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OSMAN SÖNMEZ

GARY PIERZYNSKI

CENGİZ KAYA

SALİH AYDEMİR

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The effects of phosphorus addition on phytoavailability of zinc by diffusive gradients in thin films (DGT)

Osman SÖNMEZ[1,*], Gary PIERZYNSKI[2], Cengiz KAYA[3], Salih AYDEMİR[3]
[1]Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Erciyes University, Kayseri, Turkey
[2]Department of Agronomy, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS, USA
[3]Department of Soil Science, Faculty of Agriculture, Harran University, Şanlıurfa, Turkey

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Abstract: Diffusive gradients in thin films (DGT) have been used for the assessment of Zn phytoavailability. Phosphorus is of particular interest in soils having Zn toxicity because of the Zn–P interaction. A greenhouse study was carried out to assess the influence of different P rates on the Zn phytoavailability to sorghum-Sudan grass (*Sorghum vulgare* var. sudanese) in soil systems by DGT. Soil was amended with $ZnSO_4$ at 0, 150, 300, 600, and 1200 mg Zn kg^{-1} in order to create various levels of Zn phytoavailability. Phosphorus was applied to soil at 0, 100, and 200 mg P kg^{-1} as KH_2PO_4. Plant nutrients were added as Hoagland's solution. In general, plant tissue Zn concentrations were elevated by increasing Zn concentration in soils, and they decreased by increasing P concentrations. However, this reduction was not found in C-DGT results. In other words, DGT did not predict the effects of P additions compared to Zn. P application decreased plant Zn concentrations and increased plant biomass yields. The highest relative yield in shoot tissue was with $P_{200} \times Zn_{150}$ treatment, while the lowest one was with $P_0 \times Zn_{1200}$ treatment. In general, increasing P concentrations in the soil increased shoot yields relative to control, while increasing Zn concentrations decreased the relative shoot yields where no P was added. P addition might be an alternative strategy for remediation of Zn contaminated soil, since addition of P decreased Zn phytoavailability and enhanced plant growth.

Key words: DGT, metal bioavailability, metal uptake, phosphorus, zinc

1. Introduction

Zinc is an essential trace element for humans, animals, and plants. The Zn concentration in soil generally ranges between 10 and 300 mg kg^{-1} depending on parent material, with the average around 50 mg kg^{-1} (Mortvedt, 2000). However, soil Zn concentrations can be elevated by anthropogenic sources like fossil fuel combustion, microelectronic industries, mining activities, the textile industry, smelting, agricultural inputs, and waste disposal to a point toxic to plants (Kabata-Pendias and Pendias, 2001; Chaney, 1993).

Zinc availability in soils has conventionally been assessed by several extractants such as $CaCl_2$, Mehlich 1, Mehlich 3, Morgan, and solutions containing chelators (e.g., EDTA or DTPA) (Chaney, 1993; McBride et al., 2003). Unfortunately, most of the procedures separate the solid and solution phases and disrupt the chemical and physical equilibrium; thus, the extract or soil solution gained by any of these methods cannot be representative of the actual, unchanged solution. Davison and Zhang (1994) developed a simple technique, diffusive gradients in thin films (DGT), which measures the solution metal plus resupply from the solid phase with a well described geometry. Several researchers have evaluated DGT. Hooda et al. (1999) also investigated metal bioavailability by DGT at elevated soil moisture content and determined the fluxes of Zn, Cd, Ni, Pb, Co, and Cu in sludge-treated soils. They reported that DGT can quantitatively describe the fluxes of trace metals under a wide range of soil conditions. Nolan et al. (2005) conducted a study to predict Zn, Cu, Pb, and Cd availability to wheat (*Triticum aestivum* L.) by DGT, batch extraction, chemical speciation, and isotopic dilution techniques. They found that predictions of Cd, Zn, and Pb accumulation in wheat by DGT were successful. Sonmez and Pierzynski (2005) compared the performance of DGT and $CaCl_2$ extraction to assess Zn phytoavailability and Zn phytotoxicity thresholds and reported that the performances of both methods were similar.

Zinc availability and mobility in soils are controlled by its interaction with amendments and the soil matrix. Phosphorus is of particular interest in soils having elevated Zn concentrations because Zn–P interactions can affect

* Correspondence: osmansonmez@erciyes.edu.tr

availability of both elements to the plant. In addition, Zn often occurs as a cocontaminant with Pb, and the use of P for in situ immobilization of Pb in contaminated soils can potentially influence Zn. In general, with increasing P content of the soil, plant uptake and mobility of Zn decreases (Marschner, 1991; McGowen et al., 2001). The effect of P could either be soil chemical or plant related. It has been suggested that the effect is often not soil chemical, as Zn–P precipitation is not likely to occur unless solution P levels are very high, but mostly plant related. Ova et al. (2015) showed that reduction in Zn uptake by P addition only occurred in nonsterilized soil due to the reduction in mycorrhiza under high P levels. In highly contaminated soils mycorrhizal uptake is usually of little importance, but Zn–P precipitation is more likely to happen, particularly in high pH soils with low sorption capacity for P. The effect of P amendments may also be related to changes in pH induced by the amendment. However, Friessen et al. (1980) reported that high P supply caused only a slight decrease in soil extractable Zn content. Shuman (1988) reported that soil Zn was changed by KH_2PO_4 from a less available form to one that is more available and exchangeable. Further studies of the P–Zn interaction are warranted. In fact, there has not been a published study to evaluate the response of DGT to P additions in Zn contaminated soil.

Our objective was to determine the influence of different P rates on the Zn phytoavailability to sorghum-Sudan grass as evaluated by DGT.

2. Materials and methods

2.1. Soil materials

The taxonomic classification of soil is clay mixed, Mesic, Mollic Udifluvent. Initial soil samples were collected before treatment application and sieved through a stainless steel 2-mm screen, air dried, and kept in plastic bags at room temperature for analysis. For total Zn concentration, 2 g of soil (<2 mm) was digested with 20 mL of 4 M HNO_3 acid at 80–85 °C for 2 h, and using inductively coupled plasma atomic emission spectrophotometry (ICP-AES), Zn in digest solution was determined. Selected properties of soil prior to treatment applications are presented in Table 1.

Before seeding and after harvest, samples of soil were taken from pots, and pH of the samples was determined in 1:1 (soil to water) ratio with a combination pH electrode. Samples of soil were extracted in 1:1 (soil to water) ratio and 1:1 (soil to 0.01 M $CaCl_2$) ratio and filtered before analysis. Zinc analysis was carried out as above by ICP-AES. Samples were extracted with 0.5 M $NaHCO_3$ (pH 8.5), and extractable inorganic P was measured colorimetrically in an aliquot of this extract (Olsen et al., 1954). Soil porosity was calculated by the procedure described by Flints and Flint (1996). Soil texture was determined according to Bouyoucos (1951). Cation exchange capacity (CEC) and organic matter (OM) were determined by the methods of Sumner and Miller (1996) and Walkley and Black (1934), respectively. Extractions were carried out in batches of seventeen with 15 samples and two quality assurance-quality control samples as blanks and duplicates for each extraction.

2.2. Greenhouse study

To generate different levels of Zn phytoavailability, soil was amended with $ZnSO_4$. As P and Zn sources, reagent-grade KH_2PO_4 and $ZnSO_4$ were used. The experimental design was a 3 × 5 factorial design with three replications. The factors were Zn-rate with treatments of 0 (Zn_0), 150 (Zn_{150}), 300 (Zn_{300}), 600 (Zn_{600}), or 1200 (Zn_{1200}) mg kg^{-1} and P-rate with treatments of 0 (P_0), 100 (P_{100}), and 200 (P_{200}) mg P kg^{-1} using KH_2PO_4 as the P source.

Pots were bordered with a cellophane bag to prevent free drainage, and 1 kg of soil from each treatment was added. The treatments of P were applied to the soil and thoroughly mixed. For the Zn treatments, $ZnSO_4$ dissolved in deionized water was added to the soil at the desired level and mixed well. With deionized water, the gravimetric moisture content of each pot was adjusted to 20%. After an incubation period of 7 days, samples of soil were retained for analysis. After the incubation period, 30 seeds of sorghum-Sudan grass per pot were planted. Since it grows well under greenhouse conditions, produces a high forage yield, and has been confirmed to accumulate metals, sorghum-Sudan grass as a plant was preferred for the greenhouse study (Chaney, 1993). From emergence

Table 1. Selected chemical and physical properties of soil materials (<2 mm) prior to treatment applications.

	Sand (%)	Silt (%)	Clay (%) -----	CEC[a] (cmol kg^{-1})	pH	OM[b] (g kg^{-1})	Bio. P[c] (mg kg^{-1})	Zn (mg kg^{-1})
Soil	16	40	44	25	6.5	39	32	30

[a]CEC: cation exchange capacity
[b]OM: organic matter
[c]Bioavailable P
N = 3 for all measurements.

to harvest, each pot was watered daily with distilled water and twice per week 100 mL of Hoagland's nutrient solution (including 1.9 mmol P as KH_2PO_4) (Sonmez and Pierzynski, 2005) was added to pots. Plant populations were lowered to 17 plants per pot 2 weeks after seeding.

After 35 days of growth under greenhouse conditions, plants were harvested. To eliminate sticking soil particles, plant materials were washed with deionized water. Plant tissues were then oven dried at 55 °C and ground. To determine Ca, Zn, Fe, Mg, Mn, Cu, P, and K concentrations, 0.25 g of plant tissue was digested with 30% hydrogen peroxide and concentrated sulfuric acid. Digest solutions were analyzed by ICP-AES.

2.3. Deployment and retrieval of DGT

DGT available Zn was determined from a 50-g sample of the initial soil (before sowing and after amendment). The sample was weighed into a 120-mL plastic specimen container, and appropriate amounts of deionized water were added to obtain saturation. The DGT devices (DGT Research, Lancaster, UK) were placed on the surface of each soil slurry and pushed gently into the surface without leaving any air bubbles between the DGT device and the solution. The specimen cups were loosely covered with lids to decrease evaporative losses. Specimen cups were kept at room temperature for 24 h. The DGT devices were then retrieved from each cup and rinsed with deionized water to remove soil particles adhering to the filter membrane. The Chelex resin gel was retrieved and placed in 20-mL vials containing 10 mL of 1 M HNO_3 for 24 h. The samples were analyzed for Zn by using ICP-AES. Detailed information for implementation and retrieval of DGT was given in the method defined by Sonmez and Pierzynski (2005).

2.4. Data analyses

SAS for Windows version 9 (SAS Inc., Cary, NC, USA) was used for statistical analyses. Least significant difference (LSD) values were used for mean separations.

3. Results and discussion

3.1. Effects on plant biomass

Yields of sorghum-Sudan grass (shoot and root) were significantly affected by P and Zn treatments (Table 2), and significant P and Zn interaction was found. The highest relative yield in shoot tissue was with the treatment $P_{200} \times Zn_{150}$, while the lowest was with the $P_0 \times Zn_{1200}$ treatment. In general, increasing P concentrations in the soil increased shoot yields relative to the control, while increasing Zn concentrations decreased the relative shoot yields where no P was added. There was also a significant interaction between P and Zn treatments on relative yields of root (data not shown). The relative yields of root compared to control were significantly decreased by all treatments. The highest reduction among all treatments was with $P_0 \times Zn_{1200}$, and it was significantly different from all other treatments. The treatment $P_0 \times Zn_{1200}$ had the lowest relative yields in shoot (Table 2) and root. As a summary of the P × Zn interaction, at P_0 a significant reduction in yield at Zn_{150} was found, whereas at P_{100} and P_{200} a significant reduction in yield was not seen until Zn_{1200}. In addition, at Zn_{1200} significant increases in yield were observed when comparing P_0 to P_{100} and between P_{100} and P_{200}. A reddish-purple color appeared on the leaves of plants grown in pots receiving high Zn applications with no P ($P_0 \times Zn_{1200}$). However, that symptom was not present in plants receiving P. It seems that addition of P enhanced plant growth and increased tolerance of sorghum-Sudan grass to high soil Zn. Similarly, Sonmez and Pierzynski (2005) found that elevating Zn concentration in growth media decreased the yield of relative plant tissues of sorghum-Sudan grass. Additions of phosphate rock (PR), calcium magnesium phosphate (CMP), and single superphosphate (SSR) to mining-tailing–contaminated soil resulted in significantly higher biomass and lower plant Zn, Pb, and Cd uptake (Wang et al., 2008). The application of triple super phosphate (TSP) to three contaminated soils produced the highest cumulative biomass of sudax grass (*Sorghum vulgare* L. Moench) compared to control, PR, and cryptomelane (Hettiarachchi and Pierzynski, 2002).

3.2. Zn concentrations in plants

Shoot Zn concentrations changed from 25 mg kg^{-1} in $P_{200} \times Zn_0$ to 904 mg kg^{-1} in $P_0 \times Zn_{1200}$. The concentrations of Zn in roots varied from 23 mg kg^{-1} in $P_{200} \times Zn_0$ to 4331 mg kg^{-1} in $P_0 \times Zn_{1200}$ (Table 2). There were significant interactions between P and Zn treatments at a level of $P \leq 0.05$. In general, Zn concentrations in plant tissues were elevated by increasing Zn concentrations in soils and declined by increasing P concentrations in soils. That may be explained by an antagonistic effect between P and Zn or a dilution effect of plant growth. This could be the case in our experiment. This may also be related to a decrease in soil pH due to the soil amendment, since Zn solubility is very dependent on pH. In our study, soil pH decreased slightly as Zn treatments increased (Table 3). The reason may be the addition of Zn to soil as $ZnSO_4$. Decreasing soil pH may have caused an increase in Zn solubility and plant Zn uptake while leading to a decrease in soil P availability. Another explanation from the literature is Zn phosphate solid phases that formed in Zn contaminated soil in response to P additions. Baker et al. (2012) found Zn phosphate formation in Pb/Zn smelter-contaminated soil upon addition of PR, TSP, monoammonium phosphate (MAP), and fluid polyammonium phosphate. A decrease in plant tissue Zn concentrations upon P addition was reported by Pierzynski and Schwab (1993). However, Lu et al. (1998) found that increasing P availability in soil did not have any significant effect on Zn concentration in oilseed

Table 2. Selected characteristics of plant materials

		Plant Zn concentrations	
Treatments	Relative Yield[a]	Shoot (mg kg^{-1})	Root (mg kg^{-1})
P_0			
Zn_0	1±0.01 C[a]	54±5 K	108±5 K
Zn_{150}	0.78±0.09 D	249±1 H	764±27 H
Zn_{300}	0.81±0.01 D	409±8 F	986±11 F
Zn_{600}	0.66±0.05 E	579±24 D	3100±60 B
Zn_{1200}	0.34±0.04 F	904±80 A	4331±49 A
P_{100}			
Zn_0	1.15±0.08 AB	33±3 K	111±4 K
Zn_{150}	1.15±0.03 AB	179±10 I	633±36 I
Zn_{300}	1.11±0.13 ABC	304±8 G	884±39 G
Zn_{600}	1.04±0.08 BC	528±45 E	1603±80 D
Zn_{1200}	0.61±0.02 E	827±14 B	2858±59 C
P_{200}			
Zn_0	1.12±0.14 AB	25±4 K	23±2 L
Zn_{150}	1.2±0.14 A	138±2 J	384±25 J
Zn_{300}	1.19±0.06 AB	235±4 H	735±9 H
Zn_{600}	1.09±0.07 ABC	334±10 G	1080±15 E
Zn_{1200}	0.87±0.1 D	715±11 C	2825±52 C

[a] Relative yield to control. [b] Means with same letter within a column are not significantly different using least significant differences and P ≤ 0.05. N=3 for all measurements.

rape (*Brassica napus*). The addition of TSP or PR decreased Zn uptake in shoot tissue by 31.2%–47.3% (Chen et al., 2007). In the presence of soluble P, plant tissue Zn, Pb, and Cd concentrations were consistently reduced, possibly due to mixed-metal phosphate formation (Hettiarachchi and Pierzynski, 2002). Wang et al. (2008) studied the effects of P fertilizers applied as PR, CMP, and SSP on availability and uptake of Zn, Pb, and Cd by cabbage (*Brassica chinensis* L.). They reported that only PR and CMP reduced shoot Zn concentration (31.8%–73.2%). Coa et al. (2003) indicated that while P addition is effective for Pb immobilization, it often has no or little effect on Zn. In our study, Zn solution concentrations would have been very high because of the metal salt spiking; so Zn–P precipitation may indeed have occurred.

Increasing total Zn concentrations and P in soils elevated the P concentrations in shoots (Figure). Concentrations of P in shoots in this research varied between 3.3 and 7.1 g kg^{-1}. For optimum yield, a crucial shoot P concentration in sorghum-Sudan grass was 1.3 g kg^{-1} (Hardin et al., 1989). Therefore, our data revealed that there was no P deficiency because P was added in the Hoagland's solutions. Others have stated that increasing Zn application to soil reduced the P concentration of shoots (Sonmez and Pierzynski, 2005; Germa and Minhas, 1987).

3.3. Zinc concentration measured by DGT

There were significant interactions (P ≤ 0.05) between P and Zn treatments for C-DGT in the soils (Table 3). The C-DGT increased by increasing total Zn concentrations, while P did not have any effect on it. Phytoavailability of Zn by DGT was assessed by Sonmez and Pierzynski (2005), and they presented higher C-DGT values for Zn in comparison to the C-DGT values found in this research. This could be a reflection of the high capacity for absorption in the soil used in this study due to high organic matter content and CEC. Furthermore, mineralogy plays a crucial role in mineral solubility and can affect solubility of Zn.

Plant Zn concentration increased as Zn concentrations increased and decreased as P concentrations increased. However, this reduction was not found in C-DGT results.

Table 3. Selected characteristics of soil after P and Zn treatment applications

Treatments	Soil pH	Bio. P (mg kg[-1])	C-DGT (µmol L[-1])
P_0			
Zn_0	6.2±<0.1 D[a]	32.1±0.9 F	1.0±0.1 H
Zn_{150}	6.1±<0.1 E	27.6±1.7 F	1.6±0.1 FGH
Zn_{300}	6.1±<0.1 E	30.5±1.3 F	3.3±0.2 EF
Zn_{600}	5.9±<0.1 G	24.6±4.0 F	9.0±0.9 C
Zn_{1200}	5.8±<0.1 H	25.4±1.2 F	39.0±3.2 B
P_{100}			
Zn_0	6.4±<0.1 B	58.7±2.3 E	1.1±0.1 H
Zn_{150}	6.4±<0.1 B	60.3±0.9 E	2.2±0.1 EFGH
Zn_{300}	6.3±<0.1 C	57.3±0.2 E	3.2±0.4 EFG
Zn_{600}	6.2±<0.1 D	62.6±2.1 E	8.2±0.8 D
Zn_{1200}	5.9±<0.1 G	57.1±1.0 E	42.4±2.2 A
P_{200}			
Zn_0	6.5±<0.1 A	90.3±5.8 C	1.3±0.1 GH
Zn_{150}	6.5±<0.1 A	98.6±1.7 B	1.8±0.1 FGH
Zn_{300}	6.4±<0.1 B	94.9±14.2 BC	3.9±0.5 E
Zn_{600}	6.2±<0.1 D	106.9±10.6 A	9.4±0.1 CD
Zn_{1200}	6±<0.1 F	81.8±3.7 D	41.4±2.2 A

[a]Means with same letter within a column are not significantly different using least significant differences and P ≤ 0.05. N=3 for all measurements.

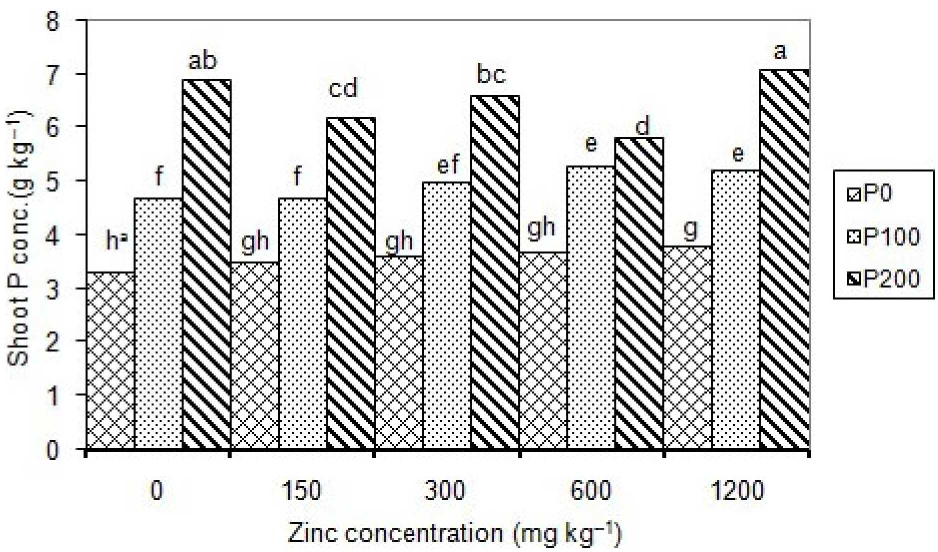

Figure. Shoot P concentrations.

In other words, DGT did not predict the effects of P additions compared to Zn.

Degryse et al. (2009) reported that the relationship between DGT and plant uptake depends on conditions. It is stronger if the diffusive transport of the element from soil to plant roots is rate-limiting. Even if uptake is not limited by diffusive transport, the relationship might still be strong as long as plant uptake is not saturated. When essential elements are in high supply plant uptake follows Michaelis–Menten kinetics which produces a saturation type curve, and the plant reduces uptake as it has too much Zn. This may be the case in our experiment. Moreover, competitive cations do not have any effect on the DGT flux. However, plant uptake under these conditions may be affected. Indeed, DGT measures labile complexes, although it is expected that they have no contribution to plant uptake. Interpretation of DGT measurement by a dynamic model assumes a constant intrinsic rate of release from solid phase to solution; however, it is much higher in soils receiving fresh supplies of Zn (Zhang et al., 2004), such as the soil in this study.

The DGT concentrations that were not affected by P level would normally suggest that no Zn–P precipitates formed. Another point is that Zn phosphate precipitations are unlikely to form in soil if DGT is not seeing a reduction in Zn with increasing P. The DGT should not measure insoluble precipitates. However, in this case the high Zn and Ca solution concentrations induced by spiking with Zn salts may have resulted in saturation of the Chelex resin with a 24-h deployment time. When saturation is approached, the resin does not act as a zero-sink anymore, which complicates interpretation and may mask differences between treatments. It is therefore possible that in situ soil solution concentrations were affected by P additions (explaining the lower Zn uptake at high P), but that this was not reflected by the DGT measurements due to saturation of the Chelex resin. As for the hypothesized effect of P addition on in situ soil concentrations, Zn–P precipitation is less likely to happen, but given the high addition rates of $ZnSO_4$, Zn concentrations in the in situ soil solution would have been high, which may have induced Zn–P precipitations. Furthermore, some of this hypothesized effect may also be pH related. The pH was generally higher at the higher P levels, which may have resulted in lower in situ Zn solution concentrations.

Adsorption of Zn by DGT was elevated by increasing Zn applied to soil. The addition of Zn decreased plant biomass yields, whereas they increased by increasing P applications. Unlike internal plant concentrations, DGT Zn concentrations were not affected by P additions. Results emphasize that phosphorus addition might be an alternative strategy for remediation of Zn contaminated soil.

References

Baker LR, Pierzynski GM, Hettiarachchi GM, Scheckel, Newville M (2012). Zinc speciation in proximity to phosphate application points in a lead/zinc smelter- contaminated soil. J Environ Qual 41: 1865-1873.

Bouyoucos GJ (1951). A recalibration of the hydrometer method for making mechanical analysis of the soil. Agron J 43: 434-438.

Cao RX, Ma LQ, Chen M, Singh SP, Harris WG (2003). Phosphate-induced metal immobilization in a contaminated site. Environ Pollution 122: 19-28.

Chaney RL (1993). Zinc phytotoxicity. In Robson AD, editor. Zinc in Soils and Plants. Dordrecht, the Netherlands: Kluwer, pp. 135-150.

Chen SB, Xu MG, Ma YB, Yang JC (2007). Evaluation of different phosphate amendments on availability of metals in contaminated soil. Ecotox Environ Safe 67: 278-285.

Davison W, Zhang H (1994). In situ speciation measurements of trace components in natural waters using thin-films gels. Nature 367: 546-548.

Degryse F, Smolders E, Zhang H, Davison W (2009). Predicting availability of mineral elements to plants with DGT technique: a review of experimental data and interpretation by modeling. Environ Chem 6: 198-218.

Flints LE, Flint AL (1996). Porosity. In: Duane JH, Topp GC, editors. Methods of Soil Analysis: Part 4—Physical Analyses. Madison, WI, USA: Soil Science Society of America, pp. 241-254.

Friessen DK, Juo ASR, Miller MH (1980). Liming and lime-phosphorus-zinc interactions in two Nigerian Ultisols. I. Interactions in the soils. Soil Sci Soc Am J 44: 1221-1226.

Germa TS, Minhas RS (1987). Zinc and phosphorus interaction in wheat-maize cropping system. Fert Res 13: 77-86.

Hardin SD, Howard DD, Wolt J (1989). Critical soil P of a low-P loess-derived soil as affected by storage temperature. Commun Soil Sic. Plant Anal 20: 1525-1543.

Hettiarachchi GM, Pierzynski GM (2002). In situ stabilization of soil lead using phosphorus and manganese oxide: influence of plant growth. J Environ Qual 31: 564-572.

Hooda PS, Zhang H, Davison W, Edwards AC (1999). Measuring bioavailable trace metals by diffusive gradients in thin films (DGT): soil moisture effects on its performance in soils. Eur. J Soil Sci 50: 285-295.

Kabata-Pendias A, Pendias H (2001). Trace Elements in Soils and Plants. 3rd ed. Boca Raton, FL, USA: CRC.

Lu ZG, Grewal HS, Graham RD (1998). Dry matter production and uptake of zinc and phosphorus in two oilseed rape genotypes under differential rates of zinc and phosphorus supply. J Plant Nutr 21: 25-38.

Marschner H (1991). Root-induced changes in the bioavailability of micronutrients in the rhizosphere. Plant roots. In: Waisel Y, Eshel A, Kafkafi K, editors. The Hidden Half. New York, USA: Marcel Dekker, pp. 503-528.

McBride MB, Nibarger EA, Richards BK, Steenhuis T (2003). Trace metal accumulation by red clover grown on sewage sludge-amended soils and correlation to Mehlich 3 and calcium chloride-extractable metals. Soil Sci 168: 29-38.

McGowen SL, Basta NT, Brown GO (2001). Use of diammonium phosphate to reduce heavy metal solubility and transport in smelter-contaminated soil. J Environ Qual 30: 493-500.

Mortvedt JJ (2000). Bioavailability of micronutrients. In: Chief Sumner ME, editor. Handbook of Soil Science. Boca Raton, FL, USA: CRC, pp. D71-D86.

Nolan AL, Zhang H, McLaughlin MJ (2005). Prediction of zinc, cadmium, lead, and copper availability to wheat in contaminated soils using chemical speciation, diffusive gradient in thin films, extraction, and isotopic dilution techniques. J Environ Qual 34: 496-507.

Olsen SR, Cole CV, Watanabe FS, and Dean L (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939: 1-19.

Ova EA, Kutman UB, Ozturk L, Cakmak I (2015). High phosphorus supply reduced zinc concentration of wheat in native soil but not in autoclaved or nutrient solution. Plant and Soil 393: 147-162.

Pierzynski GM, Schwab AP (1993). Bioavailability of zinc, cadmium, and lead in a metal-contaminated alluvial soil. J Environ Qual 22: 247-254.

Shuman LM (1988). Effect of phosphorus level on extractable micronutrients and their distribution among soil fraction. Soil Sci Soc Am J 52: 136-141.

Sonmez O, Pierzynski GM (2005). Assessment of zinc phytoavailability by diffusive gradient in thin films (DGT). Environ Toxicol Chem 4: 934-941.

Sumner ME, Miller WP (1996). Cation exchange capacity and exchange coefficients. In: Sparks DL et al., editors. Methods of Soil Analysis Part 3: Chemical Methods. SSSA Book Series No: 5. Madison, Wisconsin, USA: Am Soc of Agronomy and Soil Sci Soc of Am Inc. pp. 1201-1230.

Walkley A, Black LA (1934). An examination of the Degtjareff method for determining soil organic matter and proposed modification of the chromic acid titration method. Soil Sci 37: 29-38.

Wang B, Xie Z, Chen J, Jiang J, Su Q (2008). Effects of field application of phosphate fertilizers on the availability and uptake of lead, zinc and cadmium by cabbage (*Brassica chinensis* L.) in a mining tailing contaminated soil. J Environ Sci 20: 1109-1117.

Zhang H, Lombi E, Smolders E, McGrath S (2004). Kinetics of Zn release in soils and prediction of Zn concentration in plants using DGT. Environ Sci Technol 38: 3608-3613.