

Comparison of *in situ* DGT measurement with *ex situ* methods for predicting cadmium bioavailability in soils with combined pollution to biotas

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ABSTRACT

To assess the capabilities of the different techniques in predicting Cadmium (Cd) bioavailability in Cd-contaminated soils with the addition of Zn, one *in situ* technique (diffusive gradients in thin films; DGT) was compared with soil solution concentration and four widely used single-step extraction methods (acetic acid, EDTA, sodium acetate and CaCl_2). Wheat and maize were selected as tested species. The results demonstrated that single Cd-polluted soils inhibited the growth of wheat and maize significantly compared with control plants; the shoot and root biomasses of the plants both dropped significantly ($P < 0.05$). The addition of Zn exhibited a strong antagonism to the physiological toxicity induced by Cd. The Pearson correlation coefficient presented positive correlations ($P < 0.01$, $R > 0.9$) between Cd concentrations in two plants and Cd bioavailability indicated by each method in soils. Consequently, the results indicated that the DGT technique could be regarded as a good predictor of Cd bioavailability to plants, comparable to soil solution concentration and the four single-step extraction methods. Because the DGT technique can offer *in situ* data, it is expected to be widely used in more areas.

Key words | bioavailability, cadmium, chemical methods, combined pollution, diffusive gradients in thin films (DGT), soil

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INTRODUCTION

Cadmium (Cd) is one of the most toxic heavy metals. Due to Cd's relatively high mobility in soils and highly widespread toxicity to biota at low concentrations, it has been listed in the blacklist of the International Agency for Research on Cancer. The metal is a serious environmental and occupational contaminant and may represent a severe health hazard to humans and animals. Numerous studies have shown that pronounced amounts of Cd were often found to accompany lead (Pb)/zinc (Zn) or other metals in mineralization (Nogueira *et al.* 2010). Such combined effects may be synergistic, antagonistic, additive, or independent. These combined interactions clearly affect plant growth and other processes (Davison & Zhang 2012).

The ecological effects of heavy metals in soils depend on the metals' chemical speciation (biological availability), not the total dissolved concentrations. Biological availability refers to the heavy metals that can be taken up by plants.

The most impressive *ex situ* methods for estimating bioavailability of Cd in soils, such as the conventional single and sequential extraction method, soil solution concentration and the isotope dilution exchange method, failed to provide *in situ* data. However, diffusive gradients in thin films (DGT) is a dynamic and high-resolution technique that could predict Cd bioavailability well in an *in situ* environment where physical and chemical properties are dynamic (Zhang *et al.* 1995). DGT is now an established technique that is used for morphological analysis in different fields of research, including water quality monitoring, soils and sediment geochemistry. DGT measures the labile, dissolved fraction of analytes and pre-concentrates them *in situ*, processes that can reduce the risk of introducing contamination and chemical transformation of the sample. However, the DGT technique perturbs a solution or soil system and imposes the diffusion limiting case, so that

metal can rapidly dissociate from solution complexes or from the solid phase while being accumulated by the device. Nolan *et al.* (2005) found that DGT was a convenient and relatively easy technique to predict metal concentrations in plants. Tandy *et al.* (2011) as well as Tian *et al.* (2008) both found that DGT was more accurate than currently available *ex situ* methods at predicting the bioavailability of heavy metals, specifically plant-available Cu, Zn and P. However, Soriano-Disla *et al.* (2010) explained that the performance of the DGT method was poor for assessing the transfer of Cd to the root. Almás *et al.* (2006) confirmed there was no good linear relationship between heavy metals absorbed in plants and the content measured by the DGT technique. Consequently, there is no universal consensus about the reliability of the DGT technique for evaluation of heavy metals bioavailability in soils.

In this study, Cd bioavailability in soils with Zn combined pollution was systemically investigated by comparing the DGT technique with soil solution concentrations and traditional extraction methods (HAc, EDTA, NaAc and CaCl₂). Two typical plants (wheat and maize) were examined for Cd uptake. The Pearson correlation coefficient between Cd concentrations in the two plants and the Cd bioavailability was used to confirm the best method for predicting the concentration of labile Cd in Cd-Zn complex contaminated soils.

MATERIALS AND METHODS

Soil sample and incubation

Bulk samples of topsoil (0–20 mm) were collected from Jiang Xinzhou vineyard of Nanjing using the diagonal method and were later reduced by quartering. After being mixed evenly, the soil sample was air-dried at room temperature and sieved with a 3-mm stainless steel mesh. The basic chemical properties of the uncontaminated soil samples were pH (6.5), organic matter (2.5%), cation exchange capacity

(CEC, 26.9 cmol·kg⁻¹), and total metal concentrations (0.22 mg Cd·kg⁻¹, 130.8 mg Zn·kg⁻¹, 19.04 mg Pb·kg⁻¹ and 64.4 mg Cu·kg⁻¹). Bainong AK58 (wheat) and Zhengdan 958 (maize) were selected as test materials.

A typical DGT device consists of a Chelex-100 resin-impregnated binding phase overlaid with a well-defined diffusion phase, with functional groups selecting the target ions in a layer of hydrogel of known thickness, which serves as the diffusive layer; a protective outer membrane with a known pore size; and a plastic base and cap (Figure 1). Among these components, the cross-linker that was used to make the membrane, the plastic base and the cap were purchased from DGT Research Limited (Lancaster, UK). In the DGT assembly, the resin gel was covered sequentially by a diffusion gel and a 0.13-mm cellulose nitrate filter membrane (Whatman, 0.45-μm pore size). The diffusive gel was prepared with 15% acrylamide and 0.3% AcrylAide agarose cross-linker (Flow Gen Instruments Ltd) following a published procedure (Zhang *et al.* 1995).

Pot experiment

Two widely used plant species (wheat, *Triticum aestivum*; maize, *Zea mays*) were selected as the test species. Separate subsamples were amended with either Cd stock solutions only or Cd-Zn complex solutions. The added concentrations of Zn were 0, 50, 100, 200, 300, 400, 600 and 700 mg·kg⁻¹. CdCl₂·2.5H₂O and ZnSO₄ were selected as mother liquor, were added to soil solutions and then blended adequately with equal base fertilizers. Base fertilizers included urea, KH₂PO₄ and K₂SO₄; whereas ω (total nitrogen), ω (P₂O₅) and ω (K₂O) were 0.15, 0.10, 0.15 g·kg⁻¹, respectively. The soil was turned over every three or four days to ensure exogenous heavy metals and nutrient elements mixed evenly. After six weeks of aging (Alexander 2000), the pot experiment could be processed. Fifteen seeds of wheat and five seeds of maize were sown in pots filled with 0.75 kg of the added soils with different doses of Cd. Each soil sample at a given metal concentration was in triplicate.

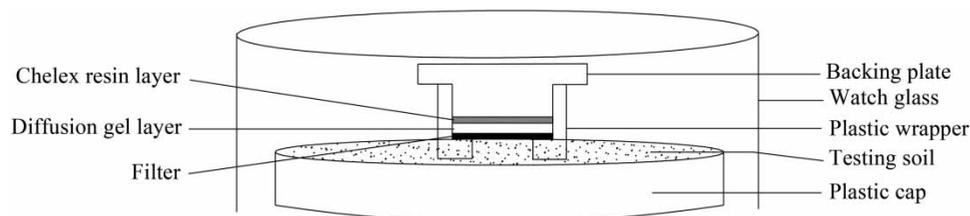


Figure 1 | Schematic view of DGT deployment in soil.

After germination, the number of plants per pot was reduced to ten seedlings for wheat and three seedlings for maize. All plants were grown without nutrient addition in a greenhouse under the natural day-night cycle. During the process of plant growth, deionized water was added to maintain 60 percent of the soil moisture every day. After 35 days of growth, all plants were harvested and separated into shoots and roots. The roots of the plants were rinsed with deionized water before they were placed in a solution of 20 mmol·L⁻¹ EDTA for 15 min to remove the heavy metals that were adsorbed onto the root surface. Afterwards, the roots were washed with deionized water again and put into numbered envelopes. The shoots and roots of the plants were dried in an oven at 105°C for 20 minutes de-enzyming. The temperature was reduced to 70°C until the weight was constant. The dry sample weights were recorded and prepared to measure Cd bioavailability. After harvest, the remaining soils were air-dried at room temperature and sieved with a 2-mm stainless steel mesh for the following analysis of Cd bioavailability and total Cd in soils.

Analytical methods of the bioavailable Cd in soils

DGT measurement

The DGT procedure was divided into four steps, as follows:

- **Soil pre-equilibrium.** Prior to DGT deployment, the maximum water holding capacity (MWHC) of each soil subsample was measured. Soil samples of 80 g were placed into a plastic pot with 40% MWHC and maintained for 48 h, followed by 80% MWHC for 24 h.
- **DGT measurements.** The appropriate amount of soil sample was put into each plastic cap (8-mm diameter). The assembled DGT devices were deployed on the soil paste of each plastic pot with gentle pressure to ensure complete contact between DGT and the soil paste. Three replicates per soil were used. The petri plate was upended to maintain water-holding capacity. The temperature was maintained exactly.
- **DGT retrieval and elution.** The devices were deployed for 24 h. On retrieval, the devices were jet-washed with deionized water to remove the adhered soil particles before they were disassembled. The resin gels were removed and immersed in 1 mL of 1 mol·L⁻¹ HNO₃ for at least 16 h prior to analysis after appropriate dilution.
- **DGT calculation.** The mass of accumulated Cd (M) in the binding gel was calculated according to formula (1) when it was eluted using a known volume of eluting

solution (V_e).

$$M = \frac{C_e(V_g + V_e)}{f_e} \quad (1)$$

where C_e is the free concentration of Cd²⁺ in acid eluent, V_g is the volume of the gel, and f_e is the elution factor (0.8) (Zhang *et al.* 2002; Luo *et al.* 2013). The concentrations of Cd measured by DGT (C_{DGT}) were calculated using formula (2):

$$C_{DGT} = \frac{M\Delta g}{DA t} \quad (2)$$

where M is the accumulated mass of Cd²⁺ over the deployment time, Δg is the thickness of the diffusive layer, D is the diffusion coefficient of Cd²⁺ in the diffusive layer, A is the area of exposure window, t is the deployment time, and the value of D for Cd²⁺ has been reported elsewhere (Scally *et al.* 2006).

Soil solution concentration

The soil solutions were collected by centrifuging the paste soils. After retrieval of the DGT deployments, soils were transferred into 50 mL PTFE tubes and then centrifuged at 4,000 r·min⁻¹ for 20 min. The resulting supernatants were filtered through a 0.45- μ m pore size cellulose nitrate filter membrane and then acidified using HNO₃. The measured concentration of Cd was recorded as C_{sol} .

Single extraction methods

Acetic acid (HAc) (Luo *et al.* 2012), EDTA (Feng *et al.* 2005), sodium acetate (NaAc) (Kaplan *et al.* 2009) and CaCl₂ (Qasim *et al.* 2015) extractions were selected to extract the bioavailable fractions of Cd in this study. The original air-dried rhizosphere soils were used as subsamples for the chemical extractions.

- HAc: 0.5 g of soil was extracted with 20 mL of 0.11 mol·L⁻¹ HAc in a 50-mL centrifuge tube, and shaken for 16 h (overnight) (Luo *et al.* 2012).
- EDTA: 2.0 g of soil was extracted with 20 mL of 0.05 mol·L⁻¹ EDTA adjusted using an ammonia solution to pH = 7.0 in a 50-mL centrifuge tube and shaken for 2 h (Feng *et al.* 2005).
- NaAc: 4.0 g of soil was extracted with 20 mL of 1 mol·L⁻¹ NaAc in a 50-mL centrifuge tube and shaken for 2 h (Kaplan *et al.* 2009).

- CaCl_2 : 2.0 g of soil was extracted with 20 mL of $0.01 \text{ mol}\cdot\text{L}^{-1}$ CaCl_2 in a 50-mL centrifuge tube and shaken for 2 h (Qasim *et al.* 2015).

All the extracting solutions were centrifuged at $3,000 \text{ r}\cdot\text{min}^{-1}$ for 20 min followed by filtering through a $0.45 \mu\text{m}$ cellulose nitrate filter membrane. Filtrates were used to measure Cd availability of soil. All of the operations were processed in triplicate at room temperature.

Cd measurements in plants

The shoots and roots of the plants were smashed by pulverizer and sieved through a 2-mm sifter. $\text{HNO}_3\text{-HClO}_4$ was added to the subsamples as a wet digestion reagent. The control test was conducted at the same time. A decomposition reagent was applied to analyze Cd in plants (dry mass was used for calculations).

Statistical analyses

A graphite furnace atomic absorption spectrophotometer (A3AFC-12, Beijing Purkinje General Instrument Co., Ltd) was used to determine Cd of solution samples. The lowest detectable limit is $0.001 \mu\text{g}\cdot\text{L}^{-1}$. Excel 2007 was used to analyze experimental data and draw charts, and SPSS statistical package (version 10.0 for Windows) was used to evaluate significance analysis and Pearson correlations.

RESULTS AND DISCUSSION

Effects of Cd along or combined with Zn in soils on the biomass and biota accumulation

Cd is one of the most toxic heavy metals and is easily assimilated and accumulated by plants because of its high mobility in soils. Zn is the essential trace element for plant growth. Because Zn and Cd have similar chemical characters, they often interact with each other. As presented in Figure 2, wheat and maize growth were inhibited compared with control plants by a single Cd addition in soils. The shoot and root biomasses (calculated as dry weight) decreased by 26.2% and 23.4%, respectively, for wheat, and by 23.5% and 30.9%, respectively, for maize, which was significant ($P < 0.05$). However, Lin *et al.* (2007) experimented on the strong tolerance of wheat to Cd stress, and the results showed that wheat could grow well under a wide range of Cd concentrations ($0.3\text{--}33 \text{ mg}\cdot\text{kg}^{-1}$). The growth of both

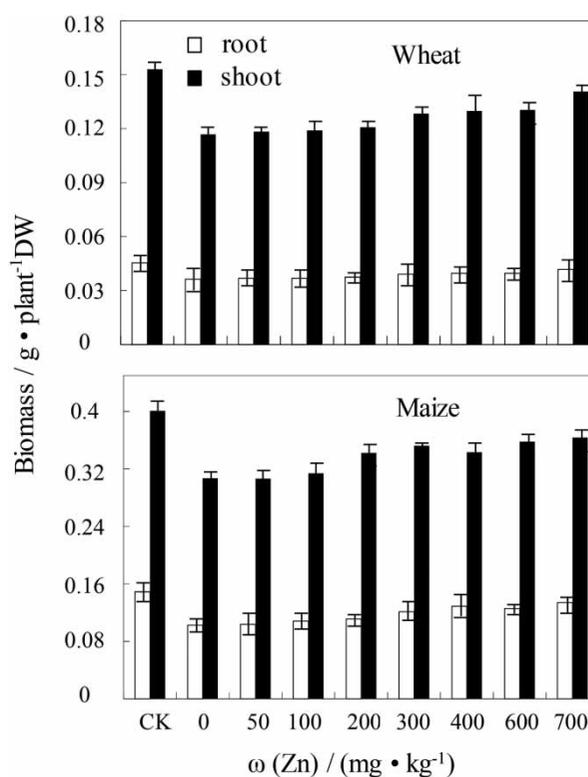


Figure 2 | Effects of Cd along or combined with Zn in soils on the biomass of wheat and maize.

plants could be promoted with the addition of Zn in Cd-contaminated soils. As the amount of Zn increased, the shoot and root biomasses also increased. The result was similar to a previous study, which reported that applied Zn increased plant Zn, while added Cd had a negative effect on plant Zn (antagonism) (Smilde *et al.* 1992). Physiological toxicity could be vigorously facilitated with wheat and maize when the plants were exposed to high ω (Cd), while Zn could decrease this effect. As seen from Figure 3, the amount of Cd in plants decreased after more Zn was added. The additional Zn could combine more Zn enzymes to make Zn enzymes remain active and restrain Cd toxicity. With more Zn accumulated in plants, the synthesis of Cd complexes was accelerated, and Cd complexes could be immobilized by the sulfhydryls in cells of plants to reduce the toxic effects.

Wheat and maize that were grown in Cd single-contaminated soils accumulated more Cd compared with those grown in the control groups. Cd could be accumulated obviously by both the shoots and roots of the plants, but the accumulated mass of Cd was mainly distributed in the roots (Figure 3). Jiang *et al.* (2001) studied *Allium sativum* and found similar conclusions as this study. The concentration of Cd decreased in both the shoots and roots when Zn was added (Figure 3). Zn in soils also restrained the absorption of

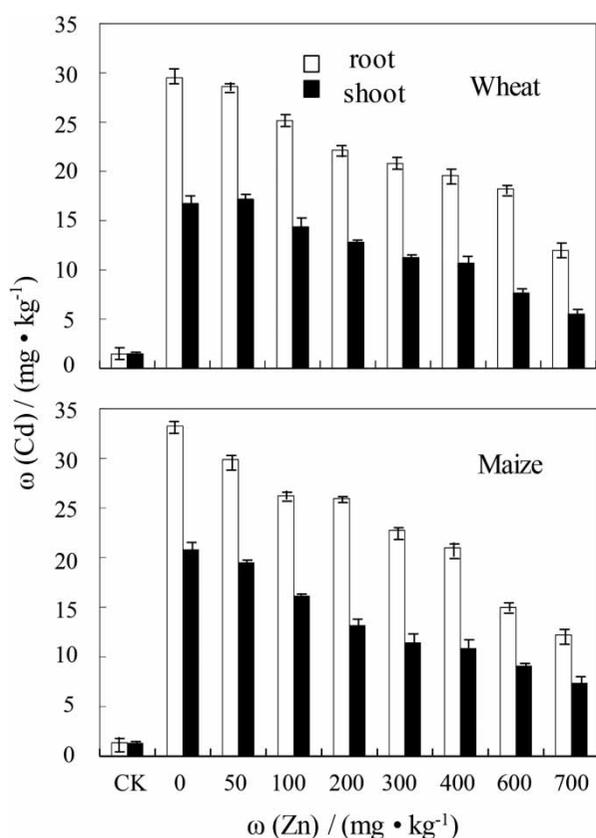


Figure 3 | Effects of the Zn addition on Cd concentrations in the shoots and roots of wheat and maize in the Cd-contaminated soils.

Cd by wheat and maize, indicating that Zn has an antagonistic action on Cd absorption. This result is because Zn and Cd have similar configurations of extra-nuclear electron and ionic radii, elements of similar types that are competing for the same transport and storage sites in the cell and which therefore displace each other from reactive enzymatic and receptor proteins. These actions and reactions might explain why the addition of Zn leads to the decreasing absorption of Cd.

DGT measurement and soil solution concentration

Cd soil concentrations measured by soil solution (C_{sol}) and DGT (C_{DGT}) both decreased distinctly as increasing concentrations of Zn were added to soils growing wheat and maize (Figure 4). The bioavailability of Cd was restrained remarkably by the addition of Zn in Cd-polluted soils. A substantial fraction of elements taken up by plants have been confirmed to come from pore water rather than solution. In most cases, the DGT-measured concentrations of metals in soils could reflect not only the concentrations of metals in soil solutions but also those related to the dynamic resupply of elements from complexes in soil solutions and solid phases. Moreover,

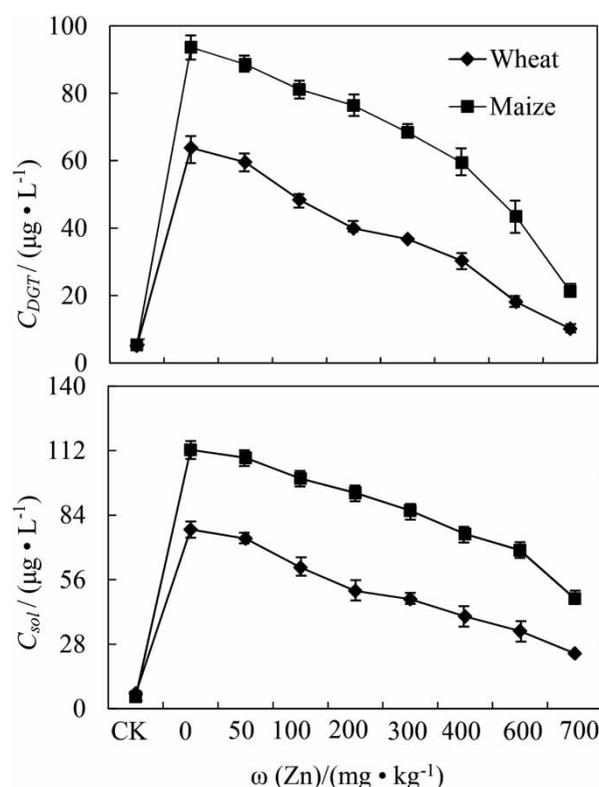


Figure 4 | Soil solution (C_{sol}) and DGT-measured (C_{DGT}) concentrations of Cd in soils growing wheat and maize with the addition of Zn.

the experiment reaction process was controlled exclusively by diffusion. Conversely, the soil solution concentration was often directly related to the free ion concentration. C_{DGT} reflected the fraction derived from pore water and the further release of the solute from the sediment solids to resupply the pore water, whereas C_{sol} reflected the concentrations of metal ions in soil solution alone. This study further verified the conclusion that C_{DGT} , which is an average value between DGT measurement and soil interface solution, would normally be less than C_{sol} because of soil buffering capability, because soil particles have the ability to supply heavy metals when they are transformed or consumed in soil solutions.

Extractable Cd using single extraction methods

The bioavailable concentrations of Cd extracted by HAc, EDTA, NaAc, and CaCl₂ in soils are shown in Figure 5. The extractable concentrations of Cd measured by these four different extractants all decreased as Zn concentrations increased. This decreasing trend aligned with those measured by the DGT technique and soil solution concentration (see Figure 4). An analysis of Figure 4 shows an antagonism of Zn on Cd toxicity from the perspective of solid to liquid static equilibrium.

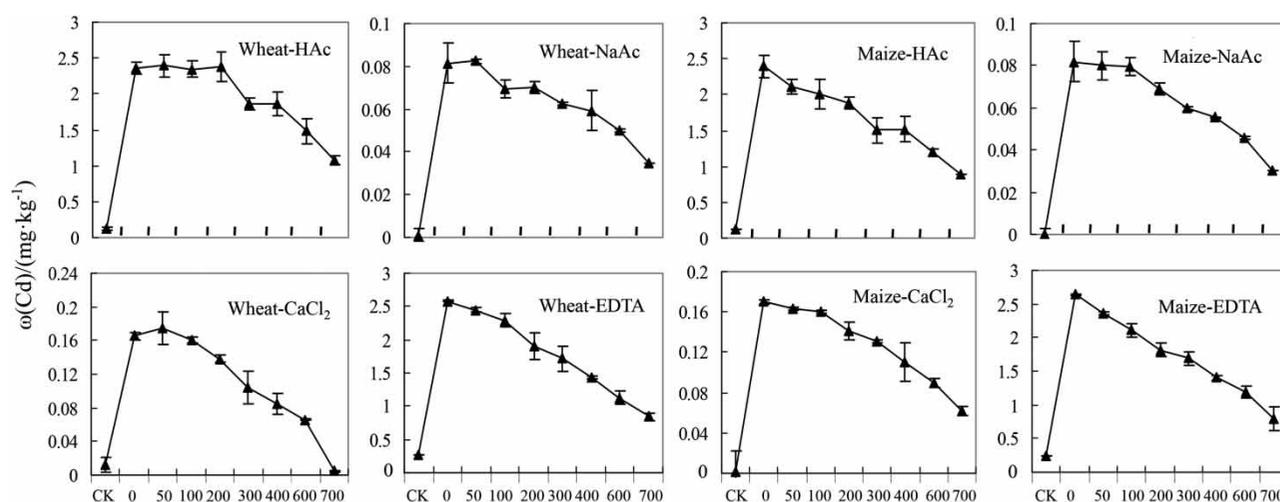


Figure 5 | The bioavailable concentrations of Cd measured by four extraction methods in soils growing wheat and maize.

As the common *ex situ* method, single extraction is understood to be influenced by many factors, including extractant properties and extracted heavy metal species (Feng et al. 2005). Among the four extractants, EDTA extracted the largest amounts of Cd from soils. Because EDTA is a chelating agent, Cd in oxides, the organically bound Cd and secondary clay minerals can be extracted by it. Following EDTA, HAc extracted the second largest amounts of Cd from soils because HAc could extract the most Cd associated with carbonate minerals, as well as Cd bound to organic matter. Compared with EDTA and HAc, CaCl₂ and NaAc extracted lower amounts of Cd. Feng et al. (2005) reached the same conclusion.

Correlation between Cd concentrations in plants and bioavailable concentrations of Cd

The relationship between plant Cd concentrations and Cd concentrations in particular soils has been applied widely to select the most suitable method of assessment for bioavailable heavy metal testing procedures in soils (Muhammad et al. 2012). In general, the results showed that Cd

concentrations in plants had extremely significant correlations ($P < 0.01$) with the extractable concentrations of Cd by all extraction methods, C_{DGT} and C_{sol} . As listed in Table 1, the R-values between Cd uptake in plants and bioavailable concentrations of Cd all presented above 0.9, indicating that each method used in this study could be a useful substitute for determining plant-available Cd, and the DGT technique could be regarded as a robust tool when predicting Cd bioavailability in soils. Good predicting results have also been found previously, using pot experiments for wheat (Nolan et al. 2005).

The conventional single extraction method is a common evaluation method. As a strong chelating reagent, EDTA has been reported to extract both the organically bound metals and the metals occluded in oxides as well as to partly extract secondary clay minerals, and EDTA could overestimate the bioavailability of the target elements. Previous studies have reported that EDTA was an ideal indicator in assessing the bioavailability of Cd (Zhu et al. 2012). HAc can extract organic matter-bound metals. As the weaker two neutral salts, NaAc and CaCl₂ are able to extract exchangeable Cd with the

Table 1 | Linear correlation coefficients between Cd concentrations in plant tissues and bioavailable concentrations of Cd using six methods in soils

Item		C_{DGT}	C_{sol}	ω (Cd)			
				HAc	EDTA	NaAc	CaCl ₂
Wheat	shoots	0.984	0.947	0.903	0.912	0.914	0.906
	roots	0.963	0.963	0.918	0.918	0.929	0.909
Maize	shoots	0.942	0.950	0.915	0.908	0.917	0.926
	roots	0.983	0.986	0.902	0.919	0.932	0.914

All results are significantly correlated (two tailed).

desorbing cations easily in soil mineral surfaces. The capacity of CaCl_2 -extractable metal concentrations to predict plant availability is metal dependent (Nolan *et al.* 2005). Many studies have also observed good relationships between Cd uptake by plants and CaCl_2 -extractable concentrations (Qasim *et al.* 2015), suggesting that CaCl_2 might be a useful extractant for determining plant-available Cd. Similarly, it has been shown that extraction with CaCl_2 in various concentrations gives a good indication of the bioavailability of different heavy metals. C_{sol} is often used to indicate bioavailability of heavy metals in soils. Generally, the metal compartments in soils are comprised of inorganic complexes, mineral colloids, organic complexes, and the free metal ion, while the solid phase is represented by tightly bound and exchangeable fractions. The soil solution concentration is able to determine not only the heavy metal fractions that could be absorbed by plants (the total dissolved) but also the inertia fractions that could not be used by plants. It is commonly understood that not only metal speciation at equilibrium but also both dynamic chemical interactions and resupply of metal from the soil solid phase determine metal bioavailability. The DGT technique could measure the concentrations of trace metals, including the dissolved fractions in soil solution and those resupplied from the solid phase *in situ*. This technique could measure inorganic complexes, a proportion of organic complexes and free metal ions, any of which could be assimilated by the roots from the soil system. pH, dissolved organic carbon, CEC, and texture had no influence on the process of metal uptake when the DGT technique was used. This study verified that the DGT technique was a robust tool for predicting Cd bioavailability in soils. Based on its theoretical advantages, the technique could provide more credible monitoring data and it could be widely used in more areas than other *ex situ* methods. A previous study has reported that the DGT technique was a useful tool for assessing metal bioavailability in clean and contaminated sediments that were being subjected to varying degrees of sediment disturbance (Amato *et al.* 2016).

The values of C_{DGT}/C_{sol} ranged from 0 to 1. High values represented an increasing ability for applied soil particles to supply heavy metals to soil solutions. The value of C_{DGT}/C_{sol} decreased with the addition of Zn in the soils growing both wheat and maize. The variation of decrease increased as the addition neared 600–700 $\text{mg}\cdot\text{kg}^{-1}$ (Table 2), indicating that the ability of soil particles to supply Cd was limited as a result of the Zn addition. This result also revealed the protective effect Zn had over Cd poisoning in Cd-Zn combined exposure in terms of kinetics. However, the ability of soils growing wheat to supply heavy metals was greatly restrained by the addition of Zn, as indicated by the larger decreasing values of wheat than

Table 2 | C_{DGT}/C_{sol} in soils growing wheat and maize with Zn added to Cd concentrations

$w(\text{Zn})/(\text{mg}\cdot\text{kg}^{-1})$	C_{DGT}/C_{sol}	
	Wheat	Maize
Control check	0.896	0.845
0	0.891	0.826
50	0.882	0.815
100	0.794	0.807
200	0.773	0.807
300	0.771	0.802
400	0.764	0.774
600	0.542	0.627
700	0.416	0.453

those of maize (Table 2). The apparent effect was an antagonism: Cd content was reduced because of the Zn addition. Zn could hinder soil particles from releasing Cd, which would reduce Cd concentrations in plants as well as soil solution C_{sol} .

Consequently, the study showed the effectiveness of the DGT technique and soil solution concentration as well as the four single-step extraction methods (HAc, EDTA, NaAc and CaCl_2) for predicting the bioavailability of Cd to wheat and maize in Cd-Zn complex-contaminated soils. Since the DGT technique could provide *in situ* data, it could be regarded as a more reliable tool than *ex situ* methods in predicting the presence of heavy metals.

CONCLUSIONS

Single Cd-polluted soils inhibited the growth of plants. The shoot and root biomasses all significantly decreased for wheat and maize ($P < 0.05$). The addition of Zn could decrease Cd bioavailability in Cd-contaminated soils and suppress the absorption of Cd so as to alleviate the physiological toxicity that was facilitated with wheat and maize. As more Zn was added, a greater effect could be observed during the experiment. Positive correlations ($P < 0.01$, $R > 0.9$) were presented between Cd concentrations in two plants and Cd bioavailability, indicating that the DGT measurement predicts Cd bioavailability in Cd-Zn contaminated soils well. The results were comparable to traditional *ex situ* methods, including soil solution concentration and four widely used single-step extraction methods (HAc, EDTA, NaAc and CaCl_2). Based on the DGT technique's theoretical advantages, it could be regarded as a more robust tool than those currently available.

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