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Determination of poultry manure and plant residues effects on Zn bioavailable fraction in contaminated soil via DGT technique

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Abstract

A greenhouse experiment was aimed at assessing the effects of poultry manure, sorghum, and clover residues (0 and 15 g kg^{-1}) on the zinc (Zn) bioavailable fraction in contaminated calcareous soil using two chemical assay, including diffusion gradient in thin films (DGT) and diethylene triamine pentaacetic acid-triethanolamine (DTPA-TEA), and a bioassay with corn (*Zea mase* L.). The results showed that poultry manure, clover, and sorghum residues application increased dissolved organic carbon (DOC) by 53.6 and 36.1, and 9.2%, respectively, with respect to unamended soils, as well as decreasing soil pH by 0.42, 0.26, and 0.06 units, respectively. These changes did result in increases of Zn effective concentration (C_E) and DTPA-Zn, and plant Zn concentration as a result of the increased exchangeable form of Zn. In the sorghum residues-amended soils, a reverse trend was observed for C_E-Zn compared to the DTPA method. Correlation analyses revealed that unlike C_E-Zn, DTPA-Zn had a significant positive correlation with organic fractions that can be considered as an equivalent to the fact that the DTPA method had been overestimated Zn available to the plant. The best correlations between corn metal concentrations and soil metal bioavailability were obtained for C_E-Zn using DGT technique, which also provided the best Zn bioavailability estimate. It is concluded that sorghum residues could be used to reduce the phytotoxicity risk of Zn in calcareous contaminated soil, and DTPA method is the less robust indicator of Zn bioavailability than DGT technique.

Introduction

Metals present in different concentrations in all soils, most play a major role at relatively low levels for biological life such as Zinc (Zn). Release of Zn to the soils occurs predominantly through industrial activities such as smelting and mining (Chen et al. 2009). The focus on metal contamination has been aimed mainly at preventing the accumulation of metal in the food chain and its toxic influences on plants and humans (Alloway 2013). Conversely, organic amendments have widely been used as a remediation method to reduce the metals phytotoxicity and bioavailability in contaminated soils (Palansooriya et al. 2020). Organic amendments affects the distribution of chemical forms of metals by modifying some soil properties (Ashraf et al. 2019). The use of organic matter as a soil amendment is based on the hypothesis that metal precipitation and sorption reactions induced by organic matter may result in an increase in metal retention on the solid phase, and consequently a decrease in the metal availability (Nwachukwu and Pulford 2009), while it is not always the case. Dodangeh et al. (2018) reported that the compost of municipal solid waste affected the absorption properties of heavy metals through affecting the soil mineral fractions. This study indicated that organic amendment increased soil absorption phases and reduce the metals availability. On the other hand, Aziz et al. (2017) found that the application of farmyard manure to contaminated soils can produce organo-metal complexes, which facilitate metal solubility and mobility. The organic amendment efficacy at reducing the heavy metals bioavailability is mostly dependent on the type of organic amendment and metal, as well as the potential measures of heavy metals.

In principle, measurement of total metal concentration is not generally considered to be an adequate estimate for metal available fraction (McLaughlin et al., 2000). In contrast, some researches have shown that chemical extractants such as buffer salts (NH_4OAc), mineral acids (HCl, HNO_3), and chelating agents (EDTA, DTPA-TEA) may be suitable for the estimation of exchangeable metal fraction (Meers et al. 2007). These extraction methods may provide an insight into the geochemical fractions of metals in the soil. However, these methods do not consider the soil ability to sustain the metals concentration in soil solution following the metals depletion by plant uptake (Zhang et al. 2001).

The diffusive gradient in thin-film technique (DGT) was initially developed by Zhang and Davison (1995), to measure in *situ* heavy metal concentration in soils, sediments, and aqueous solutions (Li et al. 2019). This technique relies on the solutes accumulation onto the cation exchange resin after passing through the diffusion hydrogel layer (Zhang et al. 1998). Since the mobility of heavy metals in the soil highly depends on its resupply from the solid phase of the soil and their concentration in the soil solution (Nolan et al. 2005), if the resupply and metals availability from the solid phase are kinetically limited or is not fast enough to occur, uptake by plant is reduced. DGT method is based on kinetic principles and can reflect the process of resupply of heavy metals from the soil and the relationship between plant roots and soil. kinetic information of heavy metal resupply process in soil can also be obtained using DGT induced fluxes (DIFS) model (Harper et al 2000). A high correlation between the elements collected by DGT and their bioavailability in plants has been concluded by various researchers (Degryse et al. 2009; Li et al. 2016). However, despite the growing use of this method to evaluate metal availability in soil, insufficient rigorous work has established the organic amendments effects on the validity of assay for metal bioavailability. Therefore, this experiment was used to determine the organic residues effects on the validity of metal bioavailability assays in contaminated calcareous soils. The overall purpose of this study was to evaluate measures of bioavailable Zn across contrasting presence of organic residues in corn -planted soil by DTPA and DGT methods.

Materials And Methods

Soil sampling and analysis

In this study, samples of contaminated soil (0-30 cm) were collected from zinc mine at 6 km in Zanjan province, Iran. After sampling, soil samples were air-dried and sieved through a 2 mm sieve. Soil chemical and physical analyses were done by a soil testing method, including total Zn concentration by acid digestion (HNO_3) (Sposito et al. 1982), Zn available concentration by DTPA-TEA solution (Lindsay and Norvell 1978), soil texture by hydrometer (Gee and Bauder 1986), Calcium carbonate equivalent (CCE) by titration (Loeppert and Suarez 1996), exchange capacity using a methodology described by Chapman (1965), organic carbon content by the Walkley-Black method (Nelson and Sommers 1996), pH and EC by pH meter (Mettler Toledo, U.S.A.) and EC meter (4010 conductivity meter, Jenway Inc, England), respectively, in a 1: 5 soil-to-water ratio (Thomas 1996; Rhoades et al. 1989).

Chemical analysis of organic amendments

Poultry manner and plant residues (sorghum and white clover) were collected from a poultry farmer at the University of Tabriz, Tabriz, Iran and Agricultural Research, Education and Extension Organization, Karaj, Iran respectively. The organic amendments were ground and sieved using a 2 mm sieve. The sieved samples were then characterized for total Zn by wet digestion (Chen et al. 2001), EC and pH using a 1:5 sewage sludge to water ratio (Thomas 1996; Rhoades 1996), dissolved organic carbon (DOC) using total organic carbon analyzer (TOC-VCPH, Shimadzu) (Mohseni et al. 2018), organic carbon content by dichromate oxidation (Nelson and Sommers 1996), and total N by the Kjeldahl method (Bremner and Mulvaney 1982).

Greenhouse experiment

To investigate the effect of organic amendments on Zn bioavailability, a factorial experiment was conducted in a completely randomized block design with three replications. Treatments including: contaminated calcareous soil, two levels of poultry manure, clover, and sorghum residues (0 and 15 g kg^{-1}). Organic amendments were mixed thoroughly in pot soil (three kg), wetted to field moisture capacity, and incubated for 90 days at a constant temperature (25°C ± 2). After incubating, six seeds of corn (*Zea mase* L.), cultivar 704, were planted into each pot (except unamended soils). After a week, seedlings were thinned to three plants per pot. Soil moisture was maintained at about field capacity, and pots irrigated every two days during the growing season according to the control pot. All required nutrients except micronutrients were applied according to soil exam and conventional fertilizer recommendations. The plants were grown under 14 hours light /10 hours dark at temperature of 28°C ± 2. After eight weeks, the shoots and roots were harvested from the soil, eluted with deionized water, and dried at 80 °C ± 5 to constant weight. Then, the samples were milled and digested by wet oxidation (Lozano-Rodríguez et al. 1995).

After harvest, two potential measures of Zn bioavailability, including DTPA-TEA extractant (the solution consisting of 0.005 M diethylenetriaminepentaacetic acid, 0.1 M triethanolamine, and 0.01 M $CaCl_2$ at pH 7.3, Sigma-Aldrich, UK) and DGT technique were used in the soil subsamples from each pot. Concentration of DOC and pH value were also measured according to the above-mentioned methods in the section of chemical analysis of organic amendments. In addition, sequential extraction procedure described by Tessier et al. (1979) was used to determine the chemical distribution of Zn. This method separated Zn chemical forms into the following five fractions: exchangeable Zn (EX-Zn), Zn-binding to carbonates (CAR-Zn), organically bound Zn (OR-Zn), Zn-binding to oxides (OX-Zn), and residual Zn (RES-Zn) (Table 1).

Table 1 sequential extraction procedure used in this study

Step	Chemical form of Zn	Extraction method
1	Exchangeable (EX-Zn)	8 ml of 1 M $MgCl_2$ was added to 1 g soil and then shaken for two h
2	Carbonate-bound (CAR-Zn)	8 ml of 1 M NaOAc was added to the sediment and shaken for five h
3	oxide-bound (OX-Zn)	20 ml of NH2OH·HCl, 0.04 M was added to 25% w/v HOAc, the solution was mixed with sediment and then shaken for 0.5 h at 50 °C in a water bath.
4	Organically bound (OR-Zn)	5 ml of 30% m/v H2O2 + 3 ml of 0.02 M HNO3 was mixed with the sediment and shaken for one h in boiling water bath. 3 ml of 30% m/v H2O2 was added to the mixture and shaken for three h at 85 °C. 5 ml of 3.2M NH4OAc + 20 mM deionized water was added to the mixture and shaken for three min.
5	Residual (RES-Zn)	The sediment was mixed with 20 ml HCl:HNO3 (3:1 v/v), incubated for 16 h at 25 °C, and then heated to 130 °C by a hot plate.

Note: All the chemicals were purchased from Sigma-Aldrich, UK.

DGT technique

The assembled DGT devices consisted of a polyethylene backing plate and a polyethylene cap were cast in the laboratory of Agricultural Research, Education and Extension Organization, Karaj, Iran according to several standard DGTs provided from DGT Research Ltd, Lancaster, UK. A chelating gel layer (0.4 mm thick), a diffusive gel layer (0.85 mm thick) and a nitrate membrane filter (0.45-µm pore size, 100-µm-thick, Sigma-Aldrich, UK) were placed on top of the polyethylene backing plate, respectively, and held in place by the polyethylene cap (Heidari et al. 2016).

Gel preparation

The chemicals used to prepare diffusive and chelex resin gels, including ammonium persulfate, agarose, acrylamide, allylglycidyl ether, sodium hydroxide (NaOH) sodium borohydride, methanol were purchased from Merck Millipore (Darmstadt, Germany), and N,N,N,N-Tetramethylethylenediamine (TEMED), and chelex 100 sodium form were provided by Sigma-Aldrich, UK.

Diffusive and chelex resin gels were made from a gel preparation solution, which consists of a 15% w/v acrylamide mixed with a 0.3% w/v allyl agarose cross-linker solution (33 mg sodium borohydride, 33 mL of 0.3M NaOH solution, and 1.6 mL allylglycidyl ether were mixed with l g agarose, shaken for 12 hours, dehydrated with methanol, and then dried in an oven set at 35°C (Heidari et al. 2016).

For the diffusive gels 70 µl ammonium persulphate solution (10% w/v) was added to 10 ml of gel preparation solution, followed by 20 µl TEMED catalyst. The prepared diffusive gel solution was poured into glass plates held 0.85 mm apart and then placed in an oven set at 45° C for 1 hour. Set diffusive gel was removed and then hydrated in deionized water for 24 hours (Heidari et al. 2016).

For the chelating resin gel 0.2 g of hydrated resin was added to 1ml of prepared gel solution and mixed with 6 µl ammonium persulphate solution (10% w/v), followed by 6 µl TEMED. The mixture was poured into glass plates held 0.4 mm apart and then placed in an oven set at 45° C for 1 hour. Set chelating gels were then removed and hydrated in deionized water for 24 hours (Davison and Zhang 2012).

DGT placement in the soil subsamples

The assembled DGT devices were left in the 50 g of the saturated soil subsamples of each pot after plant harvesting for 24 hours at a temperature of 25°C ± 2 (Hooda et al. 1999). Upon retrieval, the chelating gel layer was removed from the assembled DGT device and Zn extracted into 5 ml of 1M HNO_3 prior to analysis using ICP-OES (PerkinElmer-Optima 2100 DV, USA). The ICP-OES detection limit for Zn was 0.2 µg L^{-1}.

The DGT technique relies on the accumulation of elements onto the binding layer after passing through the diffusion gel layer. The binding resin acts as a localized sink for the Zn thus causing a depletion of Zn at the diffusive layer-soil interface and a re-supply from the labile metal pool on the solid phase, thereby inducing a diffusional flux to the DGT device from the solution.

The concentration of Zn at the soil-diffusive layer interface (C_{DGT}) which can be related to the accumulation of Zn onto the binding layer was calculated using following equation (Eq.1) (Sochaczewski et al. 2007).

See formula 1 in the supplementary files.

Where M (mg), Δg (mm), D, A, and t (s) are accumulated masses of metals in the resin membrane, diffusion layer thickness, diffusion coefficients of Zn in the diffusion layer (6.13×10 cm^2 s^{-1}), the effective area of the gel exposed to the soil (3.14 cm^2), and deployment time in the soil, respectively.

The mass of Zn (M) accumulated by the resin gel was calculated using following equation (Eq.2)

See formula 2 in the supplementary files.

Where C_e, V_{acid}, and V_{gel} are Zn concentration in the 1M HNO3, Volume of nitric acid (5 ml) (V_{acid}), and resin gel volume (V_{gel}) , respectively (Zhao et al. 2006).

The C_{DGT} of Zn was then converted to the effective concentration (C_E) using Equation 3. The effective concentration (C_E) is related to Zn concentration in the soil solution and Zn re-supply from the labile Zn pool on the solid phase which was completely explained by Zhang et al. (2001).

See formula 3 in the supplementary files.

Where R_{diff} was calculated using a two-dimensional numerical model of the soil-DGT system (2D-DIFS) (Harper et al, 2000). The value of R_{diff} represents the ratio of the mean interfacial concentration to the bulk solution concentration. The input parameters of DIFS software to calculate R_{diff} relies on the assumption that the movement of elements towards DGT is only based on the ion diffusion, T_C (soil response time) and K_d (distribution ratio) were determined at the maximum (10^{10} s) and the minimum value (10^{-10} gr cm^{-3}), respectively.

Statistical analyses

Comparisons of the plant shoot and root Zn concentrations, soil chemistry, Zn chemical fractions, and assays of Zn bioavailability between amended and unamended soils were analyzed using LSD tests in SAS 9.1 software. Linear regression models for determining the relationships between assays of Zn bioavailability and plant Zn concentrations were also performed in SAS 9.1 software.

Results

The soil characteristics results showed that the total concentration of Zn in the soil was in range of contaminated soil according to critical range presented by Kabata-Pendias and Pendias (1984), and the pH value of soil was also in the alkaline range (Table 2). The findings of organic amendments characteristics used in this study revealed that the highest of Zn concentration was in poultry manure, clover, and sorghum residues, respectively. In respect of carbon to nitrogen ratio (C:N), sorghum residues and poultry manure showed the highest and the lowest values, respectively (Table 2).

Table 2 Physicochemical characteristics of the studied soil and organic amendments.

Property	Soil	Poultry manure	Sorghum residues	Clover residues
Total Zn (mg kg^{-1})	976.45	32.97	8.78	10.97
DTPA-Zn (mg kg^{-1})	203.43	-	-	-
EC (dS m^{-1})	0.80	11.54	7.17	5.48
pH	7.77	7.43	7.36	7.25
Organic carbon (g kg^{-1})	8.43	256.96	484.17	391.56
DOC (mg l^{-1})	20.53	33.45	24.69	31.36
Texture	Clay loam	-	-	-
CEC ($cmol_c$ kg^{1})	16.58	-	-	-
Total N (g kg^{-1})	-	23.64	16.21	32.46
C/N	-	10.87	29.86	12.06

Soil characteristic after plant harvesting

As shown in Table 3, the application of poultry manure and plant residues significantly increased the DTPA-Zn concentration compared to the unamended soils. The concentration of DTPA-Zn in the poultry manure, clover, and sorghum residues-amended soils increased by 14.9, 11.2, and 6.1% with respect to the unamended soils, respectively. Moreover, the addition of poultry manure and clover residue significantly increased, and sorghum residues decreased the effective concentration of Zn (C_E-Zn) measured by DGT method. C_E-Zn increased by 8.7% and 3.6% in poultry manure and clover residual-amended soils with respect to the unamended soils, respectively. In contrast, a 29.5% reduction in C_E-Zn was recorded in residual sorghum-amended soil. The results indicated that the presence of organic amendments significantly increased the concentration of DOC compared to unamended soils. The DOC concentration in the poultry manure, clover, and sorghum residues-amended soils were increased by 53.6 and 36.1, and 9.2% in comparison to unamended soils, respectively. According to table 3, the lowest value of pH was observed in soil amended with poultry manure, followed by clover and sorghum residues-amended soil.

Table 3 Chemical characteristics of amended and unamended soils with organic amendments.

Treatment	DTPA- Zn (mg kg^{-1})	C_E -Zn (µg L^{-1})	DOC (mg l^{-1})	pH
Control (unamended)	212.35 ± 5.05^{d}	1187.24 ± 11.70^{c}	26.85 ± 1.22^{d}	7.64 ± 0.02^{a}
Poultry manure-amended soils	244.17 ± 4.77^{a}	1285.20 ± 9.01^{a}	41.23 ± 4.52^{a}	7.22 ± 0.03^{c}
Sorghum residues-amended soils	225.21 ± 1.79^{c}	837.33 ± 7.36^{d}	29.34 ± 2.65^{c}	7.58 ± 0.04^{a}
Clover residues-amended soils	236.21 ± 1.01^{b}	1229.74 ± 12.81^{b}	36.52 ± 3.41^{b}	7.38 ± 0.02^{b}

Note: Results are means ± standard deviations (n = 3). Values with the different lower-case letters within each column are significantly different at $p < 0.05$ according to LSD test.

Distribution of Zn chemical forms in soil

Table 4 presented the effect of organic amendments on the distribution of Zn chemical forms in soil. The results showed that the application of organic amendments significantly ($p \leq 0.05$) caused a change in Zn concentration in the exchangeable, carbonate, oxide, and organic fraction compared to the unamended soils. Meanwhile, there were no differences in the residual form of Zn in the organic-amended soils. The results exhibited that poultry manure and clover residue increased the exchangeable form of Zn by 37.7 and 8.9%, and sorghum residue reduced the exchangeable form of Zn by 25% compared to unamended soils, respectively. In the carbonate form, a considerable 8.6, 3.2, and 6.3% reduction in Zn concentration was recorded in soils amended with poultry manure, sorghum residues, and clover residue in comparison to the unamended soil, respectively.

The effect of organic amendments on the oxide form of Zn was also statistically significant ($p \leq 0.05$). The highest and lowest decreases were related to poultry manure (6.39%) and sorghum residue-amended soils (1.13%) compared to the unamended soils, respectively. Besides, results suggested a significant increase in Zn concentration in the organic fraction of amended soils compared to unamended soils. Overall, soils amended with poultry manure, sorghum residue, and clover residue showed a 23.6, 60.7, and 39.7% increase in the organic fraction of Zn with respect to unamended soil, respectively.

Table 4 Chemical fractions of Zn in amended and unamended soils with organic amendments.

Treatment	EX	CAR	OX	OR	RES
	(mg kg^{-1})				
Control (unamended)	64.62±1.62^{c}	195.66±3.85^{a}	282.24±3.01^{a}	49.31±1.62^{d}	386.72±3.36^{a}
Poultry manure-amended soils	88.98±3.35^{a}	178.66±2.20^{d}	264.20±0.95^{c}	60.97±3.39^{c}	389.14±3.05^{a}
Sorghum residues-amended soils	48.35±1.45^{d}	189.74±1.86^{b}	279.03±4.03^{a}	79.27±2.41^{a}	384.22±5.99^{a}
Clover residues-amended soils	70.38±4.44^{b}	183.41±2.84^{c}	269.36±1.93^{b}	68.89±3.53^{b}	385.03±4.95^{a}

Note: Results are means ± standard deviations (n = 3). Values with the different lower-case letters within each column are significantly different at $p < 0.05$ according to LSD test.

Plant Zn concentration and yield

As shown in Table 5, the addition of organic amendments did result in significant changes in the corn Zn concentration, shoot Zn content (uptake), and the plant dry weight (p0.05). The lowest plant Zn concentration and uptake were observed in sorghum residue-amended soils, followed by unamended soil, clover residue-amended soil, and poultry manure-amended soil. Overall, shoot and root Zn concentration, and shoot Zn uptake were decreased in the plants grown in soils amended with sorghum residue by 36.5, 21.6, and 8.5% in comparison to unamended soils, respectively. Conversely, shoot and root Zn concentration, and shoot Zn uptake were increased by 19.9, 4.9, and 33.8% in plants grown in poultry manure-amended soil, and 11.6, 2.1, and 28.9% in the plants grown in clover residue-amended soils with respect to unamended soils, respectively. Moreover, the presence of organic amendments resulted in a 43.9 and 40.8% increase in plant shoot and root dry weight grown in sorghum residue-amended soil, and 15.5 and 16% increase in plant shoot and root dry weight grown in clover residue-amended soil compared to unamended soils, respectively. In the case of poultry manure-amended soil, a significant increase was only observed in shoot dry weight (13.4%).

Table 5 Mean of dry matter yield, Zn uptake, and Zn concentration in corn grown in amended and unamended soils with organic amendments.

Treatment	Zn-Shoot (mg kg^{-1})	Zn-Root (mg kg^{-1})	Zn-Shoot uptake (μg pot^{-1})	Shoot dry weight	Root dry weight
Control (unamended)	205.46±4.01^{c}	356.14±3.66^{c}	2806.70±34.45^{c}	13.65±1.01^{c}	3.57±0.35^{c}
Poultry manure-amended soils	246.49±2.74^{a}	373.84±4.32^{a}	3757.94±19.16^{a}	15.48±1.08^{b}	3.91±0.44^{bc}
Sorghum residues-amended soils	130.34±2.52^{d}	278.96±4.70^{d}	2567.38±36.18^{d}	19.65±2.11^{a}	5.03±0.41^{a}
Clover residues-amended soils	229.35±2.73^{b}	363.84±4.15^{b}	3619.14±10.07^{b}	15.77±1.03^{b}	4.25±0.32^{b}

Note: Results are means ± standard deviations (n = 3). Values with the different lower-case letters within each column are significantly different at $p < 0.05$ according to LSD test.

Correlation between chemical forms of Zn and soil assays of Zn bioavailability

The correlation between different chemical forms of Zn in amended soils and Zn concentration measured by DTPA and DGT methods represented in table 6. In all amended soils, the organic and carbonate forms of Zn had the highest correlation with DTPA-extractable Zn with respect to C_E-Zn. While, C_E-Zn gave the higher correlation with the exchangeable and oxide forms rather than DTPA-extractable Zn. On the other hand, except for DTPA-Zn, which showed a positively significant correlation with organic fraction, C_E-Zn revealed a negatively significant correlation with organic forms of Zn. Furthermore, there were no significant correlations between the residual forms of Zn and soil extraction methods in amended soils.

Table 6 Correlations (Pearson coefficients) between Zn fractions in amended soils with organic amendments and two measures of Zn availability.

Fraction	Poultry manure		Sorghum residues		Clover residues	
	DTPA-Zn	C_E-Zn	DTPA-Zn	C_E-Zn	DTPA-Zn	C_E-Zn
EX	0.78*	0.85**	0.81**	0.89**	0.76*	0.86**
CAR	-0.81**	-0.69^{ns}	-0.78*	-0.71*	-0.78*	-0.72*
OX	-0.79*	-0.81**	-0.76*	-0.84**	-0.60^{ns}	-0.77*
OR	0.89**	-0.78*	0.86**	-0.77*	0.86**	-0.73*
RES	0.58^{ns}	0.51^{ns}	0.44^{ns}	0.59^{ns}	0.41^{ns}	0.43^{ns}

Note: * and ** p-values were significant at 0.01 and 0.05 levels, respectively. ns: Not significant.

Estimations of Zn availability

The relationship between Zn concentration in the amended and unamended soils, and the metal concentration in corn tissues was investigated by linear correlations (Figure 1). Relationships determined between pooled Zn concentrations of shoot and two measures of Zn availability taken from pooled soil results were significant ($P < 0.05$). Comparisons of the determination coefficients for tow assays of Zn bioavailability and plant Zn concentrations and uptakes, revealed that C_E-Zn gave the most robust relationship for plant and soil data overall, closely followed by DTPA extractable Zn.

Discussion

The addition of poultry manure, sorghum, and clover residues significantly increased the concentration of DOC in soil solution as well as decreasing the amount of soil pH. Microbial degradation of soil organic amendments and root exudates were the likely factor that resulted in these differences. Organic amendments and root exudates provide microorganisms with a valuable source of carbon and nitrogen, which results in considerable microbial activity (Khoshgoftarmanesh et al. 2018). Amending soils with poultry manure and plant residues provide additional DOC to the soil solution. The presence of DOC, particularly humic acids, may be the main factor contributing to the increased C_E-Zn and DTPA-extractable Zn (except for C_E-Zn in sorghum residues- amended soil) through decreased pH and formation soluble organo-Zn. Increases of metal bioavailability as a result of decreased pH can occur in different ways, including the acidic groups contained in DOC such as hydroxyl functional groups and carboxyl (Adeleke et al. 2017), and the production of CO_2 from root respiration and microbial activity, thereby increasing exchangeable metal form (Yao et al. 2020). The increase in exchangeable form of Zn may be linked to decreased oxide fractions of Zn which is likely due to the excretion of Zn^{2+}/OH^- to balance the internal charge variation as a result of increased H^+ in the soil solution. Another possible cause for increased Zn exchangeable form, is the presence of decreased amounts of carbonate form due to decreased pH (Alloway 2013). Montalvo et al. (2016) found that the increased concentration of DTPA-Zn in calcareous soil was related to the dissolution of zinc carbonate minerals ($ZnCO_3$) as a result of decreased pH in soil solution. Egene et al. (2018) observed that municipal waste compost increased C_E-Zn, which may be attributed to the significant decrease in soil pH.

The effects of these parameters present in organic amendments on C_E-Zn and DTPA-Zn extractability subsequently influenced the increase of corn Zn concentration. While dry matter biomass was not strongly influenced by increased Zn concentration, which in turn was likely affected by the presence of enhanced soil physical condition and increased concentration of macronutrients provided by organic amendments.

Another factor that may have affected the increased dry matter production, is likely the formation of phytochelatin-Zn complexes into plant cells as the primary mechanism contributing to the decrease in metals mobility in plant tissues and several studies have linked the mobility of heavy metals in plants' tissues to the synthesis of non-structural carbohydrates and phytoclates in the plants tissues (Chand et al. 2012; Sripriya et al. 2016).

Moreover, unlike poultry manure and clover residues-amended soils, the addition of sorghum residues resulted in a decrease in plant Zn concentration, which may be attributed to the significant decrease in C_E-Zn in the soil as a result of the conversion of exchangeable fraction into an organic fraction with lower mobility. Garrido Reyeset et al. (2013) found that decreased C_E-Cu in biosolids-amended soil was related to an increase in the organic fraction of Cu. The use of organic matter in the soil plays a key role on the absorption of heavy metals. Molecular-scale spectroscopic studies have shown that these metals form strong bonds with functional groups of organic matter such as carboxylic, phenolic, and thiols (Degryse et al. 2009). Therefore, an increased organic fraction of Zn in sorghum residues-amended soil may directly be attributed to the higher carbon to nitrogen ratio (C:N) value of sorghum residue than poultry manure and clover residues. Conversely, despite the reduction of the exchangeable form of Zn, the concentration of DTPA-Zn in the sorghum residues-amended soil increased with respect to C_E-Zn.

The reason that chelating agents such as DTPA-TEA might not be a good predictor of Zn bioavailability is that not all chemical fractions of metals are thought to be available for plants, and it has been revealed that free ion and labile complexes of metals are available for uptake, while a large amount of Zn organic form extracted by this extractant was relatively unavailable for plant and it appears that this extractant overestimated the phytoavailability (Soriano-Disla et al. 2010). The positive (DTPA-Zn) and negative (C_E-Zn) significant correlation obtained between organic fraction and two potential measures of Zn bioavailability partly confirmed that DTPA might be extracted some of the Zn organic forms which was not available for the plant. However, in poultry manure and clover residues-amended soils, due to the increase in Zn exchangeable forms, it was impossible to distinguish whether the increased DTPA-extractable Zn was related to the exchangeable fraction or the organic fraction.

Although DTPA extractant has been successfully used to measure deficiencies of metals in soils, the use of this method to assess phytotoxic concentrations of metals have been less so, with plant responses typically poorly correlated with DPTA (Karami et al. 2009; Zhang et al. 2010) which may be attributed to lack of metals concentration measurement partitioned to the solid phase. While, in the case of DGT method, the replenishment capacity of the solid phase is considered as a fundamental variable that resupply the amount depleted in soil solution (Mason et al. 2010).

Analytically quantifying the potential measure of metals bioavailability by DGT technique has only been attempted in a few studies, but in general, results from these predictions have not included calcareous soils treated with the organic amendments. A study by Soriano-Disla et al. (2010) found that C_E-Zn had the stronger correlation with sorghum root Zn concentration grown in sewage sludge-treated soils (r^2 = 0.91^{**}) in comparison to DTPA- extractable Zn ($r^2 = 0.89^{**}$). Another successful relationship between plant Zn concentration and C_E-Zn ($r^2 = 0.76^{**}$) was also obtained in peppermint grown in sewage sludge-treated soils, but a less robust relationship was found with Zn concentrations in the shoot and DTPA-Zn (r^2 = 0.62**) (Mohseni et al. 2020).

Conclusions

This study showed that the organic amendment efficacy at reducing the bioavailability of the metal was mostly dependent on the type of organic amendment and the potential measures of metal bioavailability for plants. Unlike poultry manure and clover residues, sorghum residues significantly accounted for the most reduction in Zn bioavailability as a result of higher carbon to nitrogen ratio. DTPA method was poorly correlated with plant metal concentrations, which may be associated with the extraction of the unavailable form of Zn, especially Zn organic form. In contrast, the DGT technique had strong potential as a measure of Zn bioavailability in amended soils. Thus the theory that DGT technique imitates important processes involved in plant metal uptake makes it more reliable for estimating the bioavailability of Zn.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable

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Figures

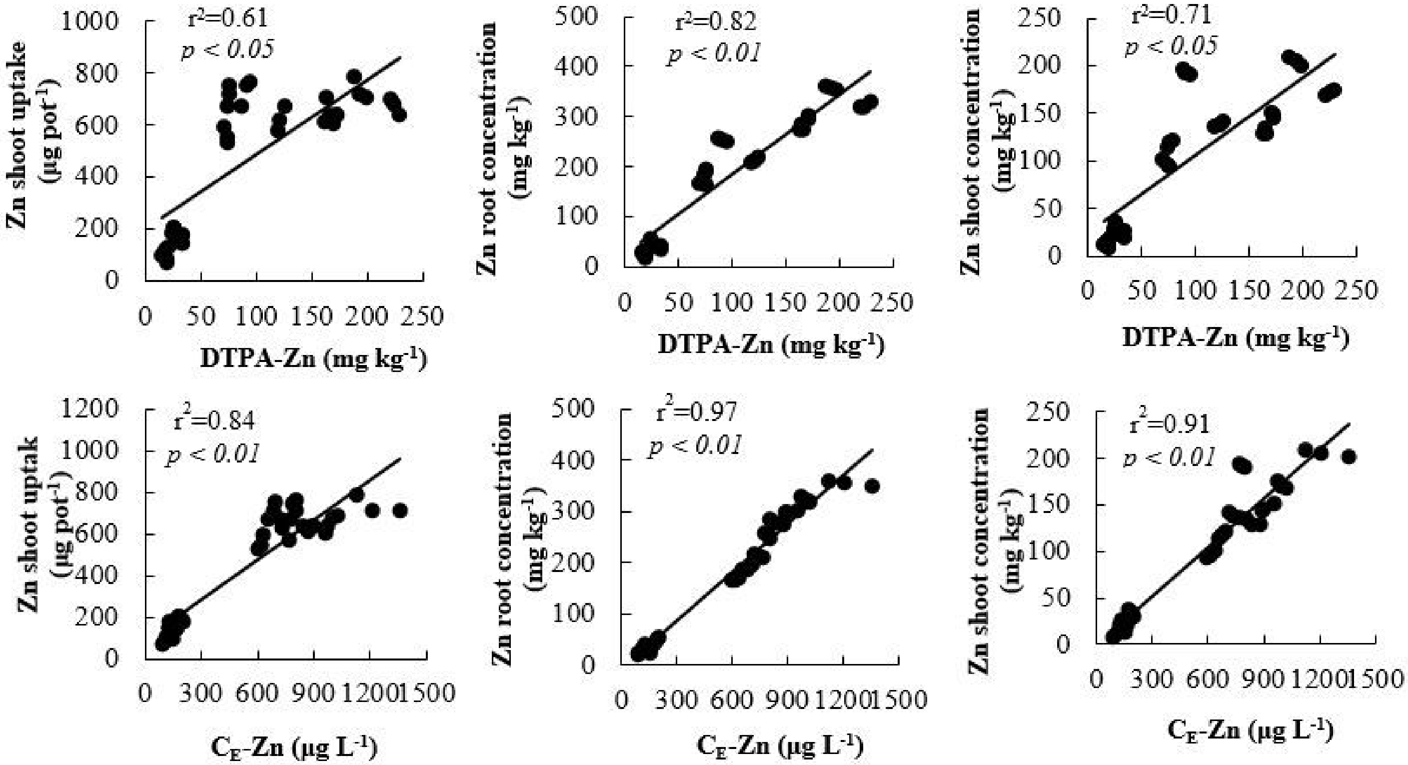

Figure 1

Relationships determined between Zn concentration in plant tissues grown in amended and unamended soils, and tow assays of Zn bioavailability (DTPA-TEA and DGT)

Supplementary Files

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