



Influence of ZnO Particle Size and Soil Characteristics on the Estimation of Long-Term Zn Bioavailability by Chemical Extraction Methods and Diffusive Gradients in Thin-Films (DGT)

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Abstract

The aim of this paper is to explore whether the long-term bioavailability of Zn in different soils can be predicted using operational extraction procedures. Green peas and beetroot were grown in two soils with contrasting physicochemical characteristics. Two Zn sources of different sizes (ZnO-nano or ZnO-bulk) were applied 1 year earlier, at different Zn application rates. The amounts of available Zn were assessed using the diffusive gradients in thin films (DGT) technique and different chemical extraction procedures: water-soluble (WS), CaCl₂, rhizosphere-based low-molecular-weight organic acid (LMWOAs), DTPA-TEA, and NH₄Ac. The different correlation and regression studies showed that the estimation of availability is dependent on the soil categorical variable, especially in the beetroot crop. Zn-DGT could be used to estimate the Zn concentration of the aerial part of the green pea using a general model for both soil and ZnO sizes. The estimation of long-term Zn bioavailability was successful using either medium-strength extractive solutions or the DGT technique. The extraction methods involving complexing agents or buffered salt solution overestimated the amount of bioavailable Zn in calcareous soil. Further studies will be necessary to know the amounts of Zn associated with the different soil fractions.

Keywords LMWOAs · DGT · Traditional chemical extraction · Aged nanoparticles · Zn nanofertilizer

1 Introduction

Zinc (Zn) is an essential trace element (micronutrient), which is required in small, but critical, amounts by both plants and animals (including humans). Nearly half of the world's soils are deficient in Zn (Das and Andrew 2016).

The causes of the Zn deficiency in soils include high Zn removal by crops due to high crop yields and intensive cropping systems, reductions in the application of organic manures, and the use of *high analysis fertilizers*, which provide only the main macronutrients. Millions of hectares of cropland are affected by Zn deficiency and approximately one third of the human population suffers from inadequate Zn intake (Alloway 2009).

Different Zn fertilizers have been used to prevent Zn deficiency in soils. In addition to traditional Zn sources (such as Zn sulfate, Zn oxide, Zn chloride, Zn nitrate, and Zn oxy-sulfate), the fertilizer market currently offers complexes and chelates of natural or synthetic origin, whose use has significantly increased in recent years (Almendros et al. 2019). However, the application of nanotechnology in different areas of agriculture has also reached fertilizers and there is now a growing field of research into the use of engineered nanoparticles (NPs) as nanofertilizers (Zahedi et al. 2020). Precise fertilization techniques (such as the use of nanofertilizers) are based on controlling nutrient delivery. These techniques aim to minimize losses to the environment

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caused by leaching to groundwater and reduce Zn changes to more stable forms, through slow reactions with soil constituents. The chemical forms used in precision agriculture aim to provide plants with the nutrients that they need at every stage of their growth (Mikula et al. 2020).

After applying Zn fertilizers, the activity and extractability of the Zn added to soils in water-soluble forms decreases slowly, but continuously, thereby increasing the retention of Zn by soils. There is an initial rapid sorption reaction followed by slower sorption reactions with soil constituents (Buekers 2007). These reactions include diffusion into soil micropores, occlusion in the solid phase by co-precipitation and co-flocculation, entrapment in cavities, and the formation of a solid solution interface (Davis and Kent 2018). The duration of this period depends on the soil characteristics, the source applied and the rate of Zn added (Almendros et al. 2013b, a; Alvarez et al. 2009; Obrador et al. 2021). Alvarez et al. (2009) found that—after 2 years—the residual effect of the application of natural Zn chelates depended on the ageing effect of each source, on Zn leaching losses and on soil characteristics. Brennan (2001) reported that the effectiveness of a ZnO treatment applied to wheat decreased by 50% over a 13-year period.

The physicochemical properties of different Zn fertilizers can influence processes such as adsorption, aggregation, dispersion and the solubility of particles in soil (Tiede et al. 2008). Nanomaterials are much more reactive than large-sized particles due to their bigger surface area per unit mass (Rickerby and Morrison 2007). This high reactivity of metal-based NPs could cause their homo-aggregation to similar NPs and the hetero-aggregation to natural colloids that are also present in pore water (Ogunkunle et al. 2021; Rodrigues et al. 2016). These aggregates could subsequently be retained in the solid matrix, or be subject to dissolution, leading to the release of metal ions (Rodrigues et al. 2016).

The total Zn concentration in a soil does not provide information about its availability, mobility, or reactivity related to chemical forms. Several chemical extraction procedures have been proposed to determine the availability of Zn in soils. The single extraction methods can be divided into four main groups, based on different strengths: (i) unbuffered salt solutions (CaCl_2 , NaNO_3 , NH_4Cl , NH_4NO_3 , AlCl_3 , BaCl_2), (ii) buffered salt solutions (NH_4 -acetate, acetic acid), (iii) chelating agents (EDTA or DTPA), and (iv) acid extractions (HCl , HNO_3 , CH_3COOH) (Rauret 1998). These different solution types and strengths determine the concentration of Zn extracted from the soil. In general, salt solutions extract weakly adsorbed metals retained on solid surfaces by a relatively weak electrostatic interaction; these metals can be released by ion-exchange processes (McLaughlin et al. 2000). Chelating agents can displace metals from insoluble organic or organometallic

complexes, in addition to those sorbed on inorganic soil components (Ure and Davidson 2002). Acid extraction dissolves soil metal forms into non-silicate bound forms. These harmonized procedures have been widely used to assess soil Zn fertility when conventional Zn fertilizers are added. Nevertheless, the properties of the NPs would influence their adsorption processes and, therefore, the recovery values of the different chemical extraction procedures.

Some studies have evaluated the suitability of chemical extraction methods and diffusive gradients in thin films for estimating Zn concentration in plants. However, these studies have been conducted on unfertilized natural soils (Tandy et al. 2011) or on recently amended soils (Almendros et al. 2020). To the best of our knowledge, there are no published studies on the evaluation and validation of extraction methods with aged Zn-NPs.

We explored the hypothesis that the predictive power of different extraction techniques used to estimate long-term Zn bioavailability from ZnO sources (bulk and nano) could depend on soil characteristics and source particle size. To verify this hypothesis, a greenhouse experiment was designed in which ZnO sources (bulk and nano) were applied to a first crop grown in two soils (acidic and calcareous), in which a second crop was subsequently grown. The specific objective of our study was to compare and validate standardized extraction techniques and to estimate the long-term Zn bioavailability from aged nano and bulk ZnO sources for two different crops (a leguminous—green pea—and a horticultural—beetroot—crop).

2 Materials and Methods

2.1 Soil Characterization

The original soils used in this study were collected a year before conducting this study. These two representative soils (an acidic soil, pH 5.4; 40° 44' N, 3° 25' W, and a calcareous soil, pH 8.5; 40° 22' N, 3° 24' W) were from different rural areas of Spain. These soils were characterized using standard analytical determinations in a previous work (Almendros et al. 2020). Both were soils commonly used to cultivate cereals and were characterized by their low organic matter contents (< 2%). Both types of soil are common in the Mediterranean area. Soil 1 was classified as a TypicPalexeralf and soil 2 as a TypicHaploxerepts (Soil Survey Staff 2014). Soil 1 was acidic (pH = 5.4), while, in contrast, soil 2 was calcareous (pH = 8.5). The soils were classified as Luvisol and Cambisol (FAO 2015). The general properties of the original soils, based on means from three replicates, are reported in Table 1.

Table 1 Main physicochemical parameters and element concentrations measured in the acidic soil and the calcareous soil

	Acidic soil	Calcareous soil
pHw (1:2.5 w:v)	5.5±0.1	8.5±0.1
Texture (USDA)	Silty loam	Silty clay loam
Sand (g kg ⁻¹)	250±8	175±6
Silt (g kg ⁻¹)	570±10	435±9
Clay (g kg ⁻¹)	180±7	390±8
Organic matter (g kg ⁻¹)	16.9±0.1 (low)	11.3±0.1 (low)
EC (μS cm ⁻¹) (1:5 w:v)	66.9±3	125.9±5
CEC (cmol kg ⁻¹)	11.4±0.8	22.1±1
Total N (g kg ⁻¹)	0.91±0.1	0.9±0.04
Total Zn (mg kg ⁻¹)	40.6±2.1	62.7±3.3
DTPA-TEA-extractable-Zn (mg kg ⁻¹)	2.49±0.07	0.28±0.01

2.2 Greenhouse Experiment

The soils used in this study were residual and obtained from experiments conducted during the previous year, which had been used to grow a cherry tomato and a common bean crop. ZnO NPs (< 100 nm) were obtained from Sigma-Aldrich (Germany) with a nominal primary particle size of less than 100 nm (i.e., $r_p \leq 50$ nm). The bulk ZnO form was purchased from Sigma-Aldrich (Germany). In the former, the different soils were treated with ZnO (bulk or NPs), applied at different Zn rates (0, 20, and 225 mg Zn kg soil⁻¹). The rate of 20 mg Zn kg⁻¹ was used because other experimental studies suggest that this dose has a beneficial effect on crop development (Reddy Pullagurala et al. 2018). A dose higher than 200 mg Zn kg⁻¹ was selected to evaluate possible toxic or inhibitory effects on crops (Zuverza-Mena et al. 2017). However, the plants did not grow in the acid soil at the higher dose (200 Zn kg⁻¹). Eventually, a rate of 3 mg Zn kg⁻¹ of NPs was also used to reflect realistic ZnO NP conditions in polluted natural soils.

The control treatment (with no added Zn) and the Zn fertilizer treatments were replicated 3 times according to a randomized complete block design (total pot number: 84). For the study, soil from each container in the previous study (Almendros et al. 2020) was mixed, air-dried and homogenized and then returned to the container.

The soil was then left to stand for 6 months to equilibrate under these greenhouse conditions. The container (each with a capacity of 10 L, a mean internal diameter of 24 cm, and a height of 24 cm) were kept in a greenhouse in which temperatures ranged from 3 °C (night) to 38 °C (day) and the relative air humidity ranged from 35 to 85%. The soils were respectively amended with macronutrients N, P, and K (100, 50 and 125 mg per kg of soil). No new additions of Zn fertilizer were made. Total Zn concentrations at the beginning of

this experiment were as follows: 40.62±1.50 mg kg⁻¹ in the acidic soil and 62.66±1.74 mg kg⁻¹ in the calcareous soil.

Green pea (*Pisum sativum* L. cv. Negret) and beetroot (*Beta vulgaris* L. cv. Detroit) were respectively grown in the same pots in which the tomato and common beans had previously been grown, until their edible parts reached maturity; this was 60 days after emergence in the case of pea and 90 days in that of beet. At the end of the cropping, the plants were removed from the pots, rinsed with deionized water, and divided in root, stem, leaf, and edible part (the beet-roots were peeled, and the pea grains were separated from their pods to obtain their edible part). The plant samples were weighed to determine their fresh weight. Their roots were successively washed with deionized water and then with 10 mmol L⁻¹ tetrasodium ethylenediaminetetraacetate (Na₄EDTA) in an ultrasound-assisted bath, at 35 kHz, for 15 min. Finally, all the plant samples were dried in an oven, at 60 °C; the only exceptions were the peeled beet roots, which were vacuum freeze-dried.

2.3 Plant and Soil Analysis

The total Zn content in the plant parts (root, stem, leaf, and edible part) was determined by wet digestion in a sample preparation block system (SPB PROBE, PerkinElmer) (Almendros et al. 2020). Different extraction methods were used to assess long-term bioavailability from Zn oxide sources: LMWOAs (rhizosphere-based extraction method), CaCl₂, DTPA-TEA, WS, and NH₄OAc. A DGT device was also used to determine diffusive fluxes of Zn in the soil (Table 2). The DGT device consisted of a cation exchange resin—Zn²⁺ binding layer—and a diffusion layer that allows solute diffusion prior to binding. It also incorporated a protective polyether sulfone filter membrane through which ions and NPs can diffuse freely. Soil samples were moistened with Milli-Q water to keep the water content at 70% of the water holding capacity (WHC). Soil samples with the DGTs were incubated in the dark at 20 °C for 24 h. When the DGT devices were recovered, they were rinsed with Milli-Q water and the binding gels were removed and eluted with 1 mL of 1 M HNO₃ for 24 h. The results were used to calculate the time-averaged solution Zn concentration at the interface between soil and DGT. The Zn concentration in all of the soil extracts obtained was determined using flame/graphite furnace atomic absorption spectrometry, with this depending on the Zn concentration range (Perkin-Elmer, AAnalyst 700).

2.4 Statistical Analysis

We studied the linear relationships between the different Zn concentrations in all the soil extracts and the Zn concentrations in all the different plant parts ($n=42$). Log-transformed

Table 2 Extraction methods used to assess long-term bioavailability from Zn oxide sources: LMWOAs (rhizosphere-based extraction method), CaCl₂, DTPA-TEA, WS, NH₄OAc, and DGT

Method	Conditions
LMWOAs (rhizosphere-based extraction method) (Feng et al. 2005)	2/20 (soil, g/extractant solution, mL) 10 mM acetic/lactic/citric/malic/formic acid molar ratio 4:2:1:1:1 Shaken end-over-end shaker for 16 h
CaCl ₂ (Houba et al. 2000)	2/20 (soil, g/extractant solution, mL) 0.01 M CaCl ₂ solution, shaken end-over-end shaker for 2 h
DTPA-TEA (Lindsay and Norvell 1978)	10/20 (soil, g/extractant solution, mL) 5•10 ⁻³ M diethylene triaminepentaacetic acid (DTPA), 0.01 M CaCl ₂ and 0.1 M triethanolamine (TEA), adjusted to pH 7.3, shaken horizontal shaker 120 cycles/min for 2 h
WS (Almendros et al. 2020)	1/10 (soil, g/extractant solution, mL) Double-deionized water, shaken end-over-end shaker for 2 h Centrifuged (4500 rpm, 15 min) Supernatant filtered 0.22 µm cellulose acetate paper
NH ₄ OAc (Van Reeuwijk 2002)	2.5/25 (soil, g/extractant solution, mL) NH ₄ OAc solution 1 M at pH=7, shaken end-over-end shaker for 2 h
DGT (Almendros et al. 2020; Degryse et al. 2009)	24 h of incubation in the dark at 20 °C The binding gels were then removed and eluted with 1 mL of 1 M HNO ₃

data were used to meet the two assumptions of normal distribution and uniform variance. According to the log-transformation that was used, it was possible to estimate the Zn concentrations in the different plant parts by applying the formula:

$$y = 10^{\beta_0 + \beta_1 \log(x)} \quad (1)$$

where β_0 is the intercept, β_1 the slope coefficient, and the variable x is the obtained soil extract (LMWOAs, CaCl₂, DTPA-TEA, WS, NH₄Ac, or DGT-Zn).

We also studied relevant relationships, including soil type (acidic vs calcareous soil) and ZnO size (bulk vs NPs) as categorical variables. The statistical studies were made using Statgraphics Centurion XVII 17.2 software (Manugistic, Rockville, MD).

To complete the study, a principal component analyses (PCA) biplot was constructed which considered two variables: (i) the concentration of Zn in the different parts (root, stem, leaf, and edible part) of each plant specie, and (ii) the soil extractable Zn amounts (Zn-DTPA-TEA, Zn-NH₄Ac, Zn-LMWOAs, Zn-CaCl₂, Zn-WS, and Zn-DGT). Classical

standardization has been carried out, centering and reducing the variables. The variables were centered and divided by their standard deviation to have variables with zero mean and standard deviation equal to 1.

3 Results

3.1 Relationships Between Long-Term Extractable Zn Concentrations in Soil and Zn Concentrations in Plant Tissues

To test whether a global linear model would be suitable for estimating long-term available Zn from applied ZnO, irrespective of particle size (bulk or NPs), simple linear regression analyses were performed. Simple linear regression analyses between the log-transformed variables of the soil Zn concentrations and the Zn concentrations in the different plant tissues (root, stem, leaf, and edible part) were estimated using the extraction methods shown in Table 3. According to Pearson's correlation coefficients (r), all

Table 3 Pearson's correlation coefficients (r) between log-transformed values of Zn concentrations in different plant tissues and Zn-extractable concentrations ($n = 29$). Zn-LMWOAs, rhizosphere-based low-molecular-weight organic-acid; Zn-WS, water-soluble; Zn-DGT, diffusive gradients in thin films

	Beetroot			Green pea				
	Stem	Leaf	Edible part	Root	Stem	Leaf	Edible part	Root
Zn-LMWOAs	0.98***	0.98***	0.97***	0.84***	0.95***	0.98***	0.89***	0.84***
Zn-CaCl ₂	0.97***	0.96***	0.95***	0.83***	0.92***	0.97***	0.85***	0.83***
Zn-DTPA-TEA	0.42*	0.44*	0.50*	0.54*	0.66**	0.53*	0.74***	0.63**
Zn-WS	0.81***	0.82***	0.79***	0.70***	0.69***	0.71***	0.64**	0.72***
Zn-NH ₄ Ac	0.84***	0.84***	0.85***	0.86***	0.88***	0.88***	0.88***	0.86***
Zn-DGT	0.97***	0.96***	0.95***	0.82***	0.88***	0.93***	0.78***	0.78***

the soil extract procedures were successful in estimating the Zn concentrations in each of the parts of the beetroot and green pea plants. In general, the correlations between the soil extractable Zn concentrations and Zn concentrations in the different plant parts were strongest when the LMWOA and CaCl₂ methods were used ($0.84 < r < 0.98$ and $0.83 < r < 0.97$, for LMWOAs and CaCl₂, respectively); they were positive and highly significant ($P < 0.0001$). The relationships between the soil extractable Zn concentrations using the DGT, NH₄Ac, and WS approaches, and the Zn concentrations found in the different plant tissues were strong ($0.78 < r < 0.97$, $0.84 < r < 0.88$, and $0.64 < r < 0.82$, for DGT, NH₄Ac, and WS, respectively), positive and significant ($0.001 < P < 0.0001$). The correlations between the Zn concentrations extracted by the DTPA-TEA method and the Zn concentrations in the different parts of the beetroot and green pea plants were positive and lower ($0.42 < r < 0.74$; $0.05 < P < 0.0001$) than those obtained applying other extraction methods.

3.2 The influence of soil type

The physicochemical parameters of soils, especially the pH value, influence the availability of Zn in soils, as shown in Table 1, the concentration of available Zn in acid soil (pH 5.5) is almost 10 times higher than that of calcareous soil (with a pH of 8.5).

Since soil characteristics, especially soil pH, could influence the model fit for different soil extraction methods, we compared the intercept (β_0) and the slope coefficient (β_1) in the regression models used to estimate Zn concentrations in the different plant parts from the Zn concentration in the soil extract obtained. A higher β_0 value indicates a higher Zn concentration in the plant for the same soil concentration. The slope coefficient represents the increase of Zn concentration in the plants in relation to the available Zn concentration in the soil. The intercept coefficients and/or slopes in the regression models were significantly different, not only as a function of soil type but also as a function of the extraction method used to predict long-term Zn bioavailability (Table 4). Soil type was significant for the intercept and/or the slope in regression models including soil type (acidic vs calcareous) as a categorical variable, except for the relationships between Zn-WS and Zn in the beet root; Zn-DGT and Zn in the pea stem; Zn-LMWOAs, Zn-CaCl₂ or Zn-DGT, and Zn in the pea leaf; and Zn-NH₄Ac or Zn-DGT and Zn in the edible part of the pea (Table 4). When soil type was significant for the intercept and/or the slope in the new regression model, the *R*-squared value improved relative to not including soil type as a categorical variable.

The regression study between LMWOAs- or CaCl₂-extractable Zn concentrations and Zn concentrations in plant tissues showed comparable behaviors for

both crops (Fig. 1). The models showed that both Zn concentrations in plant tissues and soil extractable Zn concentrations were significantly higher for acidic soil than for calcareous soil, except for root Zn concentrations. These chemical methods extracted different soil Zn concentrations, which depended on the soil type, for the same root Zn concentrations. The extractable Zn concentrations in the acidic soil were significantly higher than in the calcareous soil for the same root Zn concentration.

The relationships obtained between Zn-DTPA-TEA concentrations in soil and Zn concentrations in different plant parts showed that the regression model was dependent on soil type. Soil type was significant for the intercept and/or the slope in all the plant parts and in both plant species (Table 4). In addition, *R*-squared values increased when soil type was included as a categorical variable. Within the range of ZnO NP concentrations studied and for the same soil Zn-DTPA-TEA concentration, Zn concentrations in plant tissues were significantly higher in the acid soil than in the calcareous soil (Fig. 1). In addition, the slopes of the regression models were much greater for the acid soil than for the calcareous soil in all cases.

The regression study between WS-extractable Zn in soil and Zn concentrations in the different plant tissues showed that the soil type was only significant for the intercept for all the Zn concentrations in the plant tissues of both crops, with the exception of beet roots (Table 4). There was an increase in the *R*-squared values in the linear relationships when the soil type was significant for the intercept. In these cases, two different types of behavior as a function of Zn-WS concentrations were observed (Fig. 1). At high soil-extractable Zn-WS concentrations, the Zn concentrations in plant tissues increased. However, at Zn-WS concentration below 0.3 mg Zn kg soil⁻¹ (log Zn-WS value - 0.53), the Zn concentrations in the different plant parts were significantly higher in the acidic soil than in the calcareous soil, for the same Zn-WS concentration.

Regression models between NH₄Ac extractable Zn in soil and Zn concentrations in plant tissues obtained by including the categorical variable (soil type) gave higher *R*-squared values than using data from both soils together. This applied to all the plant species and plant tissues studied, except for the edible part of the green pea. The regression study also showed that the soil type was significant (for the intercept and/or the slope) in all the plant parts of both plant species. It would therefore be appropriate to use a model for each of the soils. Interestingly, a similar behavior was observed for this extraction in the green pea to that observed with WS-extractable Zn, with the two different forms of behavior depending on the respective Zn-NH₄Ac concentrations in the soil (Fig. 1). However, in the beetroot plants, a similar behavior to Zn-DTPA-TEA extraction was observed, with higher Zn concentrations in

Table 4 Residual standard error (RSE), intercept (β_0), and slope (β_1) of the linear relationship(s) between the log-transformed values of the Zn concentrations in the different plant tissues and the extractable Zn concentrations of all ZnO-amended soils. If the model shows differences for the intercept and/or slope depending on soil type—when

soil type is included as a categorical variable—they are denoted by AS (acid soil) and CS (calcareous soil). Zn-LMWOAs, rhizosphere-based low-molecular-weight organic-acid; Zn-WS, water-soluble; Zn-DGT, diffusive gradients in thin films

		Beetroot	<i>Beta vulgaris</i>			Green pea	<i>Pisum sativum</i>			
		Stem	Leaf	Edible part	Root	Stem	Leaf	Edible part	Root	
Zn-LMWOAs	R^2	97.68***	96.77***	95.23***	74.45***	92.60***	96.18***	89.16***	78.24***	
	RSE	0.08	0.12	0.09	0.14	0.15	0.10	0.06	0.20	
	β_0	1.63	¹	AS: 1.63 CS: 1.99	AS: 0.14 CS: 0.55	AS: 1.91 CS: 2.48	2.02	¹	¹	
	β_1	AS: 1.07 CS: 0.56		0.90	0.67	1.14	0.73	¹	¹	
Zn-CaCl ₂	R^2	97.45***	96.09***	95.58***	71.44***	90.13***	94.41***	85.92***	77.03***	
	RSE	0.08	0.13	0.08	0.15	0.18	0.12	0.07	0.20	
	β_0	¹	¹	¹	AS: 0.18 CS: 0.67	¹	2.09	¹	AS: 1.89 CS: 2.94	
	β_1	¹	¹	¹	0.60	¹	0.63	¹	1.07	
Zn-DTPA-TEA	R^2	98.83***	98.60***	96.70***	69.76***	93.77***	98.17***	92.46***	82.53***	
	RSE	0.06	0.08	0.07	0.15	0.14	0.07	0.05	0.18	
	β_0 ¹									
	β_1 ¹									
Zn-WS	R^2	84.71***	82.99***	73.95***	49.02***	76.28***	87.75***	61.77***	60.48***	
	RSE	0.20	0.27	0.20	0.19	0.27	0.18	0.11	0.27	
	β_0	AS: 2.13 CS: 1.49	AS: 2.53 CS: 1.73	AS: 2.04 AC: 1.61	0.40	AS: 2.54 CS: 1.83	AS: 2.42 CS: 1.65	AS: 1.81 CS: 1.62	AS: 2.47 CS: 2.16	
	β_1	0.34	0.49	0.31	0.41	0.31	0.26	0.10	0.40	
Zn-NH ₄ Ac	R^2	98.80***	97.34***	93.83***	74.32***	84.28***	95.89***	78.14***	77.27***	
	RSE	0.06	0.11	0.10	0.14	0.22	0.11	0.08	0.21	
	β_0	¹	¹	¹	¹	AS: 2.34 CS: 1.92	¹	1.70	2.08	
	β_1					0.82		0.39	AS: 1.68 CS: 0.51	
Zn-DGT	R^2	94.88***	93.57***	91.69***	67.35***	75.53***	87.06***	60.35***	55.25***	
	RSE	0.12	0.17	0.12	0.16	0.27	0.19	0.10	0.30	
	β_0	AS: -0.60 CS: -0.21	AS: -1.10 CS: -0.56	AS: -0.48 CS: 0.05	AS: -1.31 CS: -0.86	0.78	0.60	1.33	1.15	
	β_1	1.18	1.57	1.09	0.77	0.70	0.72	0.19	AS: 0.54 CS: 0.60	

¹The model showed differences for the intercept and slope when soil was included as a categorical variable

plant tissues for the acidic soil than for the calcareous soil, for the same soil-extractable Zn concentration.

The regression study between potentially available Zn in soil measured by the DGT technique and plant Zn concentrations showed that soil type was significant (for the intercept and/or slope) for all beet plant tissues and for green pea root (Table 4). It should be noted that in beetroot plants, these regression models showed different intercepts depending on the soil type, but the increase in plant Zn concentration in relation to the available Zn concentration in the soil is similar.

Zinc concentrations in beet shoots were significantly higher for the acidic soil than for the calcareous soil (Fig. 1). Furthermore, in these models, the R -squared values were higher than when data from both soils were used together. These increases in R -squared values ranged from 0.61–0.94 to 0.78–0.97 (with the lowest values in both ranges being for green pea root and the highest being for beetroot stem).

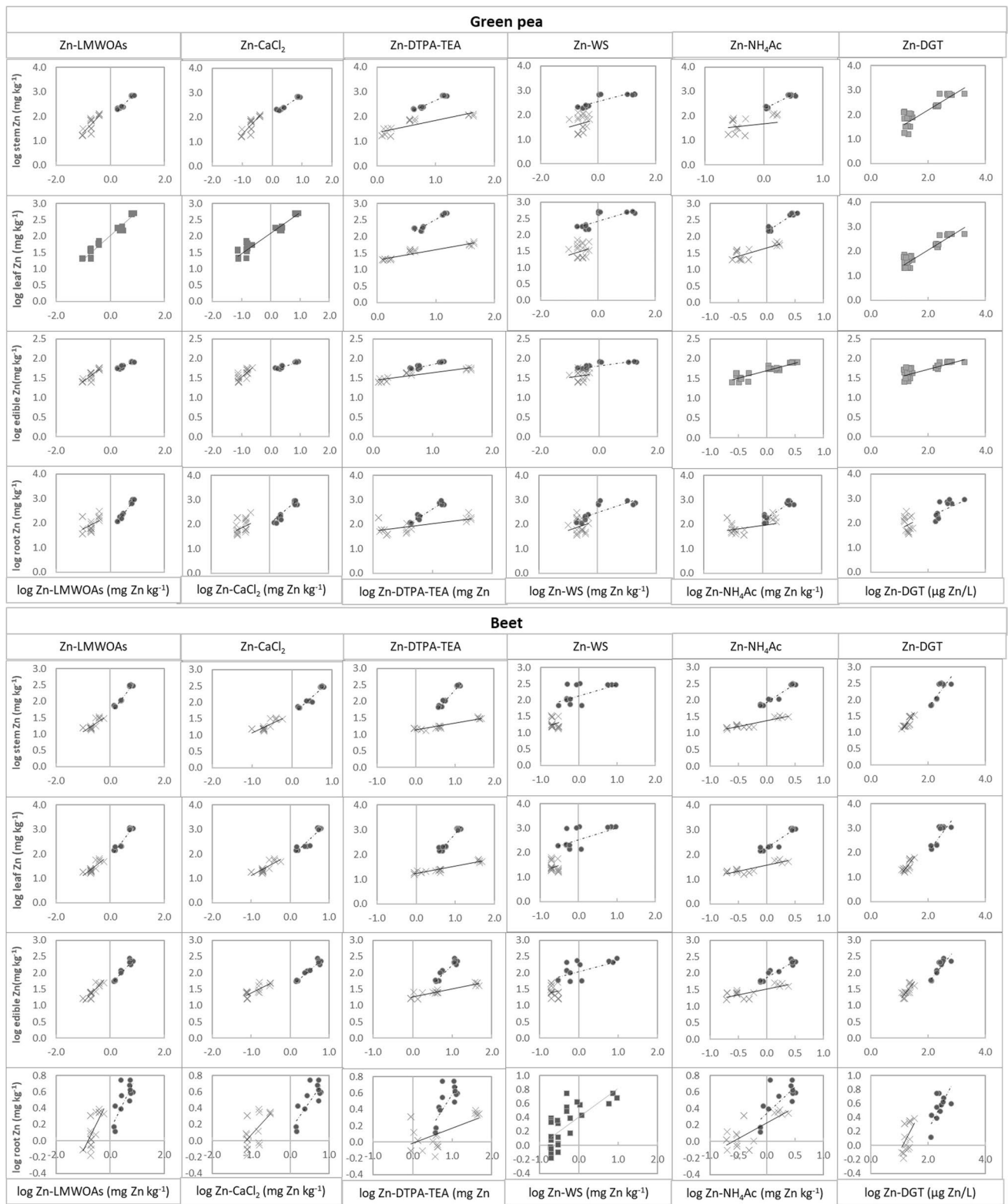


Fig. 1 Linear relationships between log-transformed values of LMWOAs-, CaCl₂-, DTPA-TEA-, WS-, NH₄-acetate- (mg Zn kg⁻¹), and DGT-extractable Zn (μg Zn L⁻¹) and the Zn concentration in plant tissues (root, stem, leaf or edible part, mg Zn kg⁻¹) for both crops. When the soil type, which was considered as a categori-

cal variable, is significant for the intercept and/or slope, the model is represented using two different lines (discontinuous and continuous, for the acidic and calcareous soils, respectively). The circles and the crosses represent the values for the acidic and calcareous soils, respectively (*n* = 72)

3.3 The Influence of ZnO Particle Size (Bulk vs NPs)

Given the possibility that the ZnO particle size (bulk vs NPs) could also have influenced the model fit, we studied a new regression model including this variable (ZnO particle size) as a categorical variable (Table 5). In this new regression model, we analyzed the two soils separately to rule out the potential influence of the soil factor. The results showed that ZnO particle size was only significant for the intercept and/or slope in the linear relationships between Zn-WS and all parts of the green pea plant and for Zn-DGT and the edible part of the beetroot.

The results also showed that WS-extractable Zn did not adequately estimate Zn concentrations in the different plant parts in calcareous soil, in either of the two crops (Table 5). Furthermore, the DGT technique failed to satisfactorily estimate Zn concentrations in the different parts of the green pea in the calcareous soil. Relatively low R -squared (R^2) values were observed in some of the regression models obtained when the soils were studied separately. These values indicated that when using WS-extractable Zn to predict the Zn concentrations in the different parts of the beetroot grown in the acidic soil, the models only explained 37.33 to 39.80% of the variability of the data. Furthermore, these results showed that, in some cases, the different models used to estimate the Zn concentrations in the roots of both crops explained less than 45% of the variability in the data (Zn-WS, Zn-DGT in both soils and Zn-DTPA-TEA in the calcareous soil, for beet root and for all the chemical extraction methods and the DGT technique for green pea root).

The result of the regression study between the logarithmically transformed values of extractable Zn in soil and Zn concentrations in plant tissues—when particle size was considered as a categorical variable—was significant for the intercept and/or slope, as shown in Fig. 2. It is notable that in the green pea grown in the acidic soil, the WS-extractable Zn at the highest Zn concentration studied increased according to the particle-size, for the same Zn concentration in the plant tissue. The extractable Zn concentrations in soils amended with bulk oxide were significantly higher than in the soils amended with NPs. However, this trend was not observed in the calcareous soil, and the overall model is valid for both particle sizes. Likewise, Zn concentrations obtained by the DGT technique from calcareous soil amended with bulk ZnO were significantly higher than those obtained from soils amended with ZnO NPs.

To study the possible relationships between the Zn concentration in the different parts of each plant species and the Zn-extractable concentration in soil, a PCA biplot was generated (Fig. 3). The variance explained by the first 2 components was 83.07% (x -axis 72.80%, y -axis 10.27%). The biplot of the first two components showed clusters of values as a function of soil type, crops, and/or applied ZnO

NP rate. The PCA biplot showed that Zn concentrations were grouped according to soil type: the calcareous soil was associated with negative values on the x -axis (except at the highest Zn rate, located at around the origin) and the acidic soil was associated with positive values on the x -axis. Clusters of points were grouped along the x -axis according to the Zn rate that was applied. The lower application rates were clustered nearer smaller x -values than the higher rates. The control, and rates 3 and 20 in the calcareous soil could not be differentiated. In contrast, the highest Zn rate in the acidic soil formed clusters that were separated according to ZnO particle size (NPs or bulk). There were also different groups on the y -axis depending on the crop: green pea on the positive y -axis values and beetroot on the negative y -axis values. Root Zn concentration influenced this clustering, as Zn concentrations in beet root were lower than in pea root. The Zn concentrations extracted by the WS, DGT, CaCl_2 , and LMWOAs methods were quite close to each other. The angle in the bi-plot between these methods and the Zn concentrations in the plant stems and edible parts of the plants implied a positive correlation between them. This suggests that under these experimental conditions the WS, DGT, CaCl_2 , and LMWOAs methods had similar extraction capabilities and successfully estimated Zn concentrations in these plant parts. In contrast, these extraction methods show no relationship with Zn concentrations in roots. The angle in the bi-plot between Zn- NH_4Ac and Zn concentrations in the edible parts of both crops implied a positive correlation between them. Conversely, this reagent did not appear to be related to Zn concentrations in the roots, as a right angle implied no correlation between the variables.

4 Discussion

The different extraction methods applied influenced the amounts of Zn that were extracted after ageing for one year in soil. The amount of metals in the soil solution represented the most available fraction (Kiekens 1995). However, the WS extraction method was not able to successfully predict Zn concentrations in all the different plant parts and particularly not for low Zn concentrations in soils. The models that were separately applied with each of the soils showed that they were not appropriate for estimating concentrations in plants based on the Zn-WS concentration in the calcareous soil. The characteristics of the calcareous soil, including its alkaline pH and high clay content, increased the retention of ZnO NPs / Zn^{2+} ions by the soil and there was thus a corresponding decrease in Zn availability to plants. The WS extraction method quantifies the free ions present in the soil solution (Zn^{2+} and ZnOH^+) and the organic Zn complexes present in the soil solution. However, in addition to this readily available fraction, plants are also able to influence

Table 5 Residual standard error (RSE), intercept (β_0), and slope (β_1) of the linear relationship(s) between log-transformed values of the Zn concentrations in different plant tissues and Zn-extractable concentrations from all the ZnO amended soils. Different intercepts and/or slopes when ZnO size is included as a categorical variable are denoted with NPs (NP) and bulk (B)

		Beta vulgaris				Pisum sativum			
		Beetroot Stem	Leaf	Edible part	Root	Green pea Stem	Leaf	Edible part	Root
Acidic soil									
Zn-LMWOAs	R^2	96.341***	93.63***	96.01***	59.60***	96.76***	88.39***	94.34***	98.19***
	RSE	0.06	0.11	0.06	0.15	0.047	0.083	0.020	0.051
	β_0	1.63	1.86	1.58	0.16	1.98	1.92	1.64	1.66
	β_1	1.08	1.48	0.99	0.63	1.00	0.89	0.31	1.47
Zn-CaCl ₂	R^2	94.76***	90.36***	96.15***	66.93**	96.52***	90.10***	92.29***	94.61***
	RSE	0.05	0.13	0.06	0.13	0.049	0.077	0.023	0.089
	β_0	1.63	1.86	1.57	0.14	2.10	2.02	1.68	1.84
	β_1	1.10	1.49	1.02	0.68	0.80	0.72	0.25	1.16
Zn-DTPA-TEA	R^2	96.23	94.36***	90.63***	58.19**	96.60***	89.88***	90.13***	95.46***
	RSE	0.06	0.10	0.09	0.15	0.05	0.078	0.03	0.08
	β_0	1.03	1.03	1.05	-0.18	1.57	1.55	1.52	1.07
	β_1	1.32	1.82	1.18	0.76	1.08	0.97	0.32	1.56
Zn-WS	R^2	37.33*	39.80*	37.53*	38.38*	93.42***	69.80***	90.54***	94.84***
	RSE	0.25	0.34	0.23	0.18	0.073	0.14**	0.03	0.09
	β_0	2.13	2.53	2.04	0.45	NP: 2.73 B: 2.46	2.46	NP: 1.88 B: 1.78	NP: 2.77 B: 2.35
	β_1	0.38	0.54	0.35	0.28	NP: 0.82 B: 0.30	NP: 0.49 B: 0.22	NP: 0.25 B: 0.09	NP: 1.22 B: 0.43
Zn-NH ₄ Ac	R^2	96.47***	93.02***	93.86***	59.46**	95.80***	95.07***	89.19***	87.36***
	RSE	0.06	0.11	0.07	0.15	0.06	0.06	0.03	0.14
	β_0	1.93	2.27	1.86	0.34	2.26	2.16	1.72	2.08
	β_1	1.14	1.55	1.04	0.66	1.21	1.12	0.37	1.66
Zn-DGT	R^2	76.59**	71.45**	78.97**	43.22*	63.04*	62.27*	59.06*	65.93**
	RSE	0.15	0.23	0.12	0.16	0.17	0.16	0.05	0.22
	β_0	-0.79	-1.25	-0.55	-1.00	0.85	0.82	1.32	0.10
	β_1	1.25	1.64	1.12	0.64	NP: 0.68 B: 0.67	0.64	0.20	0.95
Calcareous soil									
Zn-LMWOAs	R^2	62.65**	62.52**	69.16***	53.92**	68.57***	71.53***	71.45***	20.19*
	RSE	0.09	0.13	0.10	0.14	0.19	0.11	0.07	0.26
	β_0	1.65	1.97	1.89	0.59	2.58	2.04	1.93	2.34
	β_1	0.58	0.84	0.74	0.74	1.28	0.76	0.51	0.58
Zn-CaCl ₂	R^2	62.25***	59.39**	67.25***	35.86*	55.51**	52.52**	63.00**	22.80*
	RSE	0.09	0.14	0.10	0.16	0.23	0.14	0.08	0.21
	β_0	1.72	2.07	1.99	0.59	3.24	2.33	2.18	2.71
	β_1	0.49	0.69	0.62	0.51	1.65	0.87	0.65	0.83
Zn-DTPA-TEA	R^2	87.24***	92.32***	88.35***	36.66*	72.45***	89.54***	81.66***	39.74*
	RSE	0.05	0.06	0.06	0.16	0.18	0.07	0.06	0.23
	β_0	1.14	1.22	1.26	-0.01	1.35	1.30	1.43	1.73
	β_1	0.20	0.30	0.25	0.19	0.50	0.31	0.20	0.30
Zn-WS	R^2	ns	ns	ns	ns	ns	ns	ns	ns
	RSE								
	β_0								
	β_1								
Zn-NH ₄ Ac	R^2	85.86***	73.78***	55.80**	51.14**	35.36*	58.48**	49.90**	29.24*
	RSE	0.05	0.16	0.12	0.14	0.28	0.13	0.10	0.25
	β_0	1.38	1.57	1.52	0.23	1.87	1.65	1.66	2.08

Table 5 (continued)

	Beetroot	<i>Beta vulgaris</i>		Green pea	<i>Pisum sativum</i>				
Zn-DGT	β_1	0.35	0.48	0.35	0.38	0.68	0.49	0.31	0.50
	R^2	70.07***	67.05**	71.18**	39.19*	ns	ns	ns	ns
	RSE	0.08	0.13	0.10	0.17				
	β_0	0.02	-0.37	NP: -0.11 B: -0.26	-1.24				
	β_1	0.99	1.42	1.26	1.07				

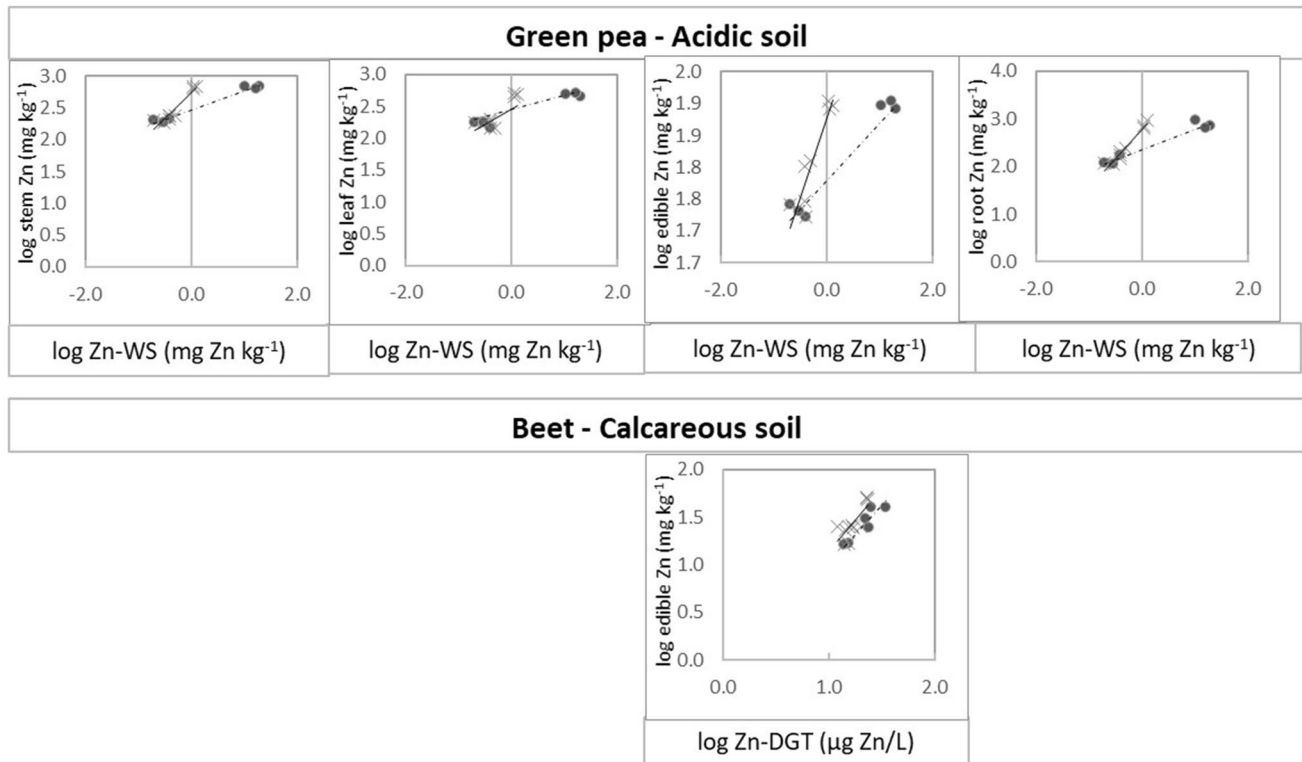


Fig. 2 Linear relationships between log-transformed values of extractable Zn (mg Zn kg^{-1}) and the Zn concentrations in plant tissues (root, stem, leaf or edible part, mg Zn kg^{-1}), when particle size (bulk vs NP), which was considered as a categorical variable, was significant for the intercept and/or slope. The model is represented using

two different lines (discontinuous and continuous, for bulk and NP, respectively). The circles and the crosses represent the bulk and NP values, respectively ($n = 15$ and 21 for the acidic and calcareous soils, respectively)

the release of some weakly bound trace metals into the soil rhizosphere (Naidu and Harter 1998).

LMWOAs is an extraction method that simulates the effect of acids released by plant roots in rhizospheric soil. These low molecular weight organic acids can dissolve Zn from the soil and promote its uptake by plant roots. The extraction procedure with LMWOAs successfully predicted Zn concentrations in plant shoots. Similarly, extraction with CaCl_2 also successfully predicted Zn concentrations in plant shoots. CaCl_2 is a neutral salt that can extract exchangeable metals from the soil. This extraction procedure is based on equilibrating the soil using surplus cations (Ca^{2+}) that

exchange a certain amount of metal ions from soil surfaces by competitive adsorption without influencing the soil pH (Duffner et al. 2013). The models obtained with both extraction methods showed higher Zn concentrations in the different plant parts when the concentration of extracted Zn increased. The models showed that both Zn concentrations in plant tissues and extractable Zn concentrations in soil were significantly higher for acid soils than for calcareous soils, except for Zn concentrations in roots. This indicates that ZnO dissolution in the acid soil was higher than in the calcareous soil. Furthermore, in calcareous soils there is a higher demand for Zn sources than in acid soils due to both

vs NPs) was evident in Zn-WS for green peas grown in the acidic soil and in Zn-DGT for the edible part of beetroot grown in the calcareous soil. In the models obtained using the Zn-WS extraction method, the Zn concentrations extracted from soils amended one year earlier at the highest application rate were significantly lower for NP size than for bulk. Although zinc oxides have lower solubility constants than other Zn fertilizer sources, the dissolution kinetics of the nanoparticles may be higher due to the higher specific surface area of the particles. In the long term, it was possible for soil components retained the Zn from the partial dissolution of the ZnO-NPs through slow sorption reactions, and it was not extracted with the WS extraction method. On the other hand, if the NPs dissolve in the soil solution, they could pass through the diffusive layer used in standard DGT devices and be retained by the binder resin layer (Pouan et al. 2014). Consequently, the DGT technique simulates Zn uptake in plants with a good level of prediction, but it would not be able to differentiate the concentration of ZnO-NPs and ionic Zn. Pouan et al. (2021) reported that to differentiate labile ZnO plus ionic Zn from labile ionic Zn it would be necessary to use the DGT technique with a 1000 molecular weight cutoff dialysis membrane in order to exclude the NPs.

5 Conclusions

The LMWOAs, and the CaCl_2 extraction methods and the DGT technique (for beetroot) could potentially be used in a similar way to predict long-term Zn bioavailability in the aerial parts (stem, leaf, and edible part) of plants from soil-applied bulk ZnO or NPs. In contrast, the WS, DTPA-TEA, and NH_4Ac extraction methods, and the DGT technique (for green pea) estimated different Zn concentrations in the plants for similar Zn concentration in both soils. While the DTPA-TEA reagent overestimated the available Zn in the calcareous soil, the WS extraction was not able to successfully predict Zn concentrations in the plant when there were low Zn concentrations in the soils. The NH_4Ac showed two different trends: in green pea plants, the models were similar to those obtained with WS, while in beetroot, the models were similar to those with DTPA-TEA. Neither method correctly assessed the internal Zn concentration in the root. In general, the methods were also unable to estimate different models as a function of particle size, except for WS in beetroot. Further research will be necessary to study the Zn associated with different soil fractions and to analyze possible differences that may depend on the ZnO particle size.

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Declarations

Conflict of Interest The authors declare no competing interests.

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References

- Alloway BJ (2009) Soil factors associated with zinc deficiency in crops and humans. *Environ Geochem Health* 31:537–548
- Almendros P, Gonzalez D, Alvarez JM (2013a) The influence of moisture on the residual effects of natural zinc chelates applied to two different soils. *J Soil Sci Plant Nutr* 13:797–807. <https://doi.org/10.4067/S0718-95162013005000063>
- Almendros P, Gonzalez D, Alvarez JM (2013b) Long-term bioavailability effects of synthesized zinc chelates fertilizers on the yield and quality of a flax (*Linum usitatissimum* L.) crop. *Plant Soil* 368:251–265. <https://doi.org/10.1007/s11104-012-1502-2>
- Almendros P, Obrador A, Alvarez JM, Gonzalez D (2019) Zn-DTPA-HEDTA-EDTA application: a strategy to improve the yield and plant quality of a barley crop while reducing the N application rate. *J Soil Sci Plant Nutr* 19:920–934. <https://doi.org/10.1007/s42729-019-00090-3>
- Almendros P, González D, Ibañez MA, Fernández MD, García-Gomez C, Smolders E, Obrador A (2020) Can diffusive gradients in thin films (DGT) technique and chemical extraction methods successfully predict both Zn bioaccumulation patterns in plant and leaching to groundwater in soils amended with engineered ZnO nanoparticles? *J Soil Sci Plant Nutr* 20:1714–1731. <https://doi.org/10.1007/s42729-020-00241-x>
- Alvarez JM, Almendros P, Gonzalez D (2009) Residual effects of natural Zn chelates on navy bean response, Zn leaching and soil Zn status. *Plant Soil* 317:277–291. <https://doi.org/10.1007/s11104-008-9808-9>
- Brennan RF (2001) Residual value of zinc fertiliser for production of wheat. *Aust J Exp Agric* 41:541–547. <https://doi.org/10.1071/EA00139>
- Buekers J (2007) Fixation of cadmium, copper, nickel and zinc in soil: kinetics, mechanisms and its effect on metal bioavailability. Thesis-dissertation Kathol Univ Leuven
- Das S, Andrew G (2016) Zinc in Crops and Human Health. In: Singh U, Praharaj CS, Singh SS, Singh NP (eds) *Biofortification of Food Crops*. pp 31–40
- Davis JA, Kent DB (2018) Surface complexation modeling in aqueous geochemistry. In: Hochella MF, White AF (eds). *De Gruyter*, pp 177–260

- Degryse F, Smolders E, Zhang H, Davison W (2009) Predicting availability of mineral elements to plants with the DGT technique: a review of experimental data and interpretation by modelling. *Environ Chem* 6:198–218. <https://doi.org/10.1071/EN09010>
- Duffner A, Hoffland E, Weng L, Zee SEATM Van Der (2013) Predicting zinc bioavailability to wheat improves by integrating pH dependent nonlinear root surface adsorption. 919–930. <https://doi.org/10.1007/s11104-013-1845-3>
- FAO (2015) Base referencial mundial del recurso suelo. Sistema internacional de clasificación de suelos. Organización de las Naciones Unidas para la Alimentación y la Agricultura, Roma
- Feng MH, Shan X, Zhang S, Wen B (2005) A comparison of the rhizosphere-based method with DTPA, EDTA, CaCl₂, and NaNO₃ extraction methods for prediction of bioavailability of metals in soil to barley. *Environ Pollut* 137:231–240. <https://doi.org/10.1016/j.envpol.2005.02.003>
- Houba VJG, Temminghoff EJM, Gaikhorst GA, van Vark W (2000) Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. *Commun Soil Sci Plant Anal* 31:1299–1396. <https://doi.org/10.1080/00103620009370514>
- Kiekens L (1995) Chapter 13: Zinc. Allow BJ
- Lindsay W (1979) Chelate equilibria. In: Lindsay W (ed) *Chemical equilibria in soils*. John Wiley and Sons, New York, pp 238–263
- Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci Soc Am J* 42:421–428. <https://doi.org/10.2136/sssaj1978.03615995004200030009x>
- McLaughlin MJ, Zarcinas BA, Stevens DP, Cook N (2000) Soil testing for heavy metals. *Commun Soil Sci Plant Anal* 31:1661–1700. <https://doi.org/10.1080/00103620009370531>
- Menzies NW, Donn MJ, Kopitke PM (2007) Evaluation of extractants for estimation of the phytoavailable trace metals in soils. *Environ Pollut* 145:121–130. <https://doi.org/10.1016/j.envpol.2006.03.021>
- Mikula K, Izydorczyk G, Skrzypczak D, Mironiuk M, Moustakas K, Witek-Krowiak A, Chojnacka K (2020) Controlled release micro-nutrient fertilizers for precision agriculture – a review. *Sci Total Environ* 712:136365. <https://doi.org/10.1016/j.scitotenv.2019.136365>
- Naidu R, Harter RD (1998) Effect of different organic ligands on cadmium sorption by and extractability from soils. *Soil Sci Soc Am J* 62:644–650. <https://doi.org/10.2136/sssaj1998.03615995006200030014x>
- Obrador A, González D, Almendros P, García C (2021) Assessment of phytotoxicity and behavior of 1-year-aged Zn in soil from ZnO nanoparticles, bulk ZnO, and Zn sulfate in different soil-plant cropping systems: from biofortification to toxicity. *J Soil Sci Plant Nutr* in Press. <https://doi.org/10.1007/s42729-021-00640-8>
- Ogunkunle CO, Oyedeji S, Okoro HK, Adimula V (2021) Interaction of nanoparticles with soil. In: Amrane A, Mohan D, Nguyen TA, Assadi AA, Yasin G (eds) *Nanomaterials for Soil Remediation*. Elsevier, pp 101–132
- Pouran HM, Martin FL, Zhang H (2014) Measurement of ZnO nanoparticles using diffusive gradients in thin films: binding and diffusional characteristics. *Anal Chem* 86:5906–5913. <https://doi.org/10.1021/ac500730s>
- Pouran H, Alkasbi M, Lahive E, Lofts S, Zhang H (2021) Measuring ZnO nanoparticles available concentrations in contaminated soils using the diffusive gradient in thin-films (DGT) technique. *Sci Total Environ* 793:148654. <https://doi.org/10.1016/j.scitotenv.2021.148654>
- Rauret G (1998) Extraction procedures for the determination of heavy metals in contaminated soil and sediment. *Talanta* 46:449–455. [https://doi.org/10.1016/S0039-9140\(97\)00406-2](https://doi.org/10.1016/S0039-9140(97)00406-2)
- Reddy Pullagurala VL, Adisa IO, Rawat S, Kim B, Barrios AC, Medina-Velo IA, Hernandez-Viezcas JA, Peralta-Videa JR, Gardea-Torresdey JL (2018) Finding the conditions for the beneficial use of ZnO nanoparticles towards plants—a review. *Environ Pollut* 241:1175–1181. <https://doi.org/10.1016/j.envpol.2018.06.036>
- Van Reeuwijk LP (2002) Procedures for soil analysis. Int Soil Ref Inf Centre, Tech Pap
- Rickerby DG, Morrison M (2007) Nanotechnology and the environment: a European perspective. *Sci Technol Adv Mater* 8:19–24. <https://doi.org/10.1016/j.stam.2006.10.002>
- Rodrigues SM, Trindade T, Duarte AC, Pereira E, Koopmans GF, Romkens PFAM (2016) A framework to measure the availability of engineered nanoparticles in soils: trends in soil tests and analytical tools. *TrAC - Trends Anal Chem Anal Chem* 75:129–140. <https://doi.org/10.1016/j.trac.2015.07.003>
- Soil Survey Staff (2014) Keys to soil taxonomy, twelfth edition. United States Dep Agric Nat Resour Conserv Serv 360
- Tandy S, Mundus S, Yngvesson J, de Bang TC, Lombi E, Schjoerring JK, Husted S (2011) The use of DGT for prediction of plant available copper, zinc and phosphorus in agricultural soils. *Plant Soil* 346:167–180. <https://doi.org/10.1007/s11104-011-0806-y>
- Tiede K, Boxall ABA, Tear SP, Lewis J, David H, Hassellöv M (2008) Detection and characterization of engineered nanoparticles in food and the environment. *Food Addit Contam - Part A Chem Anal Control Expo Risk Assess* 25:795–821. <https://doi.org/10.1080/02652030802007553>
- Ure AM, Davidson CM (2002) Chemical speciation in soils and related materials by selective chemical extraction. In: *Chemical speciation in the environment*. John Wiley & Sons, Ltd, pp 265–300
- Zahedi SM, Karimi M, da Silva JA (2020) The use of nanotechnology to increase quality and yield of fruit crops. *J Sci Food Agric* 100:25–31. <https://doi.org/10.1002/jsfa.10004>

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