

## Article

# Zinc Uptake by Plants as Affected by Fertilization with Zn Sulfate, Phosphorus Availability, and Soil Properties

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**Abstract:** Zinc (Zn) deficiency constrains crop yield and quality, but soil factors influencing Zn availability to plants and reactions of applied Zn fertilizer are not fully understood. This work is aimed at studying Zn availability in soil and the use efficiency of Zn fertilizers by plants as affected by soil properties and particularly by soil available P. We performed a pot experiment involving four consecutive crops fertilized with Zn sulfate using 36 soils. The cumulative Zn uptake and dry matter yield in the four crops increased with increased initial diethylenetriamine pentaacetic acid extraction of Zn (DTPA-Zn) ( $R^2 = 0.75$  and  $R^2 = 0.61$ ;  $p < 0.001$ ). The initial DTPA-Zn increased with increased Olsen P ( $R^2 = 0.41$ ;  $p < 0.001$ ) and with increased ratio of Fe in poorly crystalline to Fe in crystalline oxides ( $R^2 = 0.58$ ;  $p < 0.001$ ). DTPA-Zn decreased with increased cumulative Zn uptake, but not in soils with DTPA-Zn  $< 0.5 \text{ mg kg}^{-1}$ . Overall, the available Zn is more relevant in explaining Zn uptake by plants than applied Zn sulfate. However, in Zn-deficient soils, Zn fertilizer explained most of the Zn uptake by crops. Poorly crystalline Fe oxides and P availability exerted a positive role on Zn availability to plants in soil.



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**Keywords:** micronutrients; Olsen P; sorbent surfaces; Fe oxides

## 1. Introduction

Zinc (Zn) is an essential nutrient for plants whose deficiency implies low crop yields and concentrations of this micronutrient in staple food such as cereals, which may promote negative effects on human health when diets are based on this type of food [1–4]. Zn deficiency is frequent in calcareous soils, and almost 50% of the soils cultivated for cereals have low levels of bioavailable Zn [5–8]. It is assumed that of the micronutrients required by crops, Zn deficiency is likely the most relevant in terms of its negative effect on crops worldwide [9,10]. For the sustainable management of this nutritional imbalance, a knowledge base on factors governing Zn dynamics and availability to plants in soil is need.

Zinc in soils is present in the soil solution, adsorbed on different minerals—clay minerals, oxides or carbonates—or bound to organic matter [2,11,12]. Carbonates and Fe oxides play a crucial role in Zn dynamics in the soil, thus affecting its potential availability to plants [13–15]. Slow reactions of Zn in soils, which are associated with poorly crystalline Fe oxides, decrease the availability of applied Zn fertilizers with time [16–19]. Overall, Zn shows a strong affinity for colloidal particles in soil, and the adsorption on these particles increases at increased pH [20–22], resulting in a decreased Zn availability to plants [9]. Soil organic matter affects the solubility of the nutrient and its transport to roots, thus affecting its absorption by plants [3,23,24]. However, there are still unknown aspects of Zn dynamics in soils affecting its availability to plants. Although the role of clay minerals, carbonates and Fe oxides as sorbent surfaces is known, the relative contributions of carbonates and Fe oxides or the role of crystalline and poorly crystalline Fe oxides in soils with basic pH are still not fully understood [8,15].

Solubility of Zn fertilizer affects its use efficiency by crops [25]. Nowadays, Zn sulfate is the most-used fertilizer because of its high solubility and low cost. However,

the efficiency of Zn sulfate in supplying Zn to plants is assumed to be very low [26,27]. The recovery by plants of applied Zn to calcareous soils has been shown to be frequently less than 1% of applied Zn [28]. This low efficiency is ascribed to reactions of Zn in soils limiting its availability to plants or the diffusion of Zn in certain soils [29–31], processes that are not fully understood. Further knowledge about soil properties affecting reactions and availability of Zn applied to soil as fertilizer is therefore necessary for a more efficient use of Zn fertilizers with a view of overcoming Zn deficiency in soils or biofortification, i.e., increasing its concentration in staple food. In addition, there is little evidence on the relative contribution of Zn fertilizer and soil Zn in supplying the nutrient to plants, and on how this relative contribution is affected by soil properties and Zn availability to plants.

Phosphorus fertilization and its availability in soil is crucial, affecting Zn uptake by plants since both nutrients show an antagonist behaviour in plant nutrition, which may reduce the yield and quality of crops [7,32,33]. Thus, high rates of P fertilizer may negatively affect Zn absorption by crops, in particular in cereals such as wheat [34–36]. In this crop, P rates equivalent to crop uptake decreased Zn concentration in grains in Zn-deficient soils [37]. Similar results have been found in sugar beets when soil-available P levels were close to sufficiency levels [38]. On the other hand, high available P contents are common in many cultivated soils of Europe and North America [39], which may increase the risk of antagonism between P and Zn. Antagonistic effect seems to vary depending on the contents of different reactive minerals of soil that adsorb P and Zn [8,36]. The negative effect of P on Zn concentrations in plants has been shown to be more significant in substrates with a high Fe oxide to calcite ratio than in those with a low ratio [40]. However, mechanisms explaining the effect of soil P on the Zn uptake by crops are not fully understood [2,36]. Both nutrients are adsorbed on the same surfaces in soil and their interaction with adsorbent surfaces may be complex [15]. Coadsorption of P and Zn on Fe oxides may explain Zn retention and availability to plants in soils [8,15,41–43]. Consequently, the study of Zn dynamics and implications on its availability to plants should take into account the P availability and dynamics in soils.

On these grounds, further knowledge of Zn dynamics and reactions of applied Zn in soil is required for better understanding the mechanisms governing Zn availability to plants and efficiency of Zn fertilizers. Despite the current knowledge base on Zn dynamics in soil, it is still necessary to clarify aspects related to the effect of sorbent surfaces such as carbonates, poorly crystalline and crystalline Fe oxides, and the interaction with P that may affect Zn dynamics and availability to plants in soil. Thus, this work aimed at studying: (i) the relative contribution of soil Zn and Zn fertilizer to the uptake of the nutrient by plants, (ii) soil properties affecting the Zn availability to plants and the use of Zn fertilizer applied to soil, and (iii) the effect of soil P on Zn availability and uptake by plants. The study was performed by studying Zn availability change in successive crops without P supply to avoid the potential effects of freshly applied P on Zn dynamics and availability. We expect that results will contribute to estimate more accurately the risk of Zn deficiency and lead to more sustainable management of Zn fertilization for overcoming this imbalance and for crop biofortification.

## 2. Materials and Methods

### 2.1. Materials

The set of 36 soils selected was the same as that used by Recena et al. [44]. The soils embrace the following soil orders according to Soil Taxonomy 2014 [45]: Mollisols, Entisols, Inceptisols, Alfisols, and Vertisols. Each soil sample was taken from a different location in Spain (coordinates of each location in Table S1). Each location was selected by taking into account that its soil type matches a soil order typically developed under Mediterranean climate. Most of these soils were used for olive orchards or for wheat–sunflower rotation. Soil samples were taken from 0 to 20 cm depth. For the cultivation experiment described below, soil was ground to <6 mm, and for soil analysis, a subsample of the soil was ground to <2 mm.

## 2.2. Methods

### Soil Analysis

Soils were analyzed for particle-size distribution by the pipette method according to Gee and Bauder [46], organic matter (SOM) by the oxidation method of Walkley and Black [47], total CaCO<sub>3</sub> equivalent (CCE) by the calcimeter method, and pH in water at a soil:water ratio of 1:2.5. The sequential chemical fractionation of de Santiago and Delgado [48] was used to determine Fe ascribed to oxides; this scheme involved a sequential extraction with citrate-ascorbate that released Fe in poorly crystalline Fe oxides (Fe<sub>ca</sub>), and citrate-bicarbonate-dithionite that released Fe in crystalline oxides (Fe<sub>cbd</sub>). In these extracts, Fe was determined by atomic absorption spectrometry (Unicam Solaar M instrument from Thermo Fisher Scientific, Madrid, Spain). Diethylenetriamine pentaacetic acid extraction of Zn (DTPA-Zn) was performed according to Lindsay and Norvell [49] as Zn availability index in soils; in the extracts, Zn was determined by atomic absorption spectroscopy. Further information on soils can be found elsewhere [44].

### 2.3. Experimental Design

A pot experiment was performed successively growing durum wheat (*Triticum durum* L.) and sunflower (*Helianthus annuus* L.) in order to mimic a typical crop rotation in rainfed Mediterranean lands as described by Recena et al. [44]. Four crops (two durum wheat, the first and the third crop, and two sunflower, the second and the fourth) were produced under controlled conditions in a growth chamber. We used polystyrene pots (5.5 cm-diameter, 15 cm-high) filled with 300 g of soil. For each soil, five pots were used in the first crop. After harvest, the samples contained in the pots corresponding to each soil were thoroughly mixed in order to obtain homogeneous samples. From this homogeneous sample, 300 g of soil was used for analysis, and the remaining soil only allowed us to fill four pots. Consequently, we used four pots for each soil in the second cultivation. The process was repeated for the third and fourth crop. Thus, the number of pots for each soil decreased from five in the first crop to two in the fourth. The controlled conditions in the growth chamber involved 14 h of light and 10 of dark, with a relative humidity of 45% (light phase) and 60% (dark period) with an active photosynthetic radiation of 22 W m<sup>-2</sup>.

Wheat seedlings for transplanting in pots were obtained with pregermination in a petri dish on a moistened filter paper for 14 days, and after that germination using perlite as growing medium. Sunflower seeds were sown directly in perlite. Wheat and sunflower seeds were irrigated in the germination step with deionized water. After 16 days, one plant (2 true leaves stage) was transplanted to each pot. Pot irrigation was done periodically with Hoagland type nutrient solution without phosphorus at pH 6–6.5. The final volume of nutrient solution applied per crop stage was 350 mL. Thus, the cumulative Zn rate applied as sulfate in the four crops through irrigation (Zn input) was 0.63 mg kg<sup>-1</sup>. The objective with this supply was to maintain a near-zero balance, applying the Zn amount that was expected to be taken up by the four successive crops. This Zn amount was estimated on the grounds of the typical Zn concentration and the expectable dry matter production in each crop. The absence of P in the irrigation was intended to avoid interference of supplied P on Zn uptake, in order to assess the effect of the initial available P status in soil. Irrigation volume was enough to promote a slight drainage in order to avoid salt accumulation in soil. The amount of leached P is supposed to be minimal due to its sorption in soil; thus, these losses have a negligible effect on plant nutrition [44]. After three irrigations with the nutrient solution, pots were irrigated with the same volume of deionized water in order to avoid soil salinization. Nutrient concentrations (mmol L<sup>-1</sup>) in the solution were: MgSO<sub>4</sub> (2), Ca(NO<sub>3</sub>)<sub>2</sub> (5), KNO<sub>3</sub> (5), KCl (0.05), Fe-EDDHA (0.01), H<sub>3</sub>BO<sub>3</sub> (0.009), MnCl<sub>2</sub> (0.0023), CuSO<sub>4</sub> (0.0005), ZnSO<sub>4</sub> (0.006), and H<sub>2</sub>MoO<sub>4</sub> (0.0005).

## Soil and Plant Analysis after Harvesting

After each crop, DTPA extractable Zn was determined as described above and Olsen P [50], with colorimetric determination of P [51] after bicarbonate extraction. Shoots and roots were separated after each crop. To avoid the contamination of roots by soil particles, they were washed with deionized water and then introduced into an ultrasound bath for 1 min in deionized water, rinsed again with deionized water, and dried on a filter paper. Shoots and roots were dried in a forced-air oven at 65 °C for 48 h until constant weight. After drying, shoot and root dry matter (DM) was determined, and samples ground to <1 mm. Dry digestion was performed in a muffle oven at 550 °C for 8 h until the complete calcination in porcelain crucibles using 0.150 g of root and 0.250 g of shoots. The resulting ashes were dissolved in 10 mL of 1 M HCl in a heating plate, at 100 °C for 15 min. The same process was simultaneously performed with certificated plant material in order to verify the complete recovery of nutrients with this procedure. Phosphorus and Zn in the digest were determined as described above for soil extracts. The total P uptake and Zn uptake by plants was calculated as the amount of P and Zn present in shoots and roots (multiplying concentration per dry mass) minus those in seeds. This extraction was expressed as mg of nutrient extracted by kg of soil.

### 2.4. Statistical Analysis

Regression analysis was performed using Statgraphics Centurion XVI [52] in order to assess relationships among variables. Previously, normality according to the Kolmogorov–Smirnov test was checked. If variables did not meet the normality criteria, logarithmic or power transformation was performed, and the regressions were also performed with normalized variables to meet the requirements of the General Linear Model. The portion of the variance of dependent variables explained by independent variables in the relationships was considered the value of the coefficient of determination of the best relationship for untransformed variables. The threshold value for DTPA-Zn was estimated according to [53], which provides the DTPA-Zn value below which the DM yield significantly decreased from the maximum relative yield. In addition, the threshold value for Zn uptake was also estimated. Threshold values were estimated as those values of the DTPA-Zn that maximized the sum of squares between two populations of DTPA-Zn values, i.e., above and below threshold values [54], using the ANOVA procedure of Statgraphics Centurion XVI.

## 3. Results

### 3.1. Soil Properties

The studied soils ranged widely in their properties, in particular those assumed to rule Zn dynamics and availability, such as Fe oxides and carbonate concentration (summary of soil properties are shown in Table 1; detailed properties for each soil are shown in Table S1). Thirty soils were calcareous, 22 had a Ca carbonate equivalent (CCE) concentration higher than at 100 g kg<sup>-1</sup>, and six soils were not calcareous. Soil pH ranged from 6.5 to 8.3, but only two soils showed values below 7. Iron in crystalline oxides (Fe<sub>cbd</sub>) ranged from 2.0 to 13.2 g kg<sup>-1</sup>, and Fe in poorly crystalline oxides (Fe<sub>ca</sub>) ranged from 0.2 to 2.4 g kg<sup>-1</sup> (Table 1). The Fe<sub>ca</sub> and the Fe<sub>ca</sub> to Fe<sub>cbd</sub> ratio was higher in noncalcareous soils than in calcareous ones (Table 1). In calcareous soils, Fe<sub>ca</sub>/Fe<sub>cbd</sub> was negatively correlated to carbonate content ( $r = -0.56$ ;  $p < 0.01$ ). Soil organic carbon (SOC) varied from 5 to 20 g kg<sup>-1</sup>. Olsen P values ranged from 5 to 49 mg kg<sup>-1</sup>, with a higher average value in noncalcareous soils (24.2) than in calcareous ones (14.8). Ten soils were below the threshold Olsen P values for fertilizer response defined by Recena et al. [55]. DTPA-Zn ranged from 0.1 to 5.7 mg kg<sup>-1</sup>, with a higher average value in noncalcareous soils (2) than in calcareous ones (0.62). Twenty-one soils were considered Zn-deficient because DTPA extractable Zn was below 0.5 mg kg<sup>-1</sup> [49].

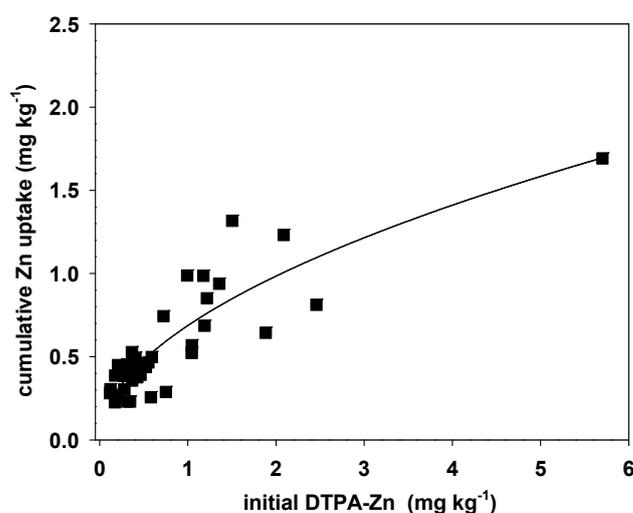
**Table 1.** General soil properties and DTPA extractable Zn before and after cultivation, and cumulative Zn uptake by crops in four consecutive cultivations in noncalcareous (6 soils) and calcareous (30 soils).

	Clay	CCE	Fe <sub>ca</sub>	Fe <sub>cbd</sub>	pH	SOC	Olsen P	Initial DTPA-Zn	Final DTPA-Zn	Cumulative Zn Uptake
	g kg <sup>-1</sup>				g kg <sup>-1</sup>			mg kg <sup>-1</sup>		
	Non-calcareous soil									
Mean	90.7	0	1.47	5.2	7.0	9.85	24.2	2.00	1.32	1.09
SD	52.1	0	0.50	1.8	0.4	4.04	16.3	1.90	1.34	0.45
Range	48–157	0	0.8–2.0	2.0–6.8	6.5–7.7	6.1–15	4.8–49.1	0.37–5.7	0.31–3.9	0.36–1.69
Median	67	0	1.60	5.5	7.0	7.80	19.6	1.43	0.96	1.11
	Calcareous soil									
Mean	334.1	311.7	0.89	5.66	7.99	10.9	14.76	0.62	0.47	0.46
SD	133.18	128.8	0.64	2.93	0.15	3.75	8.65	0.54	0.31	0.20
Range	175–640	28–723	0.2–2.4	2.1–13.2	7.7–8.3	5.5–20	5.0–32.8	0.12–2.46	0.10–1.18	0.23–0.99
Median	326.5	262	0.8	4.9	8.0	10.15	11.9	0.42	0.36	0.44

Mean, standard deviation (SD), median and range of the properties of studied soils (n = 36), including, clay content, calcium carbonate equivalent (CCE), soil organic carbon (SOC), initial Olsen P, citrate-ascorbate extractable Fe (Fe<sub>ca</sub>), citrate-bicarbonate-dithionite extractable Fe (Fe<sub>cbd</sub>); initial and final diethylenetriamine pentaacetic acid extraction of Zn(DTPA-Zn), before and after 4 cultivations, respectively. Cumulative Zn uptake expressed as mg of Zn per kg of soil.

### 3.2. Nutrient Uptake by Crops

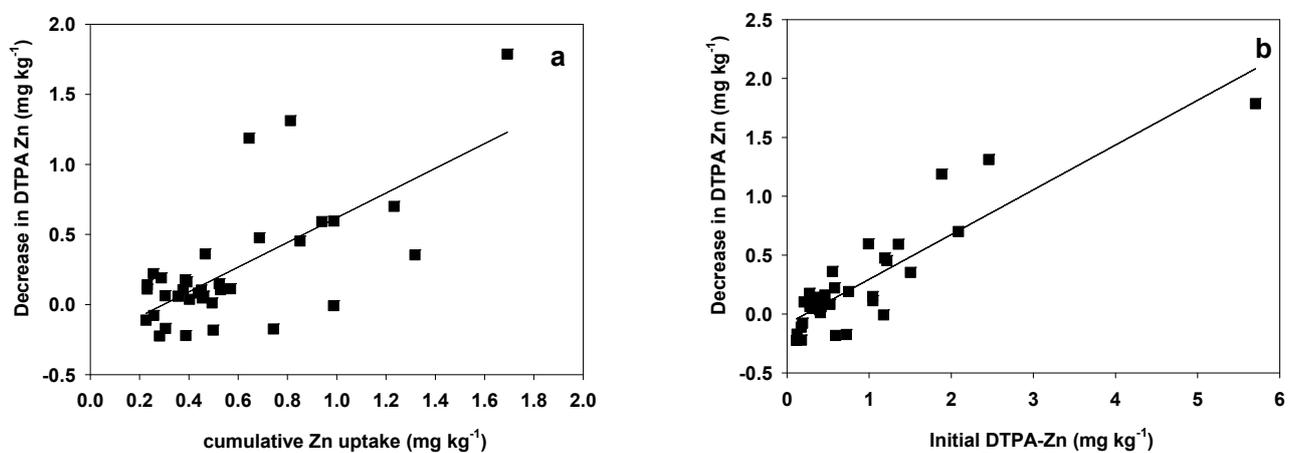
The cumulative Zn uptake of the four crops was on average 0.62 mg kg<sup>-1</sup> of soil, ranging from 0.2 to 1.7 mg kg<sup>-1</sup> (Table 1), and the mean cumulative P uptake was 15.6 mg kg<sup>-1</sup> of soil, ranging from 3.2 to 58.9 mg kg<sup>-1</sup> (not shown; previously reported by Recena et al. [44]). On average, Zn uptake in calcareous soils was much lower (0.46 mg kg<sup>-1</sup>) than in noncalcareous soils (2 mg kg<sup>-1</sup>). The applied Zn with the nutrient solution (0.63 mg kg<sup>-1</sup> soil) was equivalent to the average of that taken up by crops. The DTPA extractable Zn at the beginning of the experiment (initial DTPA-Zn) explained 75% of the variance in the cumulative Zn uptake of the four crops (Figure 1). The variance in Zn uptake explained by DTPA-Zn was similar in the first crop ( $Y = 0.05 + 0.1 X$ ;  $R^2 = 0.72$ ;  $p < 0.001$ ) and the third crop ( $Y = -0.03 + 0.13 X^{0.5}$ ;  $R^2 = 0.74$ ;  $p < 0.0001$ ). However, this explained variance was lower for the second crop ( $Y = 0.29 + 0.11 \ln X$ ;  $R^2 = 0.53$ ;  $p < 0.001$ ) and the fourth crop ( $Y = 0.07 + 0.08 X$ ;  $R^2 = 0.59$ ;  $p < 0.001$ ). Thus, variance in Zn uptake explained by the DTPA-Zn was higher in the two wheat crops than in the two sunflower crops.



**Figure 1.** Cumulative Zn uptake by the four crops (expressed in mg kg<sup>-1</sup> of soil) as a function of the initial DTPA extractable Zn (mg kg<sup>-1</sup>) in soils.  $Y = 0.01 + 0.7 X^{0.5}$ ;  $R^2 = 0.75$ ;  $p < 0.0001$ . The relationship is also significant for logarithmic transformation of both variables to meet normality criteria.  $\ln Y = 0.33 + 0.28 \ln X$ ;  $R^2 = 0.69$ ;  $p < 0.0001$ .

Cumulative Zn uptake increased with increased initial Olsen P ( $Y = 0.29 + 0.02 X$ ;  $R^2 = 0.28$ ;  $p < 0.001$ ) and with increased  $Fe_{ca}$  to  $Fe_{cbd}$  ratio ( $Y = 0.17 + 2.3 X$ ;  $R^2 = 0.29$ ;  $p < 0.001$ ) in soils. In addition, the ratio of the cumulative P uptake to Zn uptake also increased with increased Olsen P in soils ( $Y = 19.9 + 0.43 X$ ;  $R^2 = 0.26$ ;  $p < 0.01$ ).

The decrease in DTPA-Zn after 4 crops was on average  $0.24 \text{ mg Zn kg}^{-1}$ , ranging from  $-0.2$  to  $1.8 \text{ mg kg}^{-1}$ . Negative values mean that Zn fertilization covered Zn uptake by crop and even increased the value of the Zn availability index in soil. The decrease in DTPA-Zn after four crops increased with increased Zn uptake by crops (Figure 2a) and with increased initial DTPA-Zn (Figure 2b). In soils with initial DTPA-Zn below the assumed threshold value for Zn deficiency ( $\text{DTPA-Zn} = 0.5 \text{ mg kg}^{-1}$ ), the decrease in DTPA-Zn was less than  $0.2 \text{ mg kg}^{-1}$  (Figure 2b), and in some cases, this decrease was negative. For these soils, the decrease in DTPA-Zn after four crops was on average  $0.02 \text{ mg kg}^{-1}$ , and the mean cumulative Zn uptake by crops was  $0.36 \text{ mg kg}^{-1}$ , equivalent to 57% of applied Zn fertilizer. In soils above the threshold value, the decrease in DTPA-Zn after four crops was on average  $0.46 \text{ mg kg}^{-1}$ , and the cumulative Zn uptake by crops was  $0.77 \text{ mg kg}^{-1}$ .



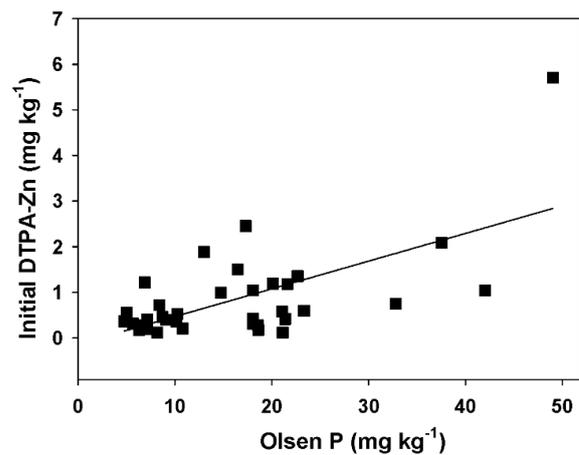
**Figure 2.** (a) Relationship between the decrease in DTPA extractable Zn ( $\text{mg kg}^{-1}$ ) in soils and the cumulative Zn uptake by the four crops (expressed in  $\text{mg kg}^{-1}$  of soil).  $Y = -0.26 + 0.88 X$ ;  $R^2 = 0.48$ ;  $p < 0.0001$ . Regression for transformed variables to meet normality criteria is:  $Y^{-0.35} = 0.71 - 0.65 \text{ Ln } X$ ;  $R^2 = 0.33$ ;  $p < 0.001$ . (b) Relationship between the decrease in DTPA extractable Zn ( $\text{mg kg}^{-1}$ ) and the initial DTPA extractable Zn in soils ( $\text{mg kg}^{-1}$ ).  $Y = -0.9 + 0.88 X$ ;  $R^2 = 0.78$ ;  $p < 0.0001$ . Regression for transformed variables to meet normality criteria is:  $Y^{-0.35} = 1.07 - 0.18 \text{ Ln } X$ ;  $R^2 = 0.63$ ;  $p < 0.0001$ .

### 3.3. Relationship between the Zn Availability Index and Soil Properties

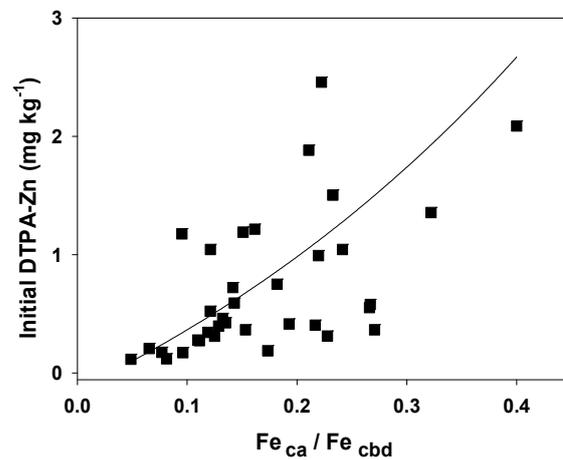
The initial DTPA-Zn increased with increased Olsen P values (Figure 3). The initial DTPA-Zn was not related to the concentration of Fe in poorly crystalline ( $Fe_{ca}$ ) or crystalline Fe oxides ( $Fe_{cbd}$ ); however, it increased with increased ratios of Fe in poorly crystalline oxides to that in crystalline oxides ( $Fe_{ca}/Fe_{cbd}$ ) (Figure 4). The ratio of DTPA-Zn to Fe in poorly crystalline Fe oxides was much lower in soils with DTPA-Zn below  $0.5 \text{ mg kg}^{-1}$  (on average 0.54) than in soils above this threshold value (on average 1.6).

### 3.4. Dry Matter Yield

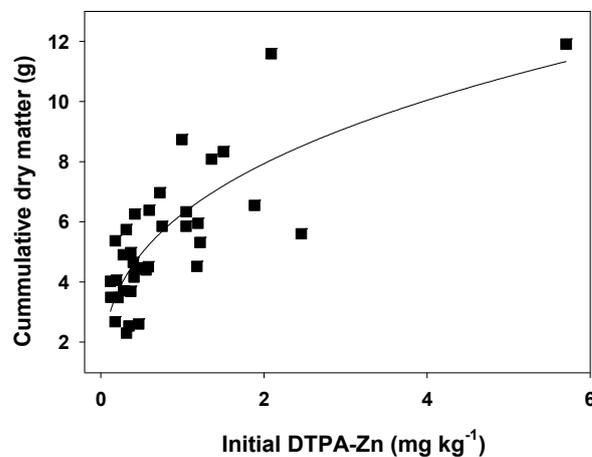
The cumulative dry matter (DM) of the four crops increased with increased initial DTPA-Zn (Figure 5), which explained 61% of the variance. The variance explained by DTPA-Zn decreased for the biomass in the first crop ( $Y = (2 + 1.5 X)^{0.5}$ ;  $R^2 = 0.41$ ;  $p < 0.001$ ). The cumulative DM was also related to Olsen P, but the explained variance was lower than that explained by the Zn availability index ( $Y = 0.49 X^{0.43}$ ;  $R^2 = 0.48$ ;  $p < 0.001$ ). In the first crop, however, variance explained by Olsen P was slightly higher than that explained by DTPA-Zn ( $Y = [0.8 + 0.15X]^{0.5}$ ;  $R^2 = 0.47$ ;  $p < 0.001$ ).



**Figure 3.** Relationship between the initial DTPA extractable Zn ( $\text{mg kg}^{-1}$ ) and the Olsen P ( $\text{mg kg}^{-1}$ ) in soils.  $Y = -0.13 + 0.06 X$ ;  $R^2 = 0.41$ ;  $p < 0.0001$ . Regression for transformed variables to meet normality criteria is:  $\text{Ln } Y = -2.47 + 0.73 \text{ Ln } X$ ;  $R^2 = 0.25$ ;  $p < 0.01$ .



**Figure 4.** Relationship between the initial DTPA extractable Zn ( $\text{mg kg}^{-1}$ ) and the ratio of citrate ascorbate extractable Fe to citrate bicarbonate dithionite extractable Fe ( $\text{Fe}_{ca}/\text{Fe}_{cbd}$ ) in soils.  $Y = (-0.53 + 0.43/X)^{-1}$ ;  $R^2 = 0.58$ ;  $p < 0.0001$ . Regression for transformed variables to meet normality criteria is  $\text{Ln } Y = 1.76 + 1.27 \text{ Ln } X$ ;  $R^2 = 0.45$ ;  $p < 0.0001$ .



**Figure 5.** Relationship between the cumulative dry matter yield in the four crops (g) and the initial DTPA extractable Zn ( $\text{mg kg}^{-1}$ ) in soils.  $Y = 1.9 + 4.2 X^{-0.5}$ ;  $R^2 = 0.61$ ;  $p < 0.0001$ . Regression for transformed variables to meet normality criteria is:  $\text{Ln } Y = 1.79 + 0.31 \text{ Ln } X$ ;  $R^2 = 0.56$ ;  $p < 0.0001$ .

The DTPA-Zn threshold value for DM yield in the first crop was  $0.5 \text{ mg kg}^{-1}$  ( $R^2 = 0.29$ ); meanwhile, that value for Zn uptake in this first crop was  $0.75 \text{ mg kg}^{-1}$  ( $R^2 = 0.37$ ). For cumulative DM and Zn uptake in the four crops, the estimated threshold value was  $0.75 \text{ mg kg}^{-1}$  ( $R^2 = 0.42$  and  $0.51$ , respectively).

#### 4. Discussion

Despite the application of Zn sulfate with nutrient solution, Zn uptake by plants was highly dependent on soil-available Zn. This is evidenced by the highly significant relationship between the cumulative Zn uptake in the four crops and the initial DTPA-Zn, and by the overall decrease in the Zn availability index after the four crops. However, below the DTPA-Zn threshold value of  $0.5 \text{ mg kg}^{-1}$  defined in the literature, the decrease in DTPA-Zn after four crops was negligible and thus, most zinc absorption depended on Zn fertilizer application. Therefore, despite the assumed low efficiency of fertilizers in supplying Zn to plants [1], their contribution to Zn nutrition depended on initial Zn availability in soil, it being very significant in Zn-deficient soils. This, however, did not guarantee enough supply to crops since the average cumulative Zn uptake in soils with  $\text{DTPA-Zn} < 0.5 \text{ mg kg}^{-1}$  was less than half of that observed in soils above this threshold value. On the other hand, the contribution of initial soil Zn to its uptake by crops increased with increased initial Zn availability in soil. This in turn explains the increased decrease in DTPA-Zn with increased Zn uptake by crops.

Zinc availability probably constrained plant growth since the cumulative DM yield in the four crops increased with increased initial DTPA-Zn. However, it is difficult to establish a cause–effect relationship because initial DTPA-Zn was related to Olsen P. In fact, dry matter yield in the first crop was related to both DTPA-Zn and Olsen P. According to Recena et al. [55], 26 of the soils had Olsen P values above the threshold for P fertilizer response. Thus, it cannot be ruled out that Zn availability may constrain plant development. Indeed, reasonable DTPA-Zn threshold values can be estimated with our dataset. When the first crop was considered, the estimated threshold value was that described in literature ( $0.5 \text{ mg kg}^{-1}$ ). However, for the cumulative DM or the cumulative Zn uptake in the four crops, the estimated threshold value was  $0.75 \text{ mg kg}^{-1}$ . This means that when a high Zn extraction is expected, the threshold value for Zn fertilizer response defined in the literature should be revised.

It should be highlighted that DTPA-Zn accurately predicted the cumulative Zn uptake in four crops (75% of the variance in Zn uptake explained). Recent works considered that DTPA-Zn was not an accurate availability index for assessing Zn availability in soils, and poor relationships between crop Zn uptake and DTPA-Zn have been described [15]. However, these results have been usually obtained at short-term scale with experiments involving only one crop. With four crops, and consequently with a significant depletion of the available pool in soil, DTPA-Zn performed accurately in predicting Zn uptake by crops.

The available Zn pool was more affected by the mineralogy of Fe oxides than by their total concentration in soils since DTPA-Zn and Zn uptake were related to the ratio of Fe in poorly crystalline oxides ( $\text{Fe}_{\text{ca}}$ ) to that in crystalline Fe oxides ( $\text{Fe}_{\text{cbd}}$ ), but not to either of these Fe fractions. The effect of the specific surface in Fe oxides, higher in poorly crystalline than in crystalline ones, is a major factor explaining the role of different Fe oxides in Zn adsorption [14]. Thus, adsorbed Zn in poorly crystalline Fe oxides may be greater than in crystalline oxides, and the available Zn pool in soil, as estimated by DTPA extraction, may increase at increased concentrations of adsorbed Zn on Fe oxides [15]. Other crucial soil properties involved in Zn adsorption, such as organic matter [56] and carbonates [13], did not affect in our case the Zn availability to plants in soil.

The relationship of the initial DTPA-Zn with  $\text{Fe}_{\text{ca}}/\text{Fe}_{\text{cbd}}$  explains the increased cumulative Zn uptake with increased  $\text{Fe}_{\text{ca}}/\text{Fe}_{\text{cbd}}$  ratios. It should be noted, however, that despite the known evidence of the effect of poorly crystalline Fe oxides on Zn adsorption in soil, the relevance of these sorbent surfaces on the availability and use by plants of soil Zn was never proved.

In Zn-deficient soils (below  $0.5 \text{ mg kg}^{-1}$  of DTPA-Zn), the efficiency of applied Zn sulfate contributing to Zn uptake by plants was much higher than in Zn-sufficient soils (i.e., above the threshold value). This may reveal that Zn fixation reactions leading to nonavailable forms occur to a lesser extent in Zn-deficient soils than in Zn-sufficient soils. In Zn-sufficient soils, a higher saturation of Zn adsorption sites in Fe oxides than in Zn-deficient soils is expected, and the DTPA-Zn to  $\text{Fe}_{\text{ca}}$  ratio may be indicative of this saturation. This may imply less contribution of adsorption on Fe oxides to Zn retention in soils relative to other reactions such as Zn oxides precipitation or precipitation/adsorption on carbonates, which contribute to a lesser extent to the available Zn pool in soil [15].

Many authors demonstrated that a high P bioavailability induce Zn deficiency in crops [24,57]. However, according to our results, it seems that there is a positive effect of soil P on Zn availability to plants since the initial DTPA-Zn and Zn uptake by crops increased with increased Olsen P. This contradicts previous results on the effect of Olsen P on Zn uptake by crops [36]. However, positive effects of P on Zn accumulation in plants has been observed in calcareous soils [58]. Thus, differences in the physicochemical soil properties would explain different P–Zn interactions in soil affecting Zn availability.

According to Sacristán et al. [8], coadsorption with P explains the Zn retention in soils and has proved crucial explaining Zn retention on poorly crystalline oxides [15,41]. The adsorption mechanism for the coadsorption of Zn and P on poorly crystalline Fe oxides is the formation of ternary complexes with P instead of Zn bound directly to the surface [43]. Zn has increased affinity for poorly crystalline oxides in the presence of phosphate under acidic conditions [40]. Ternary complexes increase binding energy and decrease Zn desorption in acidic soils [59] and consequently its potential bioavailability. However, in calcareous soils, P application may decrease Zn adsorption [60] and binding energy to sorbent surfaces [61]. This may explain an enhanced Zn desorption and uptake by plants in most of our soils. In calcareous soils, Fe oxides are also relevant in explaining Zn adsorption [60]. The effect of P on Zn adsorption on carbonates or oxides may explain the increased Zn availability. A preferential coadsorption of P and Ca forming ternary complexes with Fe oxides [62] may decrease ternary complexes involving Zn. This promotes less binding energy and affinity of Zn for Fe oxides surfaces, thus facilitating desorption from sorbent sites. The increased efficiency of applied Zn sulfate contributing to Zn uptake in Zn-deficient soils (below  $0.5 \text{ mg kg}^{-1}$  of DTPA-Zn), may be also ascribed to their lower P availability when compared with Zn-sufficient soils. This promotes less binding through the formation of ternary complexes.

Hydroxyapatite, which according to Recena [55] is a dominant P form in these soils resulting from P fertilization, may have a high Zn adsorption capacity [63] and may contribute to Zn adsorption, perhaps affecting its availability to plants.

Calcareous soils showed lower DTPA-Zn and Zn uptake than noncalcareous soils (Table 1). This may be related to the lower Olsen P and  $\text{Fe}_{\text{ca}}$  to  $\text{Fe}_{\text{cbd}}$  ratio in calcareous soils relative to noncalcareous ones. Although the  $\text{Fe}_{\text{ca}}$  to  $\text{Fe}_{\text{cbd}}$  ratio was negatively correlated to carbonate content, it seems that Zn availability was governed by the Fe oxides mineralogy since carbonate was not related to DTPA-Zn and Zn uptake in our calcareous soils. This disagrees with previous evidence showing decreased Zn desorption with increased carbonates in soils [58]. Although the relevance of Fe oxides on Zn adsorption in calcareous soils has been postulated [60], the role of poorly crystalline oxides to crystalline Fe oxides ratio was never proved. This effect of oxides on Zn availability to plants cannot be separated from that of P since the coadsorption of P and Zn on these oxides seems crucial in explaining the adsorption and availability of Zn to plants.

The ratio of P to Zn uptake by plants increased with increased Olsen P in soils, revealing that, despite the enhanced Zn uptake, the relative accumulation of both nutrients is affected by an increased P availability in soil, which may lead to physiological constraints in plants in soils with high P availability. Our results were obtained in unfertilized soils, and the available P reserve declined along the four crops in the experiment. This may also contribute to explaining why an antagonism between both nutrients was not observed.

## 5. Conclusions

Overall, soil Zn was more relevant affecting crop uptake than Zn sulfate applied as fertilizer. This reveals the need of taking into account Zn dynamics in soil for effective management of crop fertilization. The relative contribution of soil and fertilizer to Zn uptake depended on the initial Zn availability in soil, which in turns was modulated by the mineralogy of Fe oxides, i.e., the ratio of poorly crystalline Fe oxides to crystalline ones, and P concentration in soil. The increased Zn availability in soil and Zn uptake by plants with increased Olsen P seemed to contradict the well-described antagonism between both nutrients. This may be ascribed to a decreased binding energy of Zn to sorbent surfaces with increased P availability in our soils in contrast with evidence in acidic soils. On the other hand, the ratio of P to Zn uptake by plants increased with increased Olsen P in soils. This revealed that despite the effect of soil P on Zn adsorption and availability, the antagonism between P and Zn in plants may be expected at high P availability levels in soils.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/2073-4395/11/2/390/s1>, Table S1: Individual values of soil properties for the whole batch of soil profiles, including total available P (TAP), critical available P (CAP), and nutrient availability indexes for P (Olsen P and anion-exchange-resin extractable P –AER-P–), K, Ca, and Mg.

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