Determination of Poultry Manure and Plant Residues Effects on Zn Bioavailable Fraction in Contaminated Soil Via DGT Technique

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

**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, diffusion gradient in thin films (DGT) and 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 positive correlation significantly with organic fractions that can be considered as an equivalent to the fact that the DTPA method had been overestimated Zn available to plants. The best correlations between corn metal concentrations and soil metal bioavailability were 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.

**Keywords:** Chemical fraction, Clover residues, Sorghum residues, Organic amendment, Metal

**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 affect 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 (NH₄OAc), mineral acids (HCl, HNO₃), 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 (HNO₃) (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).

Organic amendment analysis

Organic amendments exerted in this study include poultry manure, sorghum, and white clover residues which 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 the methodology described by 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 experimental**

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 (DTPA-TEA extractant and DGT technique) were used in each treatment. Concentration of DOC and pH value were also measured according to the above-mentioned methods in the soil analysis section. 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 MgCl₂ 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. |

DGT technique

DGTs were prepared by Heidari et al. (2016) and evaluated for measuring the bioavailability of heavy metal using Mohseni et al. (2020). After each application, the DGTs were removed then separated and eluted with deionized water. After that, resin gel was digested with HNO₃ (2 M) for 24 hours and shaken about eight hours. Finally, the concentrations of Zn in acid were measured. When DGT is placed below the soil surface, ions (Zn) move from the diffusion layer and immobilized in the resin layer. As a result, a steady-state gradient linear concentration created in the diffusion layer. The ion flux (F) in the diffusion layer towards resin layer was determined according to Fick's first law (Eq1). As the settling time increases, the ions in the soil solution gradually deplete at the diffusive layer-soil interface, which induces the resupply of Zn from the solid phase of the soil. After the deployed time of DGT in the soil sampling, the resin layer retrieved following 24-h exposure and its total mass can be measured. The ion flux can also be explained according to Equation 2. Finally, the average ion concentration in the contact area between the soil and DGT, which
is the amount of ions absorbed by the resin layer, can be calculated according to Equation 3 (Sochaczewski et al. 2007).

\[ F(x, t) = \phi_d D_d \frac{C_a(x, t)}{\Delta g}, \quad 0 < t < T, \quad x \in (-r, r) \]  

(1)

\[ M = \phi_d D_d \int_{-r}^{r} \int_{0}^{T} C_a(x, t) \, dt \, dx \]  

(2)

\[ C_{DGT} = \frac{\int_{-r}^{r} \int_{0}^{T} C_a(x, t) \, dt \, dx}{T} = \frac{M \Delta g}{\phi_d D_d T} \]  

(3)

Where \( M \) (mg), \( \Delta g \), \( \phi_d \), \( D_d \), \( A \), and \( t \) (s) are accumulated masses of metals in the resin membrane, diffusion layer thickness, gel porosity in the diffusion layer, diffusion coefficients of Zn in the diffusion layer, the effective area of the gel which exposure with soil (3.14 cm\(^2\)), and deployment time in the soil, respectively.

The ion mass in the resin layer gel was calculated using the equation \( M = C_e (V_{gel} + V_{acid}) \). Where \( M \) calculated by the concentration of elements measured by ICP-OES after eluted with 2 M nitric acid (\( C_e \)), Volume of nitric acid (five ml) (\( V_{acid} \)) and resin gel volume (\( V_{gel} \)) (Zhao et al. 2006).

To further explanation the DGT measurements, the concentration of elements directly calculated by the DGT (\( C_{DGT} \)) was converted to the effective concentration (\( C_e \)) using Equation 4. Effective concentration (\( C_e \)) is completely explained by Zhang et al. (Zhang et al. 2001).

\[ C_e = \frac{C_{DGT}}{R_{diff}} \]  

(4)

Where \( R_{diff} \) is depleted concentration at the diffusive layer-soil interface. A two-dimensional numerical model of the soil-DGT system called 2D-DIFS was used to calculate \( R_{diff} \). Moreover, since 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. In this case, \( R_{diff} \) depends only on water holding capacity (WHC) of soils. Resultantly, soils with high water holding capacity are higher \( R_{diff} \) than soils with lower water holding capacity (Sochaczewski et al. 2007).

**Statistical analyses**
Comparisons of treatments data were statistically analyzed based on ANOVA with SAS 9.1 statistical software. Significance differences between the treatments were also determined according to LSD’s test (p < 0.05).

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, 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        | 1187.24±11.70    | 26.85±1.22   | 7.64±0.02 |
| Poultry manure-amended soils  | 244.17±4.77        | 1285.20±9.01     | 41.23±4.52   | 7.22±0.03 |
| Sorghum residues-amended soils| 225.21±1.79        | 837.33±7.36      | 29.34±2.65   | 7.58±0.04 |
| Clover residues-amended soils | 236.21±1.01        | 1229.74±12.81    | 36.52±3.41   | 7.38±0.02 |

Note: Results are means ± standard deviations. 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 ($p<0.05$) 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<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 ± SD | CAR ± SD | OX ± SD | OR ± SD | RES ± SD |
|------------------------------------------|---------|----------|---------|---------|----------|
| Control (unamended)                      | 64.62±1.62c | 195.66±3.85a | 282.24±3.01a | 49.31±1.62d | 386.72±3.36a |
| Poultry manure-amended soils             | 88.98±3.35a | 178.66±2.20d | 264.20±0.95c | 60.97±3.39c | 389.14±3.05a |
| Sorghum residues-amended soils           | 48.35±1.45d | 189.74±1.86b | 279.03±4.03a | 79.27±2.41a | 384.22±5.99a |
| Clover residues-amended soils            | 70.38±4.44b | 183.41±2.84c | 269.36±1.93b | 68.89±3.53b | 385.03±4.95a |

Note: Results are means ± standard deviations. 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 (p<0.05). 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 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±4.01c       | 356.14±3.66c      | 2806.70±34.45c            | 13.65±1.01c      | 3.57±0.35c      |
| Poultry manure-amended soils | 246.49±2.74a       | 373.84±4.32a      | 3757.94±19.16a            | 15.48±1.08b      | 3.91±0.44bc     |
| Sorghum residues-amended soils | 130.34±2.52d       | 278.96±4.70d      | 2567.38±36.18d            | 19.65±2.11a      | 5.03±0.41a      |
| Clover residues-amended soils | 229.35±2.73b       | 363.84±4.15b      | 3619.14±10.07b            | 15.77±1.03b      | 4.25±0.32b      |

Note: Results are means ± standard deviations. 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 extraction methods**

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 | CE-Zn | DTPA-Zn | CE-Zn | DTPA-Zn | CE-Zn |
| EX       | 0.78** | 0.85** | 0.81** | 0.89** | 0.76* | 0.86** |
| CAR      | -0.81** | -0.69** | -0.78* | -0.71* | -0.78* | -0.72* |
| OX       | -0.79* | -0.81** | -0.76* | -0.84** | -0.60** | -0.77* |
| OR       | 0.89** | -0.78* | 0.86** | -0.77* | 0.86** | -0.73* |
| RES      | 0.58** | 0.51** | 0.44** | 0.59** | 0.41** | 0.43** |

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 soil and different plant 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 two assays of 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, decrease pH, 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 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 the correlation for
CE-Zn and sorghum root Zn concentration grown in was higher ($r^2 = 0.91^{**}$), than DTPA-Zn concentration ($r^2 = 0.89^{**}$). Another successful relationship between Zn concentration of the shoot and CE-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 soil extractable Zn.