (*2*), which reduces not only grain yield but also the nutritional quality of the harvested grains (*3*, *4*). Although shown to be relatively tolerant to Zn deficiency, wheat crop (one of the two major staple food crops) is restricted by Zn deficiency over millions of hectares worldwide (*5*). Moreover, large applications of phosphorus fertilizers, which are the traditional way to increase wheat crop production, can increase the Zn deficiency in plants (*6*). To reduce the low Zn availability in soils, an alternative to the application of inorganic salts could be the addition of Zn complexes, as zinc lignosulfonates (ZnLS) (*7*).

Sometimes, micronutrients are applied by mixing with macronutrient fertilizers to be used at the same time (*8*) and to provide a rapid solution to Zn deficiency (*9*). Rico et al. (*10*) found that urea coated with ZnLS gave rise to a good response by maize in calcareous soil. On the other hand, Shivay et al. (*11*, *12*) in two field studies concluded that Zn enrichment of urea with ZnSO$_4$ is more effective for increasing the productivity and shoot Zn concentration of wheat and rice crops than their separate applications. However, when Zn is incorporated into NPK fertilizers, the Zn source may react with NPK components, thus affecting their efficiency as Zn fertilizer (*13*).

To evaluate the efficacy of a fertilizer, the available nutrient content for plants needs to be assessed. Total Zn measurements provide information on the Zn content of the product but not of its agronomic effectiveness. Water-soluble Zn concentration is generally used to evaluate this fact, but interactions between P and Zn in the case of NPK−Zn products could induce errors. However, ammonium bicarbonate diethylenetriaminepentaacetic (AB-DTPA) extractable Zn is considered to be one of the soil Zn fractions available for plants (*14*). Mortvedt and Giordano (*15*) found good correlation coefficients between Zn extracted by diethylenetriaminepentaacetic (0.005 M DTPA + 0.01 CaCl$_2$; pH buffered at 7.3) and the agronomic effectiveness of zinc sulfate incorporated into various macronutrient fertilizers. Thus, theoretically, both Zn extracted by AB-DTPA and total Zn of Zn-enriched NPK fertilizers could provide clues to the homogeneity and the potential Zn availability of these products.

The present work continues a previous study of the NPK + ZnLS efficiency as Zn fertilizer for maize (*16*). The results obtained showed an increase in shoot dry matter, shoot Zn concentration, and grain yield of the plants fertilized with it in comparison with plants treated with NPK and NPK + ZnSO$_4$. Moreover, its efficacy was positively correlated with the AB-DTPA Zn measured in the fertilizers prior to their application to maize plants.

In brief, the aim of this study is to prepare a homogeneous NPK + ZnLS fertilizer and evaluate its effectiveness as a Zn fertilizer for wheat grown under calcareous conditions.

MATERIALS AND METHODS

Reagents. All reagents used in the experiments were of recognized analytical grade, whereas water used for the preparation of reagents or standards conforms to EN ISO 3696 (*17*), grade I, free of organic contaminants.

Preparation of Zn Solutions and Their Adhesion to NPK. A ZnLS complex and sugar-coating ZnSO$_4$ stock solutions were prepared. ZnLS stock solution (30 mM Zn) is formed by reacting ZnSO$_4$ with ammonium fir lignosulfonate (NH$_4$-LS; kindly provided by Lignotech Iberica S.A.) on the basis of its maximum Zn complexing capacity (*7*). As ZnSO$_4$ has no adherent properties such as the LS (*18*), a sugar-coating solution was included to coat the NPK with the ZnSO$_4$ (Panreac, PA) stock solution.

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The sugar-coating solution formulation was 45% sucrose (w/w) (Panreac, PA), 30% $ZnSO_4$ (w/w) solution (30 mM Zn), and 25% talc (w/w) (Panreac, PA) (*19*). Total Zn was measured in both solutions (five replicates) by atomic absorption spectroscopy (AAs) (AAnalyst 800, Perkin-Elmer), as was complexed Zn, by following the method described by Villen et al. (*20*) for liquid formulations.

To prepare the Zn−NPK fertilizers, the adhesion method employed was as follows: 50 kg of NPK 8:24:8 (kindly provided by Fertiberia S.A.) was placed into a 190 L kneading machine. At that time, 3 L of the ZnLS stock solution was added with a spray nozzle at regular intervals of 100 mL min^{-1}. The application of the solution must be at regular intervals to avoid the formation of clusters. After this, all samples were air-dried at room temperature during 12 h. The same procedure was used in the preparation of NPK with $ZnSO_4$ adhered. As the procedure was repeated five times for each fertilizer, 250 kg of both products was finally obtained.

Eight samples of the NPK + ZnLS and the NPK + $ZnSO_4$ batches were collected at the end of the coating process, and total Zn was measured by following the CE 2003 9.1 method (*21*): 5 g of each ground product was placed into a 250 mL beaker. Fifty milliliters of 6 M HCl was added in 10 mL portions, and then 50 mL of H_2O was added. The beaker was stopped and strongly shaken. The solution was boiled for 30 min. When the solution was cooled, it was transferred to a 500 mL volumetric flask and made up to volume. The solution was filtered, discarding the first filtering portions. The final filtered solution should be clear.

AB-DTPA extractable Zn, as described by Soltanpour and Workmann (*14*), was measured in the Zn−NPK products. A half gram of each sample was placed into a 50 mL beaker, to which 20 mL of the extracting solution (1 M NH_4HCO_3 + 0.005 M DTPA adjusted to pH 7.6) was added. The solution was stirred during 15 min at 180 rpm, filtered into a 100 mL volumetric flask, and made up to volume.

Zinc concentration in both Zn extracts (total and extractable) of the fertilizer was measured by AAs.

Growth Chamber Perlite Experiment. Wheat seeds (*Triticum aestivum* L. cv. Perico) were germinated in the dark on moist filter paper at 30 °C. Seedlings were transferred to 12 L containers filled with a complete nutrient solution without Zn. The composition of Zn-deficient nutrient solution (in mM) was 4.0 KNO_3, 3.0 $Ca(NO_3)_2$, 2.0 KH_2PO_4, 1.0 $MgSO_4$, and 0.5 mM NaCl (Panreac, PA) and microelements (in μM) were 50 FeEDDHA (as Torneo, Bayer), 25 H_3BO_3, 5.0 $MnSO_4$, 0.5 $CuSO_4$, and 0.5 $(NH_4)_6Mo_7O_{24}\cdot4H_2O$ (Panreac) (*7*). This nutrient solution was buffered by a DTPA (Tritiplex V, Merck) excess solution (*22*), and the pH was kept at 8.0 ± 0.3.

Riviera 15 L polystyrene pots with 2 L solution compartments were filled with moistened expanded perlite (Projar, S.A.) to within about 5 cm of the top of the pot and left to stand during 12 h. On the top of the moistened perlite, 7 g of each NPK 8:24:8 treatment fertilizer was applied. Treatments consisted of NPK without added Zn, NPK with adhered $ZnSO_4$ (NPK + $ZnSO_4$), and NPK with adhered ZnLS (NPK + ZnLS). After the NPK addition, pots were filled to the brim and moistened to perform the seedlings transplant. Seventeen homogeneous young wheat plants grown in the Zn-deficient solution during 4 days were transplanted into each pot. Plants were irrigated with nutrient solution, taking into account that some N, P, and K have already been applied as solid NPK. Chemical composition of this nutrient solution was (in mM) 3.0 $Ca(NO_3)_2$, 1.0 KH_2PO_4, 1.0 $CaSO_4$, 1.0 $CaCl_2$, 1.0 K_2SO_4, 1.0 $MgSO_4$, and 0.5 NaCl. Microelements (in μM) were 50 FeEDDHA, 25 H_3BO_3, 5.0 $MnSO_4$, 0.5 $CuSO_4$, and 0.5 $MoO_{24}(NH_4)_6$ (*7*). In addition to this, 0.2 g of solid $CaCO_3$ was added to the lower compartment of the pots to simulate calcareous conditions. Nutrient solution was renewed weekly. The pH of the discarded nutrient solutions was 7.8 ± 0.3. Four replicates per treatment were placed in randomized blocks into a growth chamber (Dycometal), with a photoperiod light/night of 16/8 h, temperatures of 25/18 °C, and relative humidity of 50/80%. The radiation flux was 500 μmol m^{-2} s^{-1}.

After 28 days, shoots were harvested, weighed, and thoroughly washed with deionized water. Fresh and dry weights were determined. Samples were ground, and after dry digestion in a muffle furnace (480 °C), the ashes were dissolved in 6 M HCl. Concentrations of Zn, Mn, Cu, and Fe were measured by AAs (see the Supporting Information), and phosphorus was determined by a colorimetric method (*23*).

Wheat seeds that were used in the experiments were also dry mineralized to know their micronutrient concentrations. Values of Zn, Cu,

Table 1. Physicochemical Characteristics of the Soil Used for the Soil Greenhouse and Field Experiments

sand (g kg^{-1})	490
silt (g kg^{-1})	302
clay (g kg^{-1})	208
pH	7.7
CEC (cmol$_c$ kg^{-1})	13.9
OM (%)	1.3
CaCO$_3$ (%)	12.5
Olsen P (mg kg^{-1})	32
AB-DTPA Zn (mg kg^{-1})	0.20
AB-DTPA Fe (mg kg^{-1})	9.2
AB-DTPA Mn (mg kg^{-1})	7.2
AB-DTPA Cu (mg kg^{-1})	1.5

Mn, and Fe obtained were 25, 10, 18, and 25 mg g^{-1} of dry weight, respectively.

Moreover, at the end of the experiment perlite samples were taken at two different depths (10 and 20 cm) in the pots where AB-DTPA extractable Zn (*14*) was determined.

Greenhouse Soil Experiment. The experimental design of the soil experiment was similar to the one used for perlite. Wheat seeds were germinated and grown without Zn, and Riviera 15 L pots were filled with 24 kg of a soil/sand 50% (w/w) mixture. Other soil/sand mixtures were tested (100:0, 70:30, and 60:40), but due to the observed water-logging of the soil, the 50:50 mixture was selected. The soil tested was a Typic Xerofluvent obtained from the first 20 cm of an agricultural localization situated at Villamarciel, Valladolid, Spain (41° 31′ 19″ N, 4° 53′ 23″ W, altitude 679 m). Some physicochemical characteristics of this soil are shown in **Table 1**. It was a slightly alkaline loam soil with a high P content and moderate CaCO$_3$, low OM, low available Zn, and normal CEC levels (*24*). Soil pH was determined at a soil to water ratio of 1:2 (w/v); oxidized soil organic matter (OM), potassium, and exchangeable cation concentrations were measured by following the official methods of the Spanish Agricultural Ministry (*25*). The sand used was a normalized sand (0.5−2 mm of diameter), provided by Instituto Eduardo Torroja (CSIC, Spain). The soil/sand (50:50) mixture had a pH of 7.3 ± 0.5 and AB-DTPA Zn and Olsen P contents of 0.09 ± 0.01 and 23 ± 2 mg kg^{-1}, respectively.

Seven grams per pot of NPK, NPK + $ZnSO_4$, and NPK + ZnLS was applied in pots arranged in a randomized complete block design with four replicates per treatment. After 4 days of Zn deficiency, 17 wheat seedlings of similar size were transferred to the experimental pots placed in a research greenhouse at the Autonomous University of Madrid (between May 12 and June 9, 2007). Climatic conditions were (day/night) temperatures of 25/18 °C and relative humidities of 50/80%. Humidity and temperature were maintained by using a fog cooling and humidifier systems. Nutrient solution (same as in the perlite experiment) was added and renewed weekly. The pH of the solution was kept at 8.0, and 0.2 g of solid $CaCO_3$ was added to increase the Zn deficiency.

After 4 weeks, plant shoots were harvested and fresh and dry weights and Zn, P, Fe, Mn, and Cu concentrations were determined. In addition, available Zn (*14*) at two different depths (10 and 20 cm) of the soil/sand mixture was estimated at the end of the experiment.

Field Experiment. The field experiment was conducted at Villamarciel (41° 31′ 19″ N, 4° 53′ 23″ W, altitude 679 m), Valladolid, Spain, in the October 2006−July 2007 cropping season. Soil characteristics are shown in **Table 1**. Climatic data provided by the Spanish National Institute of Meteorology (INM) were (crop cycle averages) as follows: temperature, 11 °C (maximum, 17 °C; minimum, 6 °C); relative humidity, 72%; and precipitation, 38 mm. The experimental design was a split-plot with four randomized subplots per treatment. Subplots were 50 m long by 12 m wide (600 m^2). Thus, the total area of the experiment was 7200 m^2 approximately. Each plot was treated with minimum tillage consisting of two tillage operations with a chisel plow and two diskings to a depth of 5 cm with 15 cm of separation. Treatments (NPK, NPK + $ZnSO_4$, and NPK + ZnLS) were applied before sowing. Sixty kilograms of each fertilizer (equivalent to 1 Tm of NPK ha^{-1}) was applied to each of the subplots. Seeds were planted in October 2007 using a sower machine. Distance between rows was 15 cm, and plant population was approximately 250,000 seeds per subplot. At early spring, nitrogen as urea (60 kg of N ha^{-1}) was top-dressed. The experimental plot was border irrigated throughout the

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growing season. Farming practices at the experimental area were similar to those performed by local farmers.

Plants were sampled five times during the crop cycle. Shoots of 10 plants from each subplot were randomly sampled. The first sampling was made at the germination stage (December 13, 2006). The second sampling was made on February 22, 2007, with plants at seeding stage. The third was made at tillering stage (March 29, 2007). The fourth sampling was made on June 12, 2007, between spike growth and milk stages. Finally, at harvest (July 21), also grain yield was assessed. Zn, P, Fe, Mn, and Cu concentrations were determined at shoots and grains following the same procedure described previously.

Statistical Analyses. Differences among treatments were analyzed by one-way ANOVA, followed by a post hoc multiple comparison of means using the Duncan test at a significance level of $\alpha = 0.05$ for perlite and greenhouse soil experiments. For the field assay, two significance levels were tested: $\alpha = 0.05$ and 0.1. The significance of the main factors and interactions at the field experiment was evaluated by using a two-way ANOVA analysis. Pearson interclass correlation coefficients between AB-DTPA extractable Zn of the fertilizers and their agronomic efficiency were also calculated for each experiment. Statistical analyses were performed with the program SPSS 15.0.

RESULTS AND DISCUSSION

Preparation of Zn Solutions and Their Incorporation to NPK. Total Zn concentrations of the ZnLS and ZnSO$_4$ stock solutions were 26 ± 3 and 27 ± 3 mM, respectively; the Zn that remained in solution at pH 9.0 was 21 ± 5 and 2 ± 1 mM, respectively. Thus, the percentage of Zn in solution at pH 9.0 with respect to the total element was 81% for the ZnLS coating solution and 7% for the ZnSO$_4$ solution.

Once the NPK fertilizer was coated, the total Zn and AB-DTPA extracted Zn of the products were measured (**Table 2**). The extractable Zn of the NPK + ZnLS fertilizer with respect to the total metal concentration was 81 and 9% in the case of the NPK + ZnSO$_4$ product. According to these results, a lower agronomic efficiency as Zn fertilizer should be expected for the NPK + ZnSO$_4$ formulation than for the NPK + ZnLS product. This fact was in agreement with previous studies (16), in which extractable Zn concentration from a NPK + ZnSO$_4$ (0.2% of Zn) fertilizer was 74% lower than the available element from a NPK + ZnLS (0.2% of Zn) product.

Mortvedt and Giordano (15) and Mortvedt (26) pointed out that when Zn sources were incorporated into NPK, the Zn bioavailability may decrease because Zn may react with the components of the macronutrient fertilizer. Zinc included in the NPK as ZnLS could be less affected by these reactions (**Table 2**), probably due to the complexation of the Zn by the LS, which could form a highly cross-linked polymer (27).

Table 2 also shows the coefficients of variation (CV) for both total and AB-DTPA extractable Zn in fertilizers. The CV reveals the reproducibility rate of the Zn adhering method used for the preparation of NPK + ZnLS and NPK + ZnSO$_4$. As the CV of total Zn remains below 10% levels, we conclude that the coating procedure used was adequate.

Growth Chamber Perlite Experiment. No significant differences in shoot dry weight among treatments were obtained (**Table 3**), although the highest dry weight was observed for plants fertilized with NPK + ZnLS.

Plants treated with NPK + ZnLS presented the highest shoot Zn concentration followed by the wheat treated with NPK + ZnSO$_4$. Plants fertilized with NPK (without any added Zn) presented, as expected, low Zn concentration in shoots. Zinc shoot concentration followed the same trend observed in hydroponic conditions (7). In this study, ZnLS and ZnSO$_4$ were applied directly to the nutrient solution for wheat and maize crops at pH 8.0, and the efficacy of the treatments followed the sequence

Table 2. Total and AB-DTPA Extracted Zn in the NPK with ZnLS and ZnSO$_4$ Adhered[a]

	NPK + ZnLS		NPK + ZnSO$_4$	
series	total Zn (g of Zn kg^{-1})	AB-DTPA Zn (g kg^{-1})	total Zn (g of Zn kg^{-1})	AB-DTPA Zn (g kg^{-1})
1	1.01 ± 0.11	0.78 ± 0.05	0.97 ± 0.08	0.07 ± 0.02
2	1.01 ± 0.12	0.83 ± 0.06	0.88 ± 0.08	0.08 ± 0.02
3	0.99 ± 0.09	0.70 ± 0.04	1.10 ± 0.13	0.11 ± 0.03
4	0.92 ± 0.07	0.69 ± 0.08	0.92 ± 0.06	0.13 ± 0.03
5	1.00 ± 0.10	0.84 ± 0.08	1.15 ± 0.22	0.05 ± 0.02
6	0.92 ± 0.08	0.76 ± 0.05	0.99 ± 0.07	0.09 ± 0.03
7	0.89 ± 0.07	0.74 ± 0.04	0.97 ± 0.05	0.09 ± 0.04
8	0.99 ± 0.09	0.94 ± 0.07	0.95 ± 0.07	0.08 ± 0.02
mean	0.97 ± 0.05	0.79 ± 0.08	0.99 ± 0.09	0.09 ± 0.02
CV (%)	5	10	9	22

[a] Values are means $\pm$ SE of each series of samples. CV, coefficient of variation.

Table 3. Dry Weight ($\pm$SE), Zn Concentration ($\pm$SE), Phosphorus Concentration ($\pm$SE), and P/Zn Ratio in Wheat Shoots of Perlite and Soil Experiments under Controlled Conditions[a]

treatment	DW (g)	Zn (μg g^{-1} of DW)	P (mg g^{-1} of DW)	P/Zn
		Wheat Shoots Perlite Experiment		
NPK	16 ± 1 ns	10 ± 1 c	1.0 ± 0.2 a	100
NPK + ZnSO$_4$	16 ± 1	14 ± 1 b	1.5 ± 0.3 a	107
NPK + ZnLS	18 ± 2	19 ± 2 a	0.5 ± 0.1 b	26
		Wheat Shoots Soil Experiment		
NPK	14 ± 1 b	12 ± 2 b	1.2 ± 0.2 a	100
NPK + ZnSO$_4$	15 ± 1 b	16 ± 2 b	1.4 ± 0.4 a	88
NPK + ZnLS	19 ± 1 a	22 ± 3 a	0.8 ± 0.2 b	36

[a] For each column, different letters denote significant differences among the treatments according to Duncan's multiple-range test ($\alpha = 0.05$) ($n = 4$). ns, not significant.

ZnLS > ZnSO$_4 \approx 0$ Zn. The authors explained this sequence because of the formation of unavailable Zn species at alkaline pH when ZnSO$_4$ was added to the solution. In addition, similar results were obtained in the comparison of NPK + ZnLS and NPK + ZnSO$_4$ applications to maize plants grown in perlite (16). This behavior was clearly connected with the low available Zn extracted in the ZnSO$_4$−NPK fertilizers (**Table 2**).

Phosphorus and Zn deficiencies are widespread nutritional constraints on crop production in many parts of the world, and phosphorus−zinc interactions have been widely investigated (28). There are many studies dealing with the effect of P fertilization on Zn deficiency. For example, Robson and Pitman (29) showed that an increase in the P availability in the growing medium can induce Zn deficiency in plants, but little is known about the specific mechanisms. In sand-based pot culture, Singh et al. (30) and Gianquinto et al. (31) observed that an increase in P supply depressed Zn concentration in *Phaseolus vulgaris*, but they ascribed the difference to a dilution effect of plant growth. Therefore, it remains unclear whether an increase in P availability in the growth medium can reduce Zn uptake by plant roots.

However, the effect of Zn fertilization on P availability is much less reported. In the present work, this aspect was evaluated, because P nutrition remained constant for all of the treatments, and different Zn sources were tested. In this experience, the lowest P concentration in shoots was found in plants fertilized with NPK + ZnLS (**Table 3**). On the contrary, plants treated without Zn or with ZnSO$_4$ presented the highest P level in shoots. It seems that Zn supply may affect the P uptake or transport in pot assays.

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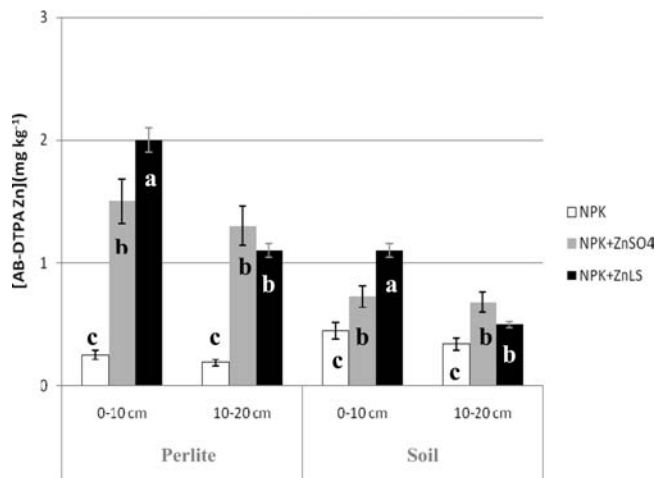*J. Agric. Food Chem.,* Vol. 58, No. 13, 2010 **7889**

Figure 1. AB-DTPA extractable Zn measured at two different depths at the end of the perlite and soil pot experiments under controlled conditions. Error bars denote standard errors of the means (*n* = 4). For each experiment, different letters denote significant differences among treatments according to Duncan's multiple-range test (α = 0.05).

Huang et al. (*32*) observed that Zn deficiency causes an increase in the expression of P transporter genes in barley roots. Second, enhancing P uptake efficiency may cause a decrease in plant uptake of Zn. Moreover, many studies have shown that a low Zn supply but a high P supply markedly enhanced P concentration in plant tissues, which may cause P toxicity and contribute to symptoms resembling Zn deficiency (*33, 34*). Nevertheless, interactions between P uptake efficiency and Zn uptake remain largely unknown (*6*).

Takkar et al. (*35*) concluded that for maize crops the P−Zn disorder was better related with the P/Zn ratio in different parts of the plant than with either the P or Zn content. In our experiment, the lowest P/Zn ratio was found for the NPK + ZnLS treated plants, followed by plants fertilized with NPK and NPK + ZnSO$_4$. However, the dry matter content did not show any positive correlation with this index. Thus, any improvement was obtained by using this relationship instead of the nutrient contents.

Figure 1 shows the available Zn concentration extracted at two depths (0−10 and 10−20 cm) of the perlite pots at the end of the experiment. The highest AB-DTPA extractable Zn concentration was found at the first 10 cm of pots fertilized with NPK + ZnLS. Differences of available Zn between the upper (2.0 mg of Zn kg^{-1} of perlite) and lower (1.1 mg of Zn kg^{-1} of perlite) depths of the perlite for these treatment were found. These data confirm previous results that showed a 98% Zn retention at the first 5 cm of perlite leaching column when it was added as NPK + ZnLS (*16*). The lower retention percentage found at this experiment (**Figure 1**) is due to plant uptake.

The Zn concentrations at the first 10 cm and between 10 and 20 cm of the NPK + ZnSO$_4$ and NPK pots were similar at the two depths and around 1.5 and 0.2 mg of Zn kg^{-1} for each treatment, respectively. In pots where NPK without any Zn source was added, the residual concentrations of Zn could be from the raw materials of NPK fertilizers (*36*). In the NPK used for these experiments, 40 mg of total Zn kg^{-1} of fertilizer was determined.

In a previous leaching experiment (*16*) with columns filled with perlite and with NPK, NPK + ZnSO$_4$, and NPK + ZnLS added at the 2 cm top of the columns, an unexpected slight mobilization of Zn to lower levels of the columns was also found in the case of NPK + ZnSO$_4$ product. For this treatment, the Zn distribution along the column was 55% in the first 5 cm, 25% between 5 and

10 cm, and 10% between 10 and 15 cm of depth. Singh (*37*) also observed a Zn movement to lower depths on calcareous and acid soils when ZnSO$_4$ was applied. However, Gangloff et al. (*38*) did not observe it on a calcareous soil. In our opinion, the kinetic aspects of ZnSO$_4$ dissolution and precipitation as metal oxides should be considered to understand its redistribution along the pots; perhaps a slow enough dissolution/precipitation process could allow a Zn fraction to move through the pot, but further research on the salt behavior at different soil−substrate conditions is required. Another hypothesis could be that after the macronutrient fertilizer dissolution, a little acidic microenvironment should appear around the granule due to the presence of phosphoric acid that allows the metal to solubilize and migrate through the pot profile. Moreover, expanded perlite is chemically and thermally inert (*39*), but it maintains a certain cation adsorption capacity (*40*), surely due to the high porosity and the presence of silicate on its structure, that could contribute to the Zn retention.

Greenhouse Soil Experiment. Shoot dry matter and Zn concentration were significantly higher in plants treated with NPK + ZnLS compared to plants fertilized with NPK or NPK + ZnSO$_4$ (**Table 3**). Rico et al. (*10, 41*) observed a similar behavior in maize grown on a calcareous soil fertilized with ZnLS coated urea in comparison with urea without added Zn at greenhouse conditions. It is assumed by some researchers that organic sources of Zn are more effective as Zn fertilizers than inorganic ones (*42, 43*). Goos et al. (*44*) showed higher efficiency of a Zn humate−lignosulfonate complex (ZnHL) as Zn source for maize grown on a calcareous soil in comparison with ZnSO$_4$ application. Ozkutlu et al. (*45*) found that a Zn humate application increased soybean shoots dry matter and Zn concentration more than ZnSO$_4$ under calcareous conditions.

Values of the P/Zn ratio of plants treated with NPK + ZnLS agree with data observed at the perlite experiment. The lowest shoot P concentration was measured in plants fertilized with NPK + ZnLS. These plants also presented the highest shoot Zn concentration and dry matter (**Table 3**). In contrast to the perlite experiment, for this assay the P/Zn ratio could be a suitable index to assess the efficiency as Zn fertilizers of the Zn−NPK products.

Available Zn in soil at two different depths (10 and 20 cm) of the experimental pots was measured when plant material was removed (**Figure 1**). Zinc mobilization to the lower level of the experimental pots was found for NPK + ZnSO$_4$ treatment (**Figure 1**). This result agrees with Singh (*37*), who observed Zn movement to a lower depth of calcareous and acid soils when ZnSO$_4$ was applied. Previous works indicated that the behavior of Zn fertilizers, when mixed with soil, is clearly influenced by soil pH, as well as the amount of CaCO$_3$ in the soil (*46, 47*). After its application, the Zn fertilizer reacts with the soil and is transformed into more stable compounds (as oxides, hydroxides, and oxyhydroxides), which are less available to plants. These reactions are likely to be rapid enough that they may limit the effectiveness of soluble inorganic Zn fertilizers (*48*). Thus, the chemical properties of Zn fertilizers that are deemed ideal for noncalcareous should be revised for highly calcareous soils (*49*).

As in the perlite experiment, the highest available Zn was found at the upper zone (10 cm) of the soil pots when plants were treated with NPK + ZnLS (1.1 mg of Zn kg^{-1} of soil). Furthermore, only when NPK + ZnLS was applied were differences in AB-DTPA Zn concentrations between upper and lower depths (1.1 and 0.5 mg of Zn kg^{-1} of soil, respectively) observed. These results could indicate the retention of the Zn added as ZnLS in the area where NPK + ZnLS was applied (first 5 cm of the pots) and agree well with those observed in the perlite experiment. Alvarez et al. (*50*) studied the relative mobility of Zn-EDTA and ZnLS complexes

added to calcareous soils columns as coated and uncoated urea fertilizers. Zn-EDTA migrated and distributed throughout the columns, but ZnLS migrated very little, especially with coated fertilizers; the Zn remained mostly in the upper zone of the columns and was not leached. Alvarez et al. (46), in a similar column experiment with two different soils at pH 6 and 7, achieved similar conclusions. These authors pointed out the possible relationship between the low mobility of Zn with the adsorption of ZnLS to some soil components. In the same way, Alvarez and Rico (4) detected that in the upper part of the columns, changes in the chemical forms of the applied Zn occurred and an increase in organically complexed and amorphous iron oxide bound fractions was detected. Gonzalez et al. (51) tested the relative efficacy of Zn chelated by a mixture of EDTA−DTPA−HEDTA in comparison with a ZnLS applied to weakly acid and calcareous soils to supply this metal to navy bean. The Zn chelate mixture showed a higher Zn mobility (11 and 32% of Zn leached from weakly acid and calcareous soils, respectively) through the soils in comparison with the ZnLS. For the ZnLS the metal leached was below 1% for both soils. The authors attributed this different mobility to, among other reasons, the lower stability of the Zn complexes compared to Zn chelates. According to Waychunas (52), the Zn retention in soil is mainly determined by the sheet structure of the surface of the iron oxides, whereas Ma and Uren (53) indicated that Zn retention is also determined by the microporosity of the soil components. Furthermore, the movement of water through the soil profile and hence that of the nutrients dissolved in it is clearly related to both the structure and texture of the soil (46). Although the calcareous soil used in the study described in ref 51 presented some physical and chemical characteristics that enhance the retention of Zn (alkaline pH, clay percentage, and free $CaCO_3$ content), this soil had a higher permeability than the acidic soil, and this could explain why the Zn leaching was greater in the calcareous soil.

Field Experiment. Figure 2 shows that plants treated with NPK + ZnLS presented a higher Zn concentration in shoots throughout the growing cycle, compared to plants treated with NPK + $ZnSO_4$ and NPK. Significant differences were observed through the first 30 weeks at an $\alpha = 0.05$ significance level. However, in the last 10 weeks, these differences were not observed, even at an $\alpha = 0.1$ significance level.

Shoots showed Zn concentration levels between 55 (early stages) and 18 mg kg^{-1} (harvest). This high concentration of the metal at the early stages was due to the base dressing (just before the crop sowing) application of the fertilizers. Zinc shoot concentration decreased during the crop cycle for all treatments due to a dilution effect (3). This fact occurred at the same time that nitrogen (as urea) was added to the crop as top-dressed fertilization, which increased plant biomass. Marschner (28) stated that in leaves the critical Zn deficiency levels are below 15−20 mg of Zn kg^{-1}. Singh et al. (54) proposed a critical Zn level of 25 mg of Zn kg^{-1} for wheat. Therefore, at the harvest, all of the treated plants were around the critical deficiency levels. Two-way ANOVA analysis showed that shoot Zn concentration values were influenced by the treatment applied and by the age of the plants ($P < 0.001$). Interaction between both factors did not occur.

Application rates of Zn fertilizers vary in different parts of the world. In Australia, wheat and other cereals are normally treated with 0.6−2.4 kg of Zn ha^{-1} on light soils and with 1.8−3.9 kg of Zn ha^{-1} on heavier soils (55). Various authors recommend applications of zinc sulfate in the range of 2.2−11.2 kg of Zn ha^{-1} depending on the soil condition, crop variety, and local agronomic factors. Recent recommendations for the Zn deficiency treatment in wheat in India are 50 kg of Zn ha^{-1} zinc sulfate for acute cases and 25 kg of Zn ha^{-1} for moderate
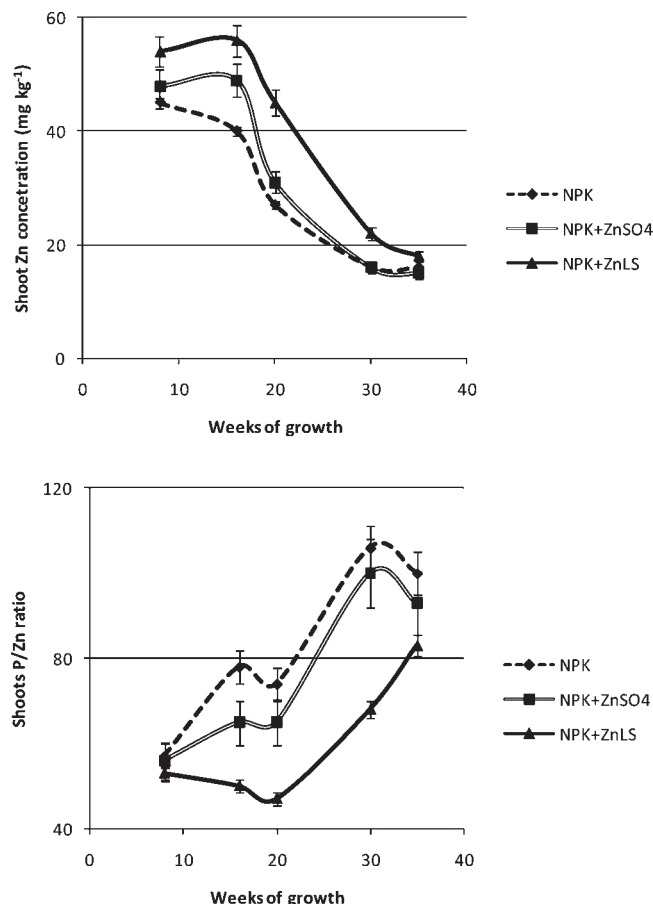

Figure 2. Zinc concentration and P/Zn ratio of wheat shoots at different growth stages in the field experiment. Error bars denote standard errors of the means ($n = 4$).

deficiencies (55). In this study, 1 kg of Zn ha^{-1} was used on the basis of the normal zinc sulfate rates applied in this area by local farmers.

For grain yield, no significant differences among treatments were obtained (around 9600 ± 100 kg of grain ha^{-1}) or Zn concentration (around 16 ± 1 mg of Zn kg^{-1}). Shivay et al. (12) recommended for spring wheat grown in calcareous soil the application of 0.65−1.30 kg of Zn ha^{-1} urea enriched with $ZnSO_4$ to increase by 8−14% the grain yield. Other authors observed a significant increase in grain yield and shoot Zn concentration of plants grown in calcareous soils and fertilized with $ZnSO_4$ compared to plants to which any source Zn was applied (2, 56−58). These authors applied Zn levels between 23 and 40 kg of Zn ha^{-1}, which are adequate for their local soil conditions. At our location, maybe, another Zn application should be recommended with the top-dressed fertilization to enhance crop response at the end of the assay.

Finally, the lowest P/Zn ratio always was measured in shoots of plants treated with NPK + ZnLS (**Figure 2**), which could indicate a higher efficiency of this product as a Zn fertilizer compared to NPK + $ZnSO_4$.

Relationship between AB-DTPA Extractable Zn and the Agronomic Efficacy of the NPK + Zn Fertilizers. The determination of the total Zn in fertilizers was not a good index of its efficacy as Zn source for crops (49). According to several authors, water-soluble Zn measurements for solid fertilizers could be an appropriate index to evaluate this efficacy for several crops (15, 26, 38, 43, 49). For the fertilizers used in this work (NPK + $ZnSO_4$ and NPK + ZnLS) also the AB-DTPA Zn was measured. It had been thought

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Table 4. Pearson Correlation Coefficients ($n = 12$) Relating Extractable AB-DTPA Zn of NPK + ZnLS and NPK + ZnSO$_4$ Fertilizers with Their Agronomic Effectiveness in Wheat Crop (in Shoot Dry Weight, Zn Concentration, and P/Zn Ratio) under the Different Experimental Designs

AB-DTPA Zn (Zn−NPK)	correlation coefficient, r^a		
	DW (g)	Zn (μg g^{-1} of DW)	P/Zn
perlite	0.57	0.91***	−0.99***
soil	0.88**	0.93***	−0.94***
field	0.16	0.42	−0.37

^{a} *, significant at 0.01 probability level; **, significant at 0.001 probability level.

that this Zn fraction, the so-called "available" for the plants in soils (*14*), could be well related with the efficacy of these fertilizers as Zn sources.

Table 4 shows the correlation between the Zn AB-DTPA extracted from the fertilizers and their agronomic efficacy in terms of shoot dry weight, Zn concentration, and P/Zn ratio. For perlite and soil experiments, a high correlation between available Zn in fertilizers and shoot Zn concentration and P/Zn ratio was observed. This fact was also observed for dry weight at the soil experiment at $\alpha = 0.01$. For the field experimental data no correlation was obtained.

As final conclusions of the present work, the NPK + ZnLS fertilizer showed evidence of being an efficient Zn source for wheat crops under calcareous conditions. This statement was supported by the results obtained in perlite and soil experiments under controlled conditions. On the other hand, when NPK + ZnLS was applied in field experiments, its efficacy to supply Zn to wheat plants decreased during the growth cycle. No significant differences in grain harvested were obtained. A top-dressed fertilization with the coated product should be recommended to increase crop production. Moreover, the AB-DTPA extracted Zn could be a good index to establish the efficacy of Zn−NPK fertilizers.

Supporting Information Available: Iron, Mn, and Cu concentrations in wheat shoots of perlite and soil experiments and field experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

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