

## Efficiency of a NPK Fertilizer with Adhered Zinc Lignosulfonate as a Zinc Source for Maize (*Zea mays* L.)

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The aim of this work was to evaluate the efficiency of a NPK fertilizer (8:15:15) with a Zn lignosulfonate (ZnLS) adhered as Zn source for maize plants. This product was compared in three experimental designs with the same NPK fertilizer with ZnSO<sub>4</sub> adhered and with no Zn adhered. The first and the second assays were carried out in a growth chamber by using perlite and a calcareous soil as substrate and the third experiment was raised in two calcareous fields. In general, growth chamber experiments showed that plants treated with NPK + ZnLS presented the highest dry weight and Zn concentrations in shoots. Also at field experiments, the Zn concentration in shoots was significantly high in plants treated with NPK + ZnLS. The grain harvested showed that this treatment gave the highest values in one location, but in the other no significant differences were observed. Although further research is required, we can conclude that NPK + ZnLS product could be a suitable source of Zn for maize crops.

**KEYWORDS:** Zinc; availability; calcareous soil; NPK fertilizer; lignosulfonate; maize

### INTRODUCTION

About fifty percent 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 the grains harvested (1). Generally, the world regions with Zn-deficient soils are also characterized by widespread Zn deficiency symptoms in humans. Cereals crops play an important role in satisfying daily calories intake in developing world, but they are inherently very low in Zn concentrations in grain, particularly when grown on Zn-deficient soils (2). High pH and CaCO<sub>3</sub> content of calcareous soils are usually referred to as the main reasons for the low availability of Zn (3). This deficiency is traditionally corrected by soil application of inorganic Zn salts, such as ZnSO<sub>4</sub>, which could be fixed to a large extent when they are applied to calcareous soils (4). The addition of synthetic chelates, such as ZnEDTA or ZnDTPA, would be a better solution for Zn deficiency but metal chelates are used only in cash crops because of their high cost (5). Furthermore, although synthetic chelates seem to be the most efficient Zn fertilizers due to their high stability in the soil solution, the great mobility through the soil profile of some of them favored their leachability (6, 7). On the other hand, this fact could also improve the Zn uptake by plants.

An alternative to inorganic salts and synthetic chelates could be the Zn complexes. Zinc lignosulfonate (ZnLS) is a complexed organic fertilizer that is formed by reacting ZnSO<sub>4</sub> with sulfonated lignin wastes (8). Lignosulfonates (LS) are a byproduct of paper and pulp industries that are rather cheap and have been used as oil well dispersants, dyestuff, and as agricultural chemicals (9).

Gangloff et al. (8) observed that ZnLS presented greater Zn availability than inorganic Zn salts and required lower application rates to satisfy plant requirements. Also, the reduction of Zn leached when ZnLS complexes were added to the soil in comparison with synthetic chelates application minimize the risk of contamination in some environmental compartments. This fact was observed by some authors when they compared the Zn lixiviation in a calcareous soil treated with ZnLS and Zn-chelate (6, 10). The authors found no micrometerutrient movement in calcareous soil when ZnLS was applied, while the addition of Zn-chelate/s showed Zn migration and leaching. The lixiviation of some synthetic chelates to the surface waters is an environmental problem found in some European rivers (11).

The incorporation of micrometerutrients into mixed fertilizers (such as NPK) is a procedure recognized as an economical mean of applying trace elements to soils and plants (12). Zn-enriched NPK fertilizers offer a rapid solution to the Zn deficiency in cereal crops (2). So, the application of NPK with ZnLS to cereal crops in calcareous soil could provide economical and environmental advantages in comparison with the addition of other Zn sources as inorganic salts or synthetic chelates.

When Zn sources were incorporated into NPK fertilizers, this element may react with the NPK components. These reactions could affect the bioavailability of the metal applied through the fertilizer. Mortvedt and Giordano (13) compared the efficiency of several chemical extractants in predicting the immediate effectiveness of Zn for maize when ZnSO<sub>4</sub> was incorporated into various macronutrient fertilizers. These authors found good correlation coefficients between Zn extracted with diethylenetriaminepentaacetic acid (0.005 M DTPA + 0.01 M CaCl<sub>2</sub>; pH buffered at 7.3) and the agronomic effectiveness of Zn sulfate incorporated to various macronutrient fertilizers. Thus, according

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to these authors, a previous ammonium bicarbonate (AB)-DTPA Zn extraction of the adhered NPK fertilizers could provide clues of their efficiency as Zn fertilizers.

The aim of the present work is to evaluate the efficacy of a ZnLS as Zn source when it is adhered to a NPK fertilizer to provide Zn to maize plants under controlled and field conditions.

## MATERIALS AND METHODS

**Reagents.** All reagents used in the experiments were of recognized analytical grade. The water used for the preparation of reagents or standards conforms to EN ISO 3696 (14), grade I, free of organic contaminants.

Zinc lignosulfonate (ZnLS) was prepared from a spruce ammonium lignosulfonate kindly provided by Lignotech Ibérica S.A. Some physico-chemical characteristics of the product given by the manufacturer were pH 4.4; total S 6.2%; carboxylic groups 2.6% and phenolic groups 1.9% while **Figure 1** illustrates the FT-IR spectrum of the  $\text{NH}_4$  spruce LS obtained on a FTIR Bruker IFS60v spectrophotometer with a MTC detector and diffuse reflectance (DRIFT) accessory. Spectra of LS samples were recorded in the region  $4000\text{--}400\text{ cm}^{-1}$  at a resolution of  $4\text{ cm}^{-1}$  in the transmittance mode, using the potassium pellet method. The main bands observed are in agreement with those reported in other works for lignins, technical lignins, and LS (15). All the LS follow a common pattern with a broadband  $3600\text{--}3300\text{ cm}^{-1}$  corresponding to hydroxyl groups in phenolic and carboxylic acids and several bands with variable intensity in the fingerprint region ( $1900\text{ to }800\text{ cm}^{-1}$ ). In this region, the main features appear at  $1770\text{ cm}^{-1}$  (aromatic acetoxy groups),  $1715$  and  $1630\text{ cm}^{-1}$  (unconjugated carbonyl–carboxyl stretching),  $1600\text{--}1500\text{ cm}^{-1}$  (C=C skeletal vibrations),  $1470\text{--}1460\text{ cm}^{-1}$  (C–H deformation combined with aromatic ring vibrations),  $1260\text{ cm}^{-1}$  (C=O stretch),  $1230\text{--}1215\text{ cm}^{-1}$  (C–C, C–O and C=O stretching),  $1140\text{ cm}^{-1}$  (C–H in plane deformation),  $1050\text{ cm}^{-1}$  (complex vibration associated with the C–O, C–C stretching and COH bending in polysaccharides) and  $840$  and  $810\text{ cm}^{-1}$  (C–H out-of-plane deformations). The band appearing at  $620\text{--}660\text{ cm}^{-1}$  is assigned to the sulfonic groups (S–O stretching vibration) formed from the reaction of sodium sulfite with the secondary OH of the aliphatic side chain of lignins.

The spruce ammonium lignosulfonate was selected based on previous data of its higher Zn complexing capacity in comparison with a spruce and an eucalyptus sodium lignosulfonate (16). On the basis of the maximum complexing capacity data (16), a ZnLS stock solution ( $60\text{ mM}$  of Zn) was prepared by mixing the suitable amount of LS and the corresponding volume of a  $100\text{ g Zn L}^{-1}$  of  $\text{ZnSO}_4$  solution. After the agitation of the mixture during one night in the dark, the solution was made up to volume. Total Zn concentration of the solution was assessed by atomic absorption spectroscopy (AAs) (AAAnalyst 800, Perkin-Elmer) and the complexed Zn concentration was estimated by using the Association of Official Analytical Chemists (AOAC) modified method (17).

**Preparation of the Zn-Adhered NPK Product.** Eight samples of  $100\text{ g}$  of NPK 8:15:15 (kindly provided by Fertiberia S.A.) were placed into a  $1.0\text{ kg}$  kneading machine (MRPI-06 SBS) where  $50\text{ mL}$  of the ZnLS solution was added with a spray nozzle at regular intervals of  $0.2\text{ mL min}^{-1}$ . After this, all samples were air dried at room temperature during  $12\text{ h}$ . The Zn concentration in the NPK products was supposed to be around  $2\text{ g}$  of Zn  $\text{kg NPK}^{-1}$  (0.2% of Zn in the NPK). The same procedure was used in the preparation of NPK with  $\text{ZnSO}_4$  adhered, but changing the ZnLS solution by one of  $60\text{ mM}$  of Zn applied as  $\text{ZnSO}_4$  sugar-coating solution. The sugar-coating solution formulation was 45% (w/w) sucrose, 30% (w/w)  $\text{ZnSO}_4$  solution, and 25% (w/w) talc.

The total Zn from each sample was measured by following the CE 2003/2003 9.1 Method (18). For that purpose,  $5\text{ g}$  of the Zn-adhered NPK was placed into a  $250\text{ mL}$  beaker. Fifty milliliters of  $6\text{ M HCl}$  was added in  $10\text{ mL}$  portions and then  $50\text{ mL}$  of  $\text{H}_2\text{O}$  was added. The beaker was stopped and vigorously shaken. The solution was first boiled during  $30\text{ min}$ , cooled, and then transferred to a  $500\text{ mL}$  volumetric flask and made up to volume. Eight replicates for each sample were done. The AB-DTPA extractable Zn, as described by Soltanpour and Workman (19), was measured in the NPK products. Half gram of product was placed into a  $50\text{ mL}$  beaker. Twenty milliliters of the extracting solution was added ( $\text{NH}_4\text{HCO}_3\ 1\text{ M} + \text{DTPA } 0.005\text{ M}$  adjusted to pH 7.6). The solution was

stirred during  $15\text{ min}$  at  $180\text{ cycles min}^{-1}$ , then filtered into a  $100\text{ mL}$  volumetric flask and made up to volume. Eight replicates for each sample were done. Zinc concentration in both portions (total and extractable Zn) was measured in AAs.

**Perlite Leaching Columns Experiment.** Polyvinyl chloride (PVC) columns ( $30\text{ cm}$  length,  $6\text{ cm}$  width) were filled up to  $17\text{ cm}$  with perlite (Projar S.A.),  $0.15\text{ g}$  of the fertilizer was applied, and then  $3\text{ cm}$  more of perlite was added. At the top of each column,  $5\text{ cm}$  of acid washed sand ( $0.5\text{--}2\text{ mm}$  of diameter) were incorporated in order to avoid evaporation. The treatments tested were NPK (8:15:15) with no Zn adhered and the same NPK with  $\text{ZnSO}_4$  (NPK +  $\text{ZnSO}_4$ ) and ZnLS (NPK + ZnLS) adhered.

Twenty milliliters of nutrient solution was added every 3 days to the upper zone of the columns. The chemical composition of the nutrient solution was as follows (mM):  $3.0\text{ Ca}(\text{NO}_3)_2$ ,  $2.0\text{ K}_2\text{SO}_4$ ,  $2.0\text{ MgSO}_4$ ,  $1.0\text{ KH}_2\text{PO}_4$ ,  $1.0\text{ CaSO}_4$ , and  $0.5\text{ NaCl}$ . Microelements in micromolar were  $50\text{ FeEDDHA}$  (Torneo, Bayer),  $25\text{ H}_3\text{BO}_3$ ,  $5.0\text{ MnSO}_4$ ,  $0.5\text{ CuSO}_4$ , and  $0.5\text{ (NH}_4)_6\text{ Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ . The N, P, and K concentrations in the solution were reduced in respect to the standard solution due to the addition of the NPK fertilizer. The nutrient solution readjustment was calculated by using the speciation program VMinteq. The pH of the nutrient solution was adjusted to  $8.0$  with  $\text{NaOH } 0.5\text{ M}$ . Leaching solutions were collected in  $50\text{ mL}$  Erlenmeyer flasks where P and Zn concentrations were measured. After 33 days, the perlite inside the columns was divided in four layers ( $5\text{ cm}$  each) and AB-DTPA extractable Zn (19) was determined on each one. Both Zn in the leachates and AB-DTPA extractable Zn from the perlite layers were measured by AAs. Phosphorus concentration was measured by an UV–visible method with a Shimadzu UV-160A spectrophotometer (20). Three replicates (columns) per treatment were conducted.

**Perlite Plant Experiment.** Maize seeds (*Zea mays* L. cv Agrano C500) sold by Syngenta-Seeds were germinated in the dark on moist filter paper at  $30\text{ }^\circ\text{C}$ . After germination, seedlings were transferred to a  $12\text{ L}$  containers with a complete nutrient solution without Zn. The composition of Zn deficient nutrient solution in mM was  $4.0\text{ KNO}_3$ ,  $3.0\text{ Ca}(\text{NO}_3)_2$ ,  $2.0\text{ KH}_2\text{PO}_4$ ,  $1.0\text{ MgSO}_4$ , and  $0.5\text{ mM NaCl}$ , and microelements in micromolar were  $50\text{ FeEDDHA}$ ,  $25\text{ H}_3\text{BO}_3$ ,  $5.0\text{ MnSO}_4$ ,  $0.5\text{ CuSO}_4$ , and  $0.5\text{ (NH}_4)_6\text{ Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ . This nutrient solution was buffered by a DTPA-excess solution (21) for precise imposition of Zn deficiency and the pH was kept at  $8.0 \pm 0.3$ .

After 4 days, maize seedlings were transferred to  $15\text{ L}$  polystyrene pots ( $1\text{ L}$  solution compartment at the bottom) filled with moistened perlite about  $5\text{ cm}$  before the top of the pot. On the top of the perlite,  $14\text{ g}$  of NPK 8:15:15 was added. Three treatments of NPK 8:15:15 were applied, NPK without added Zn, NPK with  $\text{ZnSO}_4$  adhered (NPK +  $\text{ZnSO}_4$ ), and NPK with ZnLS adhered (NPK + ZnLS). After the NPK addition, pots were filled with perlite to the brim and moistened prior to the seedlings transplant. Five maize plants per pot were placed and a diluted nutrient solution (the same as in perlite leaching columns experiment) was added. In this experiment  $0.5\text{ g}$  of solid  $\text{CaCO}_3$  was placed in the lower compartment of the pots in order to buffer the solution and simulate calcareous conditions. Four replicates per treatment were placed in a growth chamber, with a photoperiod light/night of  $16\text{ h}/8\text{ h}$ ; temperature  $25\text{ }^\circ\text{C}/18\text{ }^\circ\text{C}$  and relative humidity  $50\%/80\%$ . The radiation flux was  $500\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ .

After 28 days, shoots were collected, weighed, and thoroughly washed with deionized water. Then, plant material was dried at  $70\text{ }^\circ\text{C}$  for at least 2 days to determine dry weight. Concentration of Zn, Mn, Cu, and Fe was measured by AAs and phosphorus by a colorimetric method (20) after the dry mineralization of the samples. In addition to these determinations, substrate samples of perlite were taken at two different depths ( $10$  and  $20\text{ cm}$ ) in the pots where AB-DTPA extractable Zn (19) that remained after the experiment was determined.

**Growth Chamber Soil Experiment.** Polyethylene  $5.5\text{ L}$  pots were filled up until about  $5\text{ cm}$  from the top with a mixture of  $1.8\text{ kg}$  of soil (30%) and  $4.2\text{ kg}$  of sand (70%). The agricultural soil used was collected (first  $20\text{ cm}$  layer) from a location situated in Castronuevo de Esgueva, Valladolid, Spain ( $41^\circ 40' 50''\text{ N}$ ,  $4^\circ 36' 9''\text{ W}$ , alt.  $771\text{ m}$ ). Soil characteristics are shown in **Table 1**. Soil pH was determined at a soil-to-water ratio of  $1:2$  (w/v); oxidized soil OM, potassium, and exchangeable cations concentrations were measured by following the official methods (22). The sand used

**Table 1.** Physicochemical Characteristics of the Soils Used in the Growth Chamber Experiment and in Both Field Experiments Located in Quinto and Barrax

| soil properties                                      | 1. growth chamber | 2. Quinto | 3. Barrax |
|--|-------------------|-----------|-----------|
| sand (g kg <sup>-1</sup> )                           | 530               | 640       | 560       |
| silt (g kg <sup>-1</sup> )                           | 335               | 240       | 320       |
| clay (g kg <sup>-1</sup> )                           | 135               | 120       | 120       |
| OM (%)   | 1.5               | 1.7       | 1.5       |
| CaCO <sub>3</sub> (%)                                | 41                | 33        | 45        |
| pH   | 7.8               | 8.2       | 8.2       |
| exchangeable K (cmol <sub>c</sub> kg <sup>-1</sup> ) | 281               | 70        | 325       |
| Olsen P (mg kg <sup>-1</sup> )                       | 27                | 14        | 25        |
| AB-DTPA Zn (mg kg <sup>-1</sup> )                    | 0.5               | 0.9       | 0.4       |
| AB-DTPA Fe (mg kg <sup>-1</sup> )                    | 13                | 14        | 12        |
| AB-DTPA Mn (mg kg <sup>-1</sup> )                    | 9.2               | 12        | 10        |
| AB-DTPA Cu (mg kg <sup>-1</sup> )                    | 1.3               | 1.3       | 1.0       |

was a normalized sand (0.5–2 mm of diameter), provided by Instituto Eduardo Torroja (CSIC, Spain). The soil/sand mixture was moistened and three treatments (NPK, NPK + ZnSO<sub>4</sub>, and NPK + ZnLS) were applied with four replicates per each one. In this experiment, 2 g of NPK was added to each pot. As in the perlite experiment, the amount of Zn in the fertilizers was expected to be 0.2%. After the NPK addition, pots were completely filled with the sand–soil mixture to the brim. Maize seeds were germinated and Zn deficiency was induced as it is described in the perlite plant experiment. Then, four maize seedlings were transferred to each pot. A nutrient solution (the same as in the perlite experiments in order to make sure that Zn deficiency was the only limiting factor) was added to the pots.

Shoots were collected 28 days after the transplant. Dry weight and concentration of Zn, P, Fe, Mn, and Cu were determined in shoots. Soil samples were taken at different depths (10 and 20 cm) in the pots at the end of the experiment where AB-DTPA extractable Zn (20) was determined.

**Field Experiments.** A field experiment was carried out from May 2007 to November 2007 on a place located at Quinto de Ebro (41°25'32" N, 0°31'00" W, altitude 199 m), Zaragoza, Spain. Soil characteristics are shown in **Table 1**. The climatic data provided by the National Institute of Meteorology (INM) were (crop cycle averages) temperature 19 °C; maximum temperature 26 °C; minimum temperature 13 °C; relative humidity 56% and precipitation 27 mm. The experimental design was a split-plot design with four randomized subplots per treatment. Subplots were 18 m long by 18 m wide (324 m<sup>2</sup>). The total area of the experiment was 3900 m<sup>2</sup> approximately. Each plot was treated with minimum tillage consisted of two tillage operations with a chisel plow and two disking to a depth of 10 cm. Treatments (NPK, NPK + ZnSO<sub>4</sub>, and NPK + ZnLS) were applied before sowing. Thirty kilograms of each fertilizer were applied to each of the four subplots disposed in randomized blocks.

Preparation of the NPK products was carried out in the Agronomic Laboratory of Fertiberia S.A. following the procedure described in the preparation of the adhered NPK products but with 500 times higher scale. For each treatment, 300 kg of product were prepared in six batches of 50 kg each. For each batch, three samples were collected and the available Zn was determined. Means of available Zn measured in the NPK with ZnLS and ZnSO<sub>4</sub> adhered were 1310 and 190 mg Zn kg<sup>-1</sup> of NPK, respectively.

Maize seeds were sown in May of 2007 using a sower machine. Distance between crop rows was 0.69 m, and plant population was approximately 2600 seeds per subplot. Nitrogen as urea (500 kg ha<sup>-1</sup>) was added to the soil one month after germination of the seeds. The experimental plot was border irrigated throughout all growing season. Farming practices carried out in the experiment area were similar to those of the farmers in the region.

In November, 2007 maize plants were harvested with a harvesting machine. Grain yield, dried weight, and concentration of Zn, P, Fe, Mn, and Cu were determined in shoots and grain following the same procedure described for the previous experiments. Whole plant except ear, grains, and roots will be called as shoot.

The second field experiment was carried out in Barrax (Albacete, Spain, 39°02'34" N, 2°11'08" W, altitude 708 m) from May 2007 to November 2007. Soil characteristics are shown in **Table 1**. The climatic data provided by the INM were (crop cycle averages) temperature 18 °C; maximum

temperature 26 °C; minimum temperature 11 °C; relative humidity 58% and precipitation 15 mm. In this experiment, the plot was spray irrigated throughout all the growing season. The experimental design was the same of the previous experiment.

Maize was harvested on November, 2007. Subplots were harvested by a harvesting machine and yields were measured. Shoots and grain Zn, P, Fe, Mn, and Cu concentrations were determined following the same procedure described for the previous experiments.

**Statistical Analyses.** Differences among fertilizer treatments were analyzed by one-way ANOVA, followed by a post hoc multiple comparison of means using the Duncan test ( $\alpha = 0.05$  for growth chamber experiments and  $\alpha = 0.1$  for the field ones). Statistical analysis was performed with the SPSS 15.0 program.

## RESULTS AND DISCUSSION

**Preparation of the Zn Adhered NPK Product.** **Table 2** shows the total and AB-DTPA extractable Zn measured in the NPK with ZnLS and ZnSO<sub>4</sub> adhered. Similar total Zn concentration in both products was found, but the available Zn concentration was much lower in the NPK + ZnSO<sub>4</sub> than in the NPK + ZnLS product. The percentage of available Zn in the NPK + ZnLS product was 85% of the total Zn of the fertilizer and 19% in the case of the NPK + ZnSO<sub>4</sub>. This difference was attributed to the LS presence.

The AB-DTPA extractable Zn in soils is considered as the Zn fraction available for crops (19). Mortvedt and Giordano (13) tried to correlate the percentage of Zn incorporated as ZnSO<sub>4</sub> to various macronutrient fertilizers (NPK, urea, ammonium polyphosphate, and so forth) with its agronomic effectiveness for maize. They determined the Zn content of each fertilizer by using several extractants, that is, H<sub>2</sub>O, HCl, acid K<sub>2</sub>SO<sub>4</sub>, and solutions of different chelating agents, and concluded that there was a good correlation between the DTPA extracted Zn from the fertilizer and their agronomic efficacy for maize. Despite the higher value of total Zn concentration of the NPK + ZnSO<sub>4</sub> fertilizer, a higher efficacy of the NPK + ZnLS fertilizer was expected due to its Zn complexed fraction.

**Table 2** also shows the coefficients of variation (CV) for the total and AB-DTPA extractable Zn determinations. The CV was used to verify the reproducibility rate of the Zn coating methods used in NPK + ZnLS and NPK + ZnSO<sub>4</sub> fertilizers. This reproducibility could be related with the homogeneity of the Zn added into the fertilizers. Authors established a limit of acceptance of CV ≤ 10%. While AB-DTPA extractable Zn presented CV values higher than the total Zn, the CV of both fertilizers do not reach the threshold selected, so both fertilizers were considered as homogeneous.

**Perlite Leaching Columns Experiment.** Leached Zn was not detected from any of the columns where NPK, NPK + ZnSO<sub>4</sub>, or NPK + ZnLS fertilizers were applied. Results are similar to those obtained by Alvarez et al. (6) in a leaching column experiments done with different calcareous soils. The authors did not observe mobilization of Zn through the column when a ZnLS was applied. Moreover, in a subsequent leaching experiments Alvarez et al. (23) found that in a slightly acid soil (pH 6.2) and in a neutral soil (pH = 7.1), the Zn applied as ZnLS remained at the same depth where it was placed. In both experiments, authors pointed out the relationship between the low mobility of Zn and the fixation of ZnLS to some soil components.

In our experiment, columns were filled with expanded perlite. Although this material is chemically and thermally inert (24) it maintains a certain cation adsorption capacity (25) surely due to the high porosity and the presence of silicate on its structure.

The highest amount of available Zn in the four sections of the columns divided at the end of the leaching experiment was found

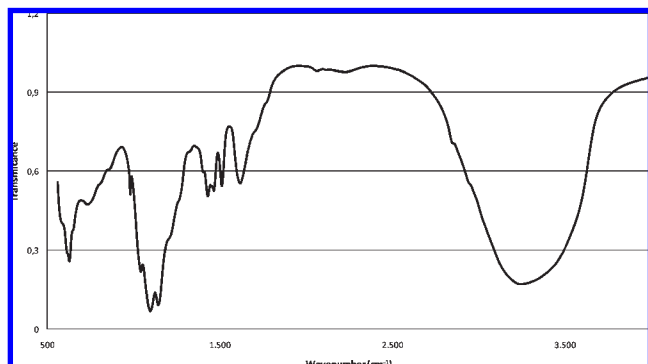


Figure 1. FT-IR spectrum of spruce ammonium lignosulfonate ( $\text{NH}_4\text{-LS}$ ).

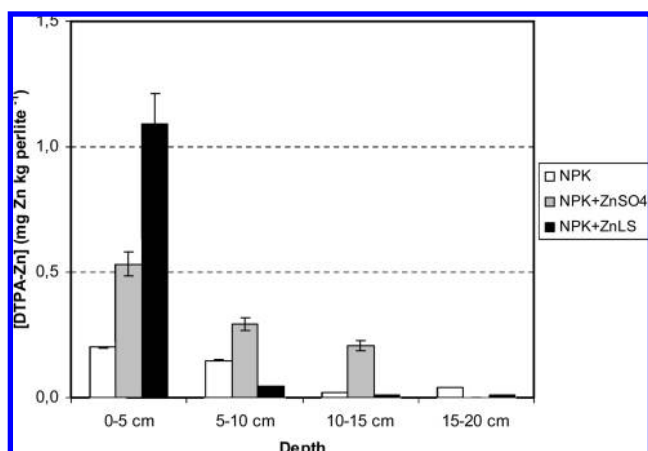


Figure 2. Ammonium bicarbonate-DTPA extractable Zn concentration ( $\text{mg L}^{-1}$ ) measured at different depths of the perlite columns at the end of the leaching experiment. Error bars denote standard errors of the means ( $n = 3$ ).

in the first 5 cm of the columns treated with NPK + ZnLS. The concentration of AB-DTPA Zn measured at the first 5 cm of perlite was two times higher at NPK + ZnLS ( $1.2 \text{ mg Zn kg}^{-1}$  of perlite) columns than in the NPK + ZnSO<sub>4</sub> ( $0.52 \text{ mg Zn kg}^{-1}$  of perlite) columns (Figure 2).

In terms of AB-DTPA Zn distribution in perlite columns, most of the Zn applied (98%) was retained in the first 5 cm when NPK + ZnLS was added (Figure 2). On the other hand, a slight mobilization of Zn to lower levels of the columns was found in the case of NPK + ZnSO<sub>4</sub>. 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 (26) observed a Zn movement to a lower depth of calcareous and acid soils when ZnSO<sub>4</sub> was applied. But Gangloff et al. (8) did not observe this mobilization when applied ZnSO<sub>4</sub> to a calcareous soil. Despite this, both Singh (26) and Gangloff et al. (8) agreed that most of the Zn added as ZnSO<sub>4</sub> was found in the area of application of the fertilizer. This fact was also observed in our experiment.

In columns where NPK without any Zn source was added, residual concentrations of Zn ( $0.1\text{--}0.2 \text{ mg Zn kg}^{-1}$  of perlite) were found in the first 10 cm (Table 2). This Zn could come from the raw materials of NPK fertilizers. In the NPK used for these experiments, 40 mg of total Zn  $\text{kg}^{-1}$  of fertilizer was determined.

The increase of the P concentration in the leaching solutions since the ninth day (Figure 3) showed the beginning of the NPK fertilizer dissolution. Thus, the decrease of P concentration since the 27th day of washings would indicate that the fertilizer was almost depleted.

Table 2. Total and AB-DTPA Extracted Zn in the NPK with ZnLS and ZnSO<sub>4</sub> Adhered Products<sup>a</sup>

| series | NPK + ZnLS                          |                                    | NPK + ZnSO <sub>4</sub>             |                                    |
|--------|-------------------------------------|------------------------------------|-------------------------------------|------------------------------------|
|        | total Zn ( $\text{mg Zn kg}^{-1}$ ) | AB-DTPA Zn ( $\text{mg kg}^{-1}$ ) | total Zn ( $\text{mg Zn kg}^{-1}$ ) | AB-DTPA Zn ( $\text{mg kg}^{-1}$ ) |
| 1      | 1210 ± 30                           | 980 ± 30                           | 1430 ± 30                           | 260 ± 20                           |
| 2      | 1250 ± 50                           | 1150 ± 30                          | 1390 ± 30                           | 260 ± 30                           |
| 3      | 1320 ± 40                           | 1130 ± 70                          | 1400 ± 50                           | 220 ± 30                           |
| 4      | 1310 ± 30                           | 1030 ± 20                          | 1420 ± 40                           | 260 ± 20                           |
| 5      | 1230 ± 30                           | 1140 ± 50                          | 1410 ± 30                           | 260 ± 30                           |
| 6      | 1310 ± 20                           | 1160 ± 70                          | 1380 ± 30                           | 290 ± 30                           |
| 7      | 1220 ± 50                           | 950 ± 50                           | 1410 ± 30                           | 280 ± 20                           |
| 8      | 1310 ± 30                           | 1070 ± 60                          | 1340 ± 20                           | 250 ± 30                           |
| mean   | 1270 ± 20                           | 1080 ± 30                          | 1400 ± 10                           | 260 ± 30                           |
| CV (%) | 4                                   | 8                                  | 7                                   | 7                                  |

<sup>a</sup>Data values are means ± SE of each series of samples. CV = coefficient of variation.

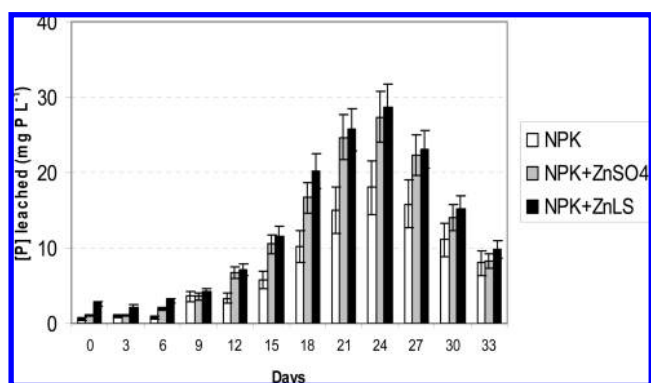


Figure 3. Phosphorus concentration ( $\text{mg L}^{-1}$ ) measured in the leached solution of the perlite columns. Error bars denote standard errors of the means ( $n = 3$ ).

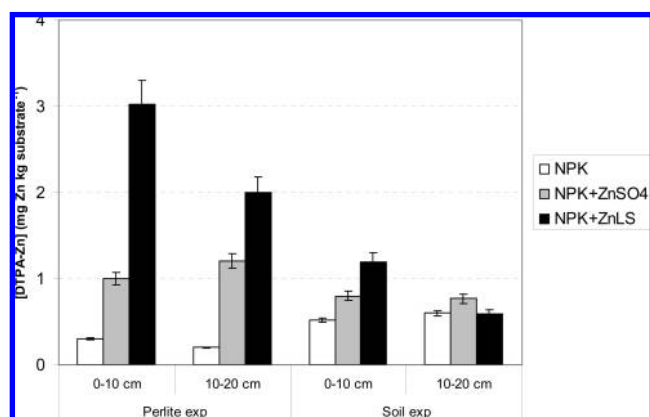
**Perlite Plant Experiment.** Maize plants treated with NPK + ZnLS rendered the highest dry weight in comparison with plants treated with NPK and with NPK + ZnSO<sub>4</sub> (Table 3). Even though, the dry weight of plants treated with adhered NPK (ZnLS and ZnSO<sub>4</sub>) did not show statistical differences between them. Moreover the highest Zn concentration in shoots was found in the plants fertilized with NPK + ZnLS, followed by plants treated with NPK + ZnSO<sub>4</sub> and the NPK-treated plants. In that case, differences were statistically significant. Plant shoots treated with NPK and NPK + ZnSO<sub>4</sub> showed Zn levels below the critical concentration for maize ( $20 \text{ mg kg}^{-1}$ ) (27, 28). Dry matter and Zn concentration in maize shoots followed the same trends observed by Martín-Ortiz et al. (16) in hydroponic conditions. These authors found that the plants treated with ZnLS in pure hydroponic conditions at pH 8.0 presented higher dry weight and Zn concentration than the ones fertilized with ZnSO<sub>4</sub> at the same conditions. On the basis of these data, NPK + ZnSO<sub>4</sub> treatment was expected to show lower efficiency as Zn fertilizer than NPK + ZnLS at our experimental conditions; this fact was confirmed by our results. The best behavior of the NPK + ZnLS as Zn fertilizer could be related with the higher ability of the ZnLS to maintain Zn in solution in comparison with ZnSO<sub>4</sub>.

For all treatments, shoots P content presented the opposite tendency than Zn content (Table 3). Antagonism between P and Zn has been recognized for many years despite that the mechanism responsible is still not completely understood. Takkar et al. (29) in a 3-year field experiment conducted with a wheat-maize rotation concluded that a high P supply could produce

**Table 3.** Dry Weights ( $g \pm SE$ ); Zn, Fe, Mn, Cu Concentration ( $\mu g g^{-1} DW \pm SE$ ); Phosphorus Concentration ( $mg g^{-1} DW \pm SE$ ) and P/Zn Ratio in Maize Shoots of Perlite and Soil Experiments<sup>a</sup>

| treatments              | maize shoots perlite experiment |                          |                      |      |                          |                          |                          |
|-------------------------|---------------------------------|--------------------------|----------------------|------|--------------------------|--------------------------|--------------------------|
|                         | DW (g)                          | Zn ( $\mu g g^{-1} DW$ ) | P ( $mg g^{-1} DW$ ) | P/Zn | Fe ( $\mu g g^{-1} DW$ ) | Mn ( $\mu g g^{-1} DW$ ) | Cu ( $\mu g g^{-1} DW$ ) |
| NPK                     | 4 $\pm$ 1 b                     | 3 $\pm$ 1 c              | 4.3 $\pm$ 0.1 a      | 1300 | 160 $\pm$ 22 a           | 46 $\pm$ 2 a             | 6 $\pm$ 1 a              |
| NPK + ZnSO <sub>4</sub> | 8 $\pm$ 1 a                     | 16 $\pm$ 3 b             | 3.5 $\pm$ 0.2 b      | 215  | 96 $\pm$ 4 b             | 25 $\pm$ 3 b             | 4 $\pm$ 1 b              |
| NPK + ZnLS              | 9 $\pm$ 1 a                     | 29 $\pm$ 2 a             | 2.8 $\pm$ 0.6 c      | 100  | 70 $\pm$ 7 c             | 21 $\pm$ 2 b             | 4 $\pm$ 1 b              |
| treatments              | maize shoots soil experiment    |                          |                      |      |                          |                          |                          |
|                         | DW (g)                          | Zn ( $\mu g g^{-1} DW$ ) | P ( $mg g^{-1} DW$ ) | P/Zn | Fe ( $\mu g g^{-1} DW$ ) | Mn ( $\mu g g^{-1} DW$ ) | Cu ( $\mu g g^{-1} DW$ ) |
| NPK                     | 5 $\pm$ 1 b                     | 10 $\pm$ 1 c             | 1.9 $\pm$ 0.1 a      | 190  | 56 $\pm$ 2 b             | 38 $\pm$ 3 ab            | 5 $\pm$ 1 ns             |
| NPK + ZnSO <sub>4</sub> | 5 $\pm$ 1 b                     | 19 $\pm$ 1 b             | 1.6 $\pm$ 0.1 b      | 85   | 60 $\pm$ 2 b             | 42 $\pm$ 2 a             | 4 $\pm$ 1                |
| NPK + ZnLS              | 7 $\pm$ 1 a                     | 34 $\pm$ 2 a             | 0.8 $\pm$ 0.2 c      | 25   | 82 $\pm$ 2 a             | 35 $\pm$ 2 b             | 5 $\pm$ 1                |

<sup>a</sup> For each column, different letters denote significant differences among the treatments according to Duncan's multiple-range test ( $\alpha = 0.05$ ) ( $n = 4$ ).



**Figure 4.** Ammonium bicarbonate-DTPA extractable Zn measured at two different depths at the end of the perlite and soil pot experiments. Error bars denote standard errors of the means ( $n = 4$ ).

severe Zn-deficiency symptoms. Thus, an increase on shoots Zn concentration could result in a significant increase of growth and a dilution of the P concentration in plant. Evidences of the Zn–P antagonism was also observed in our perlite experiment. Plants fertilized with NPK presented the highest levels of P and the lowest Zn levels in shoots. Thus, plants treated with NPK + ZnLS showed the lowest levels of P and the highest Zn concentration in shoots. The P–Zn interaction was expressed with the P/Zn ratio at different parts of the maize plant by different authors (29). According to Takkar et al. (29) values of P/Zn greater than 150 in 25 day old leaves indicated a severe Zn deficiency. Our results gave P/Zn levels of 1300 in plants treated without any Zn source, 215 in plants treated with NPK + ZnSO<sub>4</sub> and 100 in plants fertilized with NPK + ZnLS.

Moreover, results showed that the Fe and Mn concentrations decreased as the concentration of Zn increased in shoots (Table 3), although for Mn the decrease was not significant. These data were similar to those obtained by Adiloglu (30) in an experiment with maize plants grown in a Zn-deficient soil. The author concluded that Fe, Mn, and Cu plant contents decreased as well as the applied Zn concentration increased. It is known that Zn can interact with iron, copper, and manganese (31). The most marked interactions were found between Fe and Zn, which mutually antagonized in translocation (32), and between Mn and Zn, according to the speculative model of Kochian (33), Mn may influence Zn<sup>2+</sup> influx into root cells via the divalent cation channel.

At the end of the experiment, Zn retained by the perlite substrate was quantified at different depths (Figure 4). The highest AB-DTPA extractable Zn concentration was found in

the pots fertilized with NPK + ZnLS (3.0 mg Zn kg<sup>-1</sup> of perlite), especially at the upper 0–10 cm layer. This concentration was three times higher than the AB-DTPA Zn concentration found when NPK + ZnSO<sub>4</sub> treatment was applied (1.0 mg Zn kg<sup>-1</sup> of perlite). Finally, NPK + ZnLS fertilized pots presented significant differences of available Zn between the upper (3.0 mg Zn kg<sup>-1</sup> of perlite) and lower (2.0 mg Zn kg<sup>-1</sup> of perlite) layers, while NPK + ZnSO<sub>4</sub> and NPK treatments did not present these differences (Figure 4). That highest available Zn found at the NPK + ZnLS treatment could be related with the data showed in Table 2, where the AB-DTPA Zn extracted in NPK + ZnSO<sub>4</sub> columns decreased in comparison with the Zn extracted in the NPK + ZnLS ones. Moreover, when NPK + ZnLS was applied, the available Zn concentration measured at the upper level of the pots was statistically higher than the one at the lower level. The applied Zn scarcely moves throughout the perlite and most of the fertilizer remained at the zone where it was applied.

**Growth Chamber Soil Experiment.** NPK + ZnLS treated plants rendered the shoot highest dry weight in comparison with the other treatments (NPK and NPK + ZnSO<sub>4</sub>) (Table 3). Wang et al. (2004) (34) also studied the effect of ZnLS application on maize under liming soil conditions. The results of their pot experiment indicated that the maize fertilized with ZnLS showed higher biomass than plants fertilized with ZnSO<sub>4</sub>.

Concerning the Zn concentration in shoots, the plants with the highest concentration were the ones fertilized with NPK + ZnLS. Plants treated with NPK + ZnSO<sub>4</sub> showed higher Zn concentration in their shoots than the plants treated with NPK without added Zn. As in the perlite experiment, plants treated with NPK and NPK + ZnSO<sub>4</sub> presented Zn levels below the reference critical concentration (27, 28). The results obtained in our experiment were similar to those presented by Rico et al. (35) in maize plants grown in calcareous soils (pH = 8.5) when urea products coated with ZnLS were supplied. Authors observed that crop yield and the amount of Zn in plants increased when ZnLS fertilizers were applied in comparison with the control treatment (without added Zn). Moreover, Goos et al. (36) compared the availability of some granular Zn sources (ZnSO<sub>4</sub>, and a Zn humate-lignosulfonate [ZnHL]) to maize in a calcareous soil. In these experiments, the Zn source was administered without a macronutrient fertilizer. These authors concluded that Zn availability for the plant when the ZnHL complex was applied was greater than when the Zn was supplied as ZnSO<sub>4</sub>.

Plants treated only with NPK without added Zn presented the highest P concentration in the shoots in comparison with the plants treated with NPK + ZnSO<sub>4</sub> (Table 3). However, plants of NPK + ZnLS treatment showed the lowest P concentration in their shoots. Moreover, plants treated with NPK + ZnLS

**Table 4.** Grain Harvested (Mt ha<sup>-1</sup> ± SE) and Zn, Fe, Mn, Cu Concentration (μg g<sup>-1</sup> DW ± SE); Phosphorus Concentration (mg g<sup>-1</sup> DW ± SE); P/Zn Ratio in Maize Shoot and Grains in Two Field Experiments<sup>a</sup>

| treatments              | Quinto Experiment          |                           |      |                            |                            |                            |                                |                            |                           |      | Barrax Experiment          |                            |                            |                            |                           |        |                            |                            |                            |  |
|-------------------------|----------------------------|---------------------------|------|----------------------------|----------------------------|----------------------------|--------------------------------|----------------------------|---------------------------|------|----------------------------|----------------------------|----------------------------|----------------------------|---------------------------|--------|----------------------------|----------------------------|----------------------------|--|
|                         | shoots                     |                           |      |                            |                            | grains                     |                                |                            |                           |      | shoots                     |                            |                            |                            |                           | grains |                            |                            |                            |  |
|                         | Zn (μg g <sup>-1</sup> DW) | P (mg g <sup>-1</sup> DW) | P/Zn | Fe (μg g <sup>-1</sup> DW) | Mn (μg g <sup>-1</sup> DW) | Cu (μg g <sup>-1</sup> DW) | Harvest (Mt ha <sup>-1</sup> ) | Zn (μg g <sup>-1</sup> DW) | P (mg g <sup>-1</sup> DW) | P/Zn | Fe (μg g <sup>-1</sup> DW) | Mn (μg g <sup>-1</sup> DW) | Cu (μg g <sup>-1</sup> DW) | Zn (μg g <sup>-1</sup> DW) | P (mg g <sup>-1</sup> DW) | P/Zn   | Fe (μg g <sup>-1</sup> DW) | Mn (μg g <sup>-1</sup> DW) | Cu (μg g <sup>-1</sup> DW) |  |
| NPK                     | 23 ± 2 c                   | 0.20 ± 0.03 a             | 9    | 116 ± 15 b                 | 94 ± 7 a                   | 12 ± 1 ns                  | 16 ± 1 ns                      | 19 ± 3 b                   | 0.24 ± 0.05 ns            | 13   | 13 ± 1 ns                  | 35 ± 4 a                   | 3 ± 1 ns                   | 16 ± 1 ns                  | 0.25 ± 0.03               | 10     | 15 ± 1                     | 13 ± 2 b                   | 2 ± 1                      |  |
| NPK + ZnSO <sub>4</sub> | 42 ± 7 b                   | 0.15 ± 0.02 b             | 4    | 155 ± 19 a                 | 84 ± 11 a                  | 10 ± 1                     | 16 ± 1                         | 26 ± 4 ab                  | 0.22 ± 0.06               | 10   | 15 ± 1                     | 13 ± 2 b                   | 2 ± 1                      | 17 ± 1                     | 0.25 ± 0.03               | 10     | 16 ± 1                     | 11 ± 2 b                   | 2 ± 1                      |  |
| NPK + ZnLS              | 69 ± 5 a                   | 0.12 ± 0.04 c             | 2    | 150 ± 12 a                 | 61 ± 9 b                   | 12 ± 1                     | 17 ± 1                         | 30 ± 2 a                   | 0.25 ± 0.03               | 10   | 16 ± 1                     | 11 ± 2 b                   | 2 ± 1                      |                            |                           |        |                            |                            |                            |  |
| NPK                     | 9 ± 1 c                    | 0.22 ± 0.01 ns            | 24   | 94 ± 14 ns                 | 62 ± 8 a                   | 9 ± 1 ns                   | 14 ± 1 b                       | 16 ± 1 ns                  | 0.25 ± 0.02 ns            | 16   | 14 ± 1 ns                  | 33 ± 1 ns                  | 3 ± 1 ns                   | 14 ± 1 b                   | 0.26 ± 0.02               | 15     | 14 ± 1                     | 31 ± 2                     | 3 ± 1                      |  |
| NPK + ZnSO <sub>4</sub> | 19 ± 2 b                   | 0.20 ± 0.02               | 11   | 90 ± 11                    | 40 ± 1 b                   | 10 ± 3                     | 14 ± 1 b                       | 17 ± 1                     | 0.26 ± 0.03               | 15   | 14 ± 1                     | 31 ± 2                     | 3 ± 1                      | 16 ± 1 a                   | 0.26 ± 0.02               | 15     | 14 ± 1                     | 33 ± 1                     | 3 ± 1                      |  |
| NPK + ZnLS              | 28 ± 1 a                   | 0.20 ± 0.01               | 7    | 98 ± 10                    | 65 ± 2 a                   | 10 ± 1                     | 16 ± 1 a                       | 18 ± 1                     | 0.26 ± 0.02               | 15   | 14 ± 1                     | 33 ± 1                     | 3 ± 1                      |                            |                           |        |                            |                            |                            |  |

<sup>a</sup>For each column, different letters denote significant differences among the treatments according to Duncan's multiple-range test ( $\alpha = 0.1$ ). ns = not significant ( $n = 4$ ).

presented higher concentration of Fe and lower concentration of Mn in their shoots than the rest of the treated plants. The Cu concentration in plant shoots did not showed significant differences among treatments.

The AB-DTPA extractable Zn measured in upper and lower levels of the soil after the experiment is shown in the **Figure 4**. The highest concentration of AB-DTPA Zn was found in the upper level (0–10 cm) of the NPK + ZnLS treatment pots (1.2 mg Zn kg<sup>-1</sup> of soil). The extractable Zn measured in the NPK + ZnSO<sub>4</sub> pots presented Zn levels half a unit lower than the ones obtained for the NPK + ZnLS pots. The lowest AB-DTPA Zn was extracted at the NPK treatment pots (0.5 mg Zn kg<sup>-1</sup> of soil). The NPK + ZnLS treatment was the only one that presented significant differences of available Zn between the upper (1.2 mg Zn kg<sup>-1</sup> of soil) and lower levels (0.6 mg Zn kg<sup>-1</sup> of soil). It is necessary to take into account that the critical Zn level for corn was established as 0.9 mg Zn kg<sup>-1</sup> of soil (37). Ammonium bicarbonate-DTPA extractable Zn at the end of the experiment decreased 7 times in soil pots where NPK + ZnLS was added compared to the amount found in the perlite experimental pots. Both at perlite and soil experiments, the greatest concentration of AB-DTPA Zn was obtained at the upper level of pots where the NPK + ZnLS product was placed (**Figure 4**). Gonzalez et al. (10) compared the Zn mobility of a mixture of Zn chelates (Zn DTPA–HEDTA–EDTA) and a Zn–aminelignosulfonate (ZnAML) in a calcareous soil. They found that the amount of Zn leached from the soils treated with ZnAML was very small in comparison with the soils fertilized with the mixture of Zn chelates.

Although the agronomic efficiency (shoots dry weight and Zn concentration) of the products used in our perlite and soil experiments presented a similar trend, higher Zn levels in shoots where found at the soil test for the three treatments. This could be related with the lower P uptake observed at the soil experiment (**Table 3**), which is probably due to the reactions of phosphate fertilizers with some calcareous soil constituents (38). Thus, P/Zn index decreased in the soil compared with the perlite experiment.

**Field Experiments.** **Table 4** shows the crop yield and the mineral analysis of the maize (grains and shoots) harvested in both field experiments. The amount of grain collected in Quinto experiment did not presented statistical differences among treatments, even though in the plots treated with NPK + ZnLS, 1000 kg ha<sup>-1</sup> more of grain were harvested than in the others plots. At this location, plants fertilized with NPK + ZnLS presented the highest Zn concentration in shoots and grains. Shoot and grains from plots fertilized with NPK + ZnSO<sub>4</sub> showed higher Zn levels than the plants with no added Zn, although the differences were higher in shoot than in grain (**Table 4**). Furthermore the highest concentrations of P and Mn in the maize shoot were found in the plots fertilized with the NPK product with no added Zn (**Table 4**).

In Barrax experiment, statistical differences among treatments in the crop yield were found. In plots fertilized with NPK + ZnLS, an increase of crop yield was obtained in comparison with the two other treatments. Moreover, the NPK + ZnLS fertilization provided the highest Zn maize shoots concentration in comparison to NPK and NPK + ZnSO<sub>4</sub> additions (**Table 4**).

The P/Zn ratio in shoots seems to be a good index to follow the Zn uptake and its distribution inside the plant (29). This ratio showed drastic changes in treatments at both locations (**Table 4**) and was higher in plants treated with NPK and lower for the plants treated with NPK + ZnLS. Phosphorus/Zn ratio was higher in Barrax than in Quinto experiment. As the amount of P added for all treatments in both field experiments was the same, the P/Zn index changes could be mainly attributed to the differences on Zn availability at each experimental plot. Both

the P/Zn ratio in shoots and the shoot Zn content were suitable indexes of Zn nutrition of maize grown in calcareous soil (29). Our results showed that the P/Zn ratio in shoots should not exceed a value of 10 and shoot Zn content should be between 20 and 30 mg kg<sup>-1</sup> for a good maize production.

Results of field experiments showed an increase of 6% (Quinto experiment) and 12% (Barrax experiment) in maize yields when NPK + ZnLS product was applied to the plots in comparison with NPK + ZnSO<sub>4</sub>, and with NPK plots application (Table 4). No differences in grain yield were obtained between NPK and NPK + ZnSO<sub>4</sub> treatments. However, Malakouti et al. (39) showed 10% increases in wheat grain yield when a NPK fertilizer with a simultaneous ZnSO<sub>4</sub> addition was applied compared to NPK addition with no added Zn. Shivay et al. (40) obtained 10% increases of rice grain production when applied a ZnSO<sub>4</sub>-coated urea fertilizer (1.3 kg of Zn ha<sup>-1</sup>) in comparison with prilled urea addition. Although results obtained by these authors do not agree with those observed in our experiments, comparisons between experiments are difficult to do because experimental conditions are diverse and the studied crops have different sensitivities to Zn deficiency (1).

Although we did not find literature references of field experiments where a mixed NPK with ZnLS fertilizer was tested, there were numerous studies related to the benefits of Zn fertilization on cereal crops that were grown in calcareous soils (1, 41, 42). Cakmak (2) remarked that the application of Zn fertilizers or Zn-enriched NPK fertilizers could offer a good solution in the correction of Zn deficiencies and increase plant growth and yield. This fact was observed in our results, especially when the NPK + ZnLS fertilizer was applied.

Data showed that plants grown in Quinto experiment presented higher Zn concentrations than the plants grown in Barrax experiment for all treatments. Besides plants grown in Quinto presented Zn shoots levels above the critical concentration for all plots (27, 28). However, in Barrax only plants fertilized with NPK + ZnLS showed shoot Zn concentration higher than this critical level. This fact was well related with the AB-DTPA extractable Zn obtained in both soils before the addition of the fertilizers. The Quinto soil had 0.9 mg Zn kg<sup>-1</sup> of soil, while the AB-DTPA Zn measured in the soil of Barrax reached levels of 0.4 mg Zn kg<sup>-1</sup> of soil. Havlin and Soltanpour (37) established the critical level for available Zn in soils as 0.9 mg of Zn kg<sup>-1</sup> soil. The Zn bioavailability could be influenced by other properties of the soil, as the carbonate and phosphate contents. These compounds were higher in the Barrax than in the Quinto soils and could explain the lowest Zn availability for the crop grown in Barrax (32). The relative high level of available Zn observed in Quinto soil coupled with its P and carbonate contents and could explain the relative high Zn uptake by maize plants in this soil.

The NPK + ZnLS treatment presented a significant increase of Zn concentration in the grains harvested at the Quinto experiment. This tendency was also observed in the Barrax field. Zinc grain concentration in NPK treatment of Quinto and all treatments in Barrax locations did not reach the meet daily human requirement, established by FAO in at least 20 mg of Zn kg<sup>-1</sup> of grain (43, 44).

In summary, growth chamber perlite and growth chamber soil experiment showed that NPK + ZnLS addition to maize seedlings increased both yield and Zn concentration in shoots in comparison with NPK + ZnSO<sub>4</sub> in calcareous conditions. The Zn incorporated as ZnLS was fairly fixed in the zone where it was applied, avoiding leaching to the environment. In general, in field experiments NPK + ZnLS application increased the amount of grain and the Zn concentration in the shoots of the plants harvested. A great response to Zn fertilization should be expected

in soils with lower Zn content, as occurred in our field work between Barrax (extremely low AB-DTPA Zn concentration) and Quinto (low AB-DTPA Zn concentration) experiment. Results obtained show that the application of NPK + ZnLS in maize crops is an economical and ecological alternative to another Zn sources in calcareous conditions.

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