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THE EVALUATION OF DIFFUSIVE GRADIENTS IN THIN FILMS (DGT) AND $CaCI_2$ EXTRACTION ON PHOSPHORUS-ZINC INTERACTION IN SUDAN GRASS

OSMAN SONMEZ[1*], GARY PIERZYNSKI[2], CENGIZ KAYA[3] AND SALIH AYDEMIR[3]

[1]*Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Erciyes University, Kayseri 38039, Turkey*
[2]*Department of Agronomy, Throckmorton Plant Sciences Center, Kansas State University,*
Manhattan, Kansas 66506-5501, USA
[3]*Department of Soil Science, Faculty of Agriculture, Harran University, Sanlıurfa 63200, Turkey*
[*]*Corresponding author's e-mails: osmansonmez@erciyes.edu.tr. ; osharran@yahoo.com*

Abstract

Diffusive gradients in thin films (DGT) has been used for the assessment of Zn availability and offers some advantages over routine batch extractions of soil samples. To evaluate the effectiveness of DGT compared with $CaCl_2$-extraction to P additions in Zn contaminated soil, a greenhouse study was established. Zinc was added at the rate of 0, 150, 300, 600 and 1200mg Zn kg^{-1} as $ZnSO_4$ in order to create various levels of Zn phytoavailability. Soil was amended with KH_2PO_4 at 0, 100 and 200mg P kg^{-1}. Increasing application of Zn to soil elevated Zn adsorption by DGT, plant Zn concentrations, $CaCl_2$ extractable and water-soluble Zn and decreased soil bioavailable P and pH. Internal plant concentrations and $CaCl_2$-extraction were influenced by P additions, whereas DGT Zn concentrations were not affected. Phosphorus application decreased plant Zn concentrations and increased plant biomass yields.

Key words: DGT, Phosphorus, Zinc, Metal bioavailability, Metal uptake.

Introduction

Zinc is a crucial trace element for terrestrial life. The concentration of total Zn in soil is related to the parent material and soil mineralogy generally ranging from 10 to 300 mg kg^{-1} with the average around 50 mg kg^{-1} (Mortvedt, 2000). On the other hand, anthropogenic sources can elevate concentrations of soil Zn to a point toxic to plants (Chaney, 1993; Kabata-Pendias & Pendias, 2001).

There is not a strong relationship between total soil metal concentration and metal toxicity since it is associated with bioavailability (Nolan *et al*., 2005). The determination of bioavailability of metal is difficult because many soil properties affect bioavailability (Rieuwerts *et al*., 1998). These soil properties (soil organic matter (OM), mineralogy, pH, cation exchange capacity (CEC), the amount of metal, and redox potential) influence metal bioavailability by controlling the transformation of metals, the retention, and mobility in soils (Calace *et al*., 2002).

Several extractants (e.g., solutions containing chelators (e.g., DTPA or EDTA), Morgan, $CaCl_2$, Mehlich 1 and 3) has conventionally been employed to predict soil Zn availability (Chaney, 1993; McBride *et al*., 2003). The effectiveness of them is evaluated on the relationship between Zn uptake by plants and extracted Zn. Limitations of batch extractions include soil specificity, extraction of the element in amounts larger than the plant- available pool, analysis problems, and the changing of soil chemistry (Zhang *et al*., 1998). Davison & Zhang (1994) developed a simple technique, measuring the solution metal plus resupply from the solid phase with a well described geometry called DGT. The DGT has been evaluated by researchers. A good relationship between those calculated from soil solution directly and concentrations of Zn and Cd measured by DGT (r^2 =0.88 and 0.95, respectively) was reported by Zhang *et al*. (1998). The chemical speciation, DGT, isotopic dilution techniques, and batch extraction have been evaluated by Nolan *et al*. (2005) to predict Pb, Cd, Cu, and Zn availability to wheat (*Triticum aestivum* L.) and reported that DGT successfully predicted accumulation of Cd, Zn, and Pb in wheat.

Zinc interaction with amendments and the soil matrix depend on its mobility and availability in soils. In Zn contaminated soil, addition of P has been getting more attention because Zn-P interactions can affect availability of both elements to the plant. Plant uptake and mobility of the Zn generally decrease with increasing P content of the soil (Marschner, 1991; McGowen *et al*., 2001). Friessen *et al*. (1980) found that soil extractable Zn was slightly decreased by high P supply. Influence of P could either be plant or soil chemical related and this effect is mostly plant related not soil chemical as Zn-P precipitation is not likely to occur unless under very high solution P levels. Ova *et al*. (2015) reported that due to the reduction in mycorrhiza under high P level the Zn uptake was decreased by P addition in non-sterilized soil. Mycorrhizal uptake in highly contaminated soils usually is of little importance, but Zn-P precipitation might happen, particularly in soils having low sorption capacity for P and high pH, or the effect of P amendments may also be related to changes in pH induced by the amendment. In literature, there are some controversial results for P-Zn interaction. Further studies of the P-Zn interaction are needed. In fact, there has not been any published work to compare the effectiveness of DGT and $CaCl_2$ extraction to P-Zn interaction.

Our objective was to evaluate the effectiveness of DGT compared with $CaCl_2$ extraction to P additions in Zn contaminated soil.

Materials and Methods

Soil materials: Soil taxonomic classification is clay, mixed, Mesic, Mollic Udifluvent. Prior to treatment application, soil samples were gathered and sieved through a stainless steel 2-mm screen, air dried, and stored in plastic bags at room temperature for analysis. For concentration of total Zn, a 2-g soil (<2 mm) was digested with 20 ml 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. Soil had a silty clay texture with a CEC of 25 cmolc kg^{-1}, pH of 6.5, OM of 39 g kg^{-1}, 32 mg bioavailable P kg^{-1} and 30 mg total Zn kg^{-1}.

The samples of soil were collected from pots before seedling and after harvest. Soil pH was determined in 1:1 soil to water ratio with a combination pH electrode. Soil samples were extracted in 1:1 water to soil ratio and 1:1 0.01 M $CaCl_2$ to soil and filtered before being analyzed. Zinc concentration was determined as mentioned above by ICP-AES. For bioavailable P determination the Olsen method (Olsen *et al*., 1954) was used. The Bouyoucos (1951) method was used for soil texture. Soil OM and CEC were determined by the methods of Walkley & Black (1934) and Sumner and Miller (1996), respectively.

Greenhouse study: To generate various levels of Zn bioavailability, different rates of $ZnSO_4$ (0 (Zn_0); 150 (Zn_{150}), 300 (Zn_{300}), 600 (Zn_{600}), or 1200 (Zn_{1200}) mg kg^{-1}) were applied to soil. Phosphorus was applied to soil at the rates of 0 (P_0), 100 (P_{100}) and 200 (P_{200}) mg P kg^{-1} using KH_2PO_4 as the P source. The experimental design was a 3x5 factorial with three replications.

In order to prevent free drainage, pots were bordered with a cellophane bag, and 1 kg of soil from each treatment was then added. After additions of P treatments, soils were mixed comprehensively. $ZnSO_4$ dissolved in deionized water (DW) for the Zn treatments was applied to soil at the predetermined rates and mixed well. The gravimetric moisture content of each pot was adjusted to 20% with DW. After seven days (incubation period), samples of soil were taken for analysis. After the incubation period, sorghum-sudan grass was planted as 30 seeds per pot. Since it accumulates metals well and produces a high forage yield (Chaney, 1993). From emergence to harvest, twice per week 100 ml Hoagland's nutrient solution (including 1.9 mmol P as KH_2PO_4) (Sonmez & Pierzynski, 2009) was given to pots and they were watered daily with DW. Two weeks after seeding plant populations were decreased to 17 plants per pot.

After 35 days of growth under greenhouse conditions, plants were harvested and washed with DW to remove sticking soil particles. Plant tissues were then oven dried at 55˚C and ground. Digestion of plant tissues (0.25g) were done with 98% concentrated sulfuric acid and 30% hydrogen peroxide to determine Zn, P, Fe and Mn concentrations and were analyzed by ICP-AES.

Deployment and retrieval of DGT: The method defined by Sonmez & Pierzynski (2005) was followed for implementation and retrieval of DGT. To determine C-DGT, a 50-g of initial soil sample (after amendment and before sowing) was put in a plastic cup and saturated DW. On the top of the soil slurry, the surface of the DGT devices (DGT Research, Lancaster, UK) were inserted to soil without leaving any air bubbles between the solution and the DGT device. To reduce evaporation losses, the cups were loosely covered with lids and were kept at room temperature. After 24 hrs., DGT devices were recovered from cups and washed with DW to eliminate soil particles sticking to the filter membrane. In 20-ml vials containing 10 ml of 1 M HNO_3 for 24 hours, the Chelex resin gel was placed. For DGT Zn the samples were analyzed by ICP-AES.

Data analyses: For statistical analyses, SAS for Windows version 9 (SAS Inc., Cary, NC, USA) were used. Means were separated by Least Significant Difference (LSD) values. In order to find the best regression model for figures, Curveexpert version 1.3 (Install-shield Corporation, Inc.) was used.

Results and Discussion

Zn concentrations measured by $CaCl_2$ and Water extraction: Initial soil total Zn concentration was 30 mg kg^{-1}. $CaCl_2$ -extractable Zn concentrations ranged between 0.1 and 20.6 mg kg^{-1} (Table 1). Phosphorus and Zn treatments statistically affected $CaCl_2$ -extractable Zn concentrations in soils, and a significant interaction ($p \leq 0.05$) between P and Zn treatments were observed. In general, $CaCl_2$ -extractable Zn concentrations increased by elevating Zn concentrations in soils and reduced by increasing P in soils (Table 1). $CaCl_2$ extraction is thought to mimic soil solution due to its increased ionic strength compared to DW water.

Water-soluble Zn concentrations in soils were statistically influenced by P and Zn treatments (Table 1). All treatments were statistically different from the zero Zn treatments. The highest rate of P and Zn treatment (P_{200} x Zn_{1200}) had the highest water-soluble Zn concentration while the lowest was with control treatment (P_0 x Zn_0). This result was expected due to the water-soluble Zn concentration is bound to increase as Zn is added in the same form and the soil properties are same. Wang *et al*. (2008) found that addition of all three P sources (PR, CMP, and SSP) significantly reduced water soluble and exchangeable Zn in soil (11.7-75.3%).

Zinc concentration measured by DGT: Increasing total Zn concentrations elevated the C-DGT, while P did not have any effect on the C-DGT. In our study, C-DGT varied between 0.9 and 34.7 mg kg^{-1} (Table 1). These values were lower for C-DGT compared to some of the work done on this subject. Soil Zn solubility is greatly influenced by soil mineralogy and other soil properties like organic matter and CEC. The soil used in our study had high CEC and organic matter. That might be a reason for having low C-DGT values compared to other studies like Sonmez & Pierzynki (2005). Moreover, soil pH has great effect on Zn solubility. In our study, additions of Zn to soil significantly decreased soil pH. We believe this was due to $ZnSO_4$ used as a Zn source in our study.

Table 1. Selected characteristics of soil after P and Zn treatment applications.

Treatments	Bio. P (mg kg^{-1})	Extractable Zn Water-soluble (mg kg^{-1})	Extractable Zn $CaCl_2$-extractable (mg kg^{-1})	Extractable Zn C-DGT (mg kg^{-1})
P_0				
Zn_0	32.1 ± 0.9 F[a]	<0.1 ± <0.01 E	0.2 ± 0.02 I	0.9 ± 0.1 H
Zn_{150}	27.6 ± 1.7 F	0.1 ± <0.01 D	0.9 ± 0.01 GHI	1.3 ± 0.1 FGH
Zn_{300}	30.5 ± 1.3 F	0.1 ± 0.01 D	2.2 ± 0.04 F	2.7 ± 0.2 EF
Zn_{600}	24.6 ± 4.0 F	0.1 ± 0.01 D	9.3 ± 0.66 D	7.4 ± 0.9 C
Zn_{1200}	25.4 ± 1.2 F	0.3 ± 0.01 B	19.6 ± 0.81 B	31.8 ± 3.2 B
P_{100}				
Zn_0	58.7 ± 2.3 E	<0.1 ± 0.01 E	0.1 ± 0.04 I	0.9 ± 0.1 H
Zn_{150}	60.3 ± 0.9 E	0.1 ± 0.01 D	0.5 ± 0.13 HI	1.8 ± 0.1 EFGH
Zn_{300}	57.3 ± 0.2 E	0.1 ± 0.01 D	1.9 ± 0.22 FG	2.6 ± 0.4 EFG
Zn_{600}	62.6 ± 2.1 E	0.1 ± 0.01 D	8.6 ± 0.01 D	6.7 ± 0.8 D
Zn_{1200}	57.1 ± 1.0 E	0.3 ± 0.04 B	20.6 ± 2.08 A	34.7 ± 2.2 A
P_{200}				
Zn_0	90.3 ± 5.8 C	0.1 ± 0.01 D	0.1 ± 0.03 I	1.1 ± 0.1 GH
Zn_{150}	98.6 ± 1.7 B	0.1 ± 0.01 D	0.6 ± 0.09 HI	1.5 ± 0.1 FGH
Zn_{300}	94.9 ± 14.2 BC	0.1 ± 0.01 D	1.4 ± 0.05 FGH	3.2 ± 0.5 E
Zn_{600}	106.9 ± 10.6 A	0.2 ± 0.01 C	7.3 ± 0.44 E	7.7 ± 0.1 CD
Zn_{1200}	81.8 ± 3.7 D	0.4 ± 0.03 A	15.6 ± 0.37 C	33.8 ± 2.2 A

[a] Means with same letter within a column are not significantly different using least significant differences and $p \leq 0.05$

N=3 for all measurements

Zhang *et al*. (1998) reported a strong relationship between those calculated from soil solution directly and C-DGT for Zn and Cd (r^2 = 0.88 and 0.95, respectively). Hooda *et al*. (1999) also evaluated DGT technique by measuring the fluxes of soil metals (Zn, Cu, Ni, Pb, Cd), DGT can quantitatively describe the fluxes of trace metals. DGT also extracts Zn resupplied from the solid phase when the solution is depleted whereas water and $CaCl_2$ extraction do not measure it. They measure the concentrations at equilibrium with the extractant.

Correlation between soil and plant Zn concentrations: Soil $CaCl_2$ -extractable Zn increased as Zn concentrations increased and decreased as P concentrations increased. However, this reduction was not found on C-DGT results. Although C-DGT unlike plant Zn concentrations, was not influenced by P, the correlations between plant tissues (root and shoot) Zn concentrations and C-DGT were strong (Fig. 1A and 1B).

The $CaCl_2$-extractable Zn and plant Zn concentration was also correlated well (Fig. 1A and 1B) for shoots (r^2 = 0.92) and roots (r^2 = 0.90). A high correlation coefficient values (r^2) between plant Zn concentration and water-soluble Zn were also observed for shoots (0.65) and roots (0.73) (data not shown).

Both extraction methods correlated well with the C-DGT. The r^2 values were 0.97 for the $CaCl_2$-extractable Zn (Fig. 2) and 0.88 for the water-soluble Zn.

In general, the DGT concentrations of Zn were not affected by P, whereas internal plant concentrations and $CaCl_2$-extractable Zn were. In other words, the DGT did not predict the effects of P additions compared to Zn. $CaCl_2$-extraction was better correlated with plant tissue concentration compared to DGT and water-extractions. However, Nolan *et al*. (2005) stated that the DGT technique has an advantage over $CaCl_2$ extraction in that it determines soil solution metal along with parts of metal adsorbed to the solid phase. This unique property gives an advantage to DGT over batch extractions. The use of the $CaCl_2$ extraction minimizes spatial heterogeneity through the use of a homogenized soil sample.

The DGT measurement as mentioned above takes into account not only solution concentrations of Zn but also release from solid phase to solution with a constant intrinsic rate, but in our study, this rate was much higher since soil had fresh supplies of Zn. Moreover, the correlation between DGT and plant tissue concentration is generally strong when diffusive transport of the element from soil to the plant roots is rate-limiting unlike in our experiment. Furthermore, in our study, soil amended with $ZnSO_4$ at high rates, the in-situ soil solution concentrations of Zn and Ca may have been high, and saturation of the Chelex resin may have been approached with a 24-h deployment time.

As mentioned above, there could be several reasons why the DGT result was not as correlated well with plant uptake as much as the $CaCl_2$ –extraction did. In our experiment, the reason probably that the $CaCl_2$ correlation is better is that it does not extract much from the solid phase and in our situation where the plant has oversupply then the plant will not extract from solid phase although DGT will.

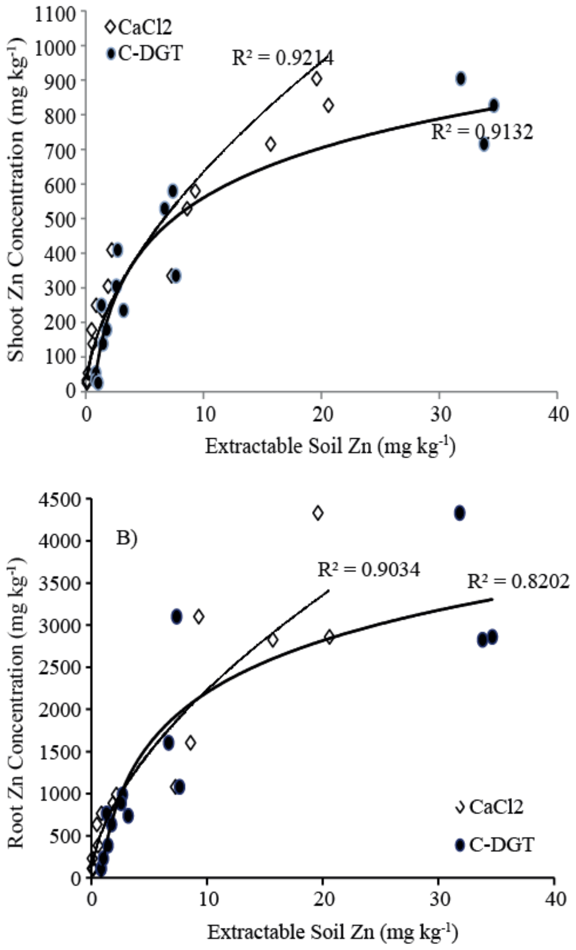

Fig. 1. Zn concentration in soil measured by DGT and soil $CaCl_2$ -extractable Zn versus, A) Zn shoot tissue concentration, the lines show the exponential association regression for C-DGT and power regression model for $CaCl_2$, B) Zn root tissue concentration, the lines show modified geometric fit for C-DGT and power regression model for $CaCl_2$. N=3 for all measurements.

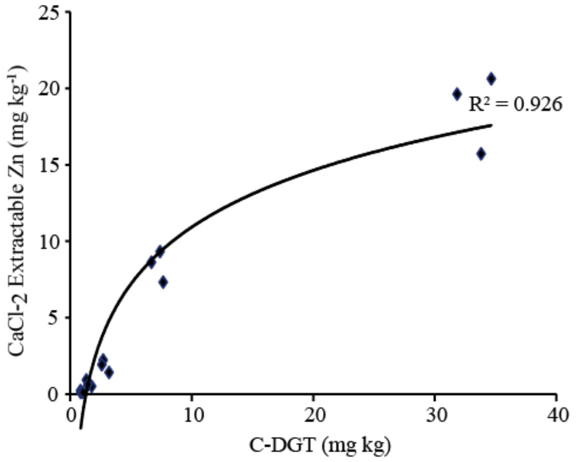

Fig. 2. Effective Zn concentration in soil measured by DGT versus soil $CaCl_2$ -extractable Zn, the line shows the modified exponential regression. N=3 for all measurements.

Conclusions

Internal plant concentrations and $CaCl_2$-extraction were influenced by P additions, whereas DGT Zn concentrations were not affected. Increasing addition of Zn to soil elevated adsorption of Zn by DGT, plant Zn concentrations, $CaCl_2$ extractable and water-soluble Zn. Soil pH was decreased by additions of Zn. In general, C-DGT, $CaCl_2$, and water-soluble extractable Zn had a strong correlation with plant Zn concentration. Since there are inconsistent results in literature, future studies are required on P-Zn interaction by DGT in soil systems.

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