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Evaluation of different methods for assessing bioavailability of DDT residues during soil remediation

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Application and validation of isotope dilution method (IDM) for predicting bioavailability of hydrophobic organic contaminants in soil☆

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A B S T R A C T

Risk assessment of hydrophobic organic contaminants (HOCs) using the total chemical concentration following exhaustive extraction may overestimate the actual availability of HOCs to non-target organisms. Existing methods for estimating HOC bioavailability in soil have various operational limitations. In this study, we explored the application of isotope dilution method (IDM) to quantify the accessible fraction (E) of DDTs and PCBs in both historically-contaminated and freshly-spiked soils. After addition of ^{13}C or deuterated analogues to a soil sample, the phase distribution of isotope-labeled and native chemicals reached an apparent equilibrium within 48 h of mixing. The derived E values in the three soils ranged from 0.19 to 0.82, depending on the soil properties and also the contact time of HOCs (i.e., aging). The isotope dilution method consistently predicted greater accumulation into earthworm (Eisenia fetida) than that by polyethylene (PE) or solid phase microextraction (SPME) sampler, likely because desorption in the gut enhanced bioavailability of soil-borne HOCs. A highly significant linear regression ($R^2 = 0.91$) was found between IDM and 24-h Tenax desorption, with a slope statistically identical to 1. The IDM-derived accessible concentration (C_e) was further shown to accurately predict tissue residues in earthworm exposed in the same soils. Given the relatively short duration and simple steps, IDM has the potential to be readily adopted for measuring HOC bioaccessibility in soil and for improving risk assessment and evaluation of remediation efficiency.

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1. Introduction

Soil contaminated with hydrophobic organic contaminants (HOCs), of which many are persistent, bioaccumulative, and toxic compounds such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs), and polycyclic aromatic hydrocarbons (PAHs), is a global issue and a long-term threat to human and ecological health (Nizzetto et al., 2010). Due to their low aqueous solubility and high hydrophobicity, the total chemical concentrations (C_{Total}) of HOCs in soil, derived usually after exhaustive solvent extraction, may not relate directly to the actual biological effects, e.g., bioaccumulation, toxicity or biodegradation (Alexander, 2000; Ehlers and Luthy, 2003; Semple et al., 2004). Risk assessment based on C_{Total} may provide overly conservative estimates of exposure, whereas evaluation of remediation performance using C_{Total} may lead to costly overtreatment (Alexander, 2000; Ehlers and Luthy, 2003). Thus, predicting bioavailability is essential for accurate risk assessments, and has been a major drive in the past decades in environmental research and engineering (Alexander, 2000; Cui et al., 2013; Ortega-Calvo et al., 2015).

Biological methods, based on direct biodegradation or bioaccumulation measurement, are traditionally considered as the most direct approach to quantifying a contaminant's bioavailability (Cachada et al., 2014). However, these methods are often time-consuming and expensive, and the results are often organism dependent (Gomez-Eyles et al., 2011; Kelsey et al., 1997; Reid et al., 2000). Thus, a wide variety of chemically-based methods have been introduced to quantify HOC bioavailability in recent years. These methods include partial extraction-based methods, e.g., mild solvent extraction (Chung and Alexander, 1998; Tao et al., 2006), Tenax extraction (Cornelissen et al., 2001; You et al., 2011), hydroxypropyl-β-cyclodextrin (HPCD) extraction (Hartnik et al., 2008; Reid et al., 2000), and supercritical fluid extraction (SFE) (Kreitinger

et al., 2007), and equilibrium sampling devices, e.g., solid phase microextraction (SPME) (Bao et al., 2013), polyethylene sampling device (PE) (Fernandez et al., 2009), and polyoxymethylene sampler (POM) (Endo et al., 2011; Muijs and Jonker, 2012). Many studies have attempted to demonstrate how these methods would provide a good prediction for the actual bioavailability of HOCs in sediments or soils. However, most methods have operational uncertainties and limitations. For example, for the mild solvent-extraction methods, the measurement invariably depends on the strength of the selected solvent and sometimes even the solvent-to-solid ratio (Mayer and Reichenberg, 2006), while Tenax sequential desorption is extremely time-consuming and tedious (Cui et al., 2013; Liu et al., 2011). Equilibrium samplers, such as SPME and PE, require equilibrium conditions, which may take weeks or even months to attain (Mayer et al., 2000; Bao et al., 2013). Therefore, a simple, rapid, and ready-to-use method is needed to accurately predict HOC bioavailability in soil.

The isotope dilution method (IDM), which is based on similar equilibrium behavior between a native HOC and its isotope-labeled analogue in the accessible phase, has been successfully developed and validated in freshwater and marine sediments (Delgado-Moreno and Gan, 2013; Jia et al., 2014). Compared to other methods, IDM only involves a few simple steps and may be easily adopted in laboratories with mass spectrometry facilities (Delgado-Moreno and Gan, 2013; Jia et al., 2014). In this study, we explored the application of IDM to both spiked and field contaminated soils. The DDT derivatives (DDTs) and PCB congeners (PCBs) were used as model HOCs. Bioaccessibility derived by IDM was further validated by correlation with bioaccumulation in earthworm (Eisenia fetida), and also compared with Tenax extraction and passive samplers. This study demonstrated that IDM may serve as an effective method for predicting the bioavailability of HOCs in soil and may be used for risk assessment and evaluation of soil remediation effectiveness.

2. Materials and methods

2.1. Chemicals and other materials

Ning model HOCs, including six DDT derivatives (p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, p,p'-DDD, and o,p'-DDD) and three PCB congeners (PCB 52, PCB 70, and PCB 153), were considered in the current study. PCB 67 and PCB 191 were used as surrogate standards to verify the method recovery, while PCB 30 and PCB 82 were used as internal standards to check for instrument drift. Stable isotope-labeled HOCs, including ^{13}C-labelled (^{13}C- o,p'-DDD and ^{13}C-PCB 153) and deuterated compounds (p,p'-DDE-d_8, o,p'-DDE-d_8, p,p'-DDD-d_8, p,p'-DDT-d_8, o,p'-DDT-d_8, PCB 52-d_3, and PCB 70-d_3), were used in IDM method development and application. The isotope-labeled standards were dissolved in acetone at 1.0 mg/L as the stock solution. Additional details of the chemical standards are given in the Supporting Information (SI).

Thin fiber (430-μm diameter) with 35 μm polydimethylsiloxane (PDMS, 51.1 μL/m) coating was purchased from Polymicro Technologies (Phoenix, AZ), and used for the SPME sampling. Low-density polyethylene film (PE, 25 μm thickness) was purchased from BBB Accredited Business (Cleveland, OH). Both fiber (1 cm long) and film (1 × 2 cm) were pre-cleaned by sonication in hexane for 1 h and cut into applicable pieces prior to use. Tenax TA resin (60–80 mesh) was purchased from Scientific Instrument Services (Ringoes, NJ).

Two surface soils, denoted as soil A (loamy sand soil) and soil B (organic soil), that were historically-contaminated with DDTs and PCBs were collected from the North Shore Restoration Area of Lake Apopka in central Florida. Long time agricultural use and a spill

from a nearby pesticide manufacturing facility resulted in the contamination of a large area of soil with DDT, which induced developmental abnormalities of the gonads and abnormal sex hormone levels in alligators in the area (Semenza et al., 1997). One relatively clean soil, denoted as soil C (clay soil), was collected from southern California and was used for the freshly spike treatment. All soils were sieved through a 2-mm mesh and stored at 4 °C before use. The background levels of the 9 HOCs in soil C are given in Table S1, and were much lower than the spiked concentrations. The procedure used for spiking soil C followed a previous study (Bao et al., 2013). Briefly, 10 g of sand was uniformly spread on the bottom of 1.5 L glass jar and spiked with an acetone solution containing the 9 HOCs. The spiked sand was then dried in a fume hood, followed by the addition of 1 kg soil. The glass jar was then rotated at 50 rpm for 2 weeks at room temperature. The C_{Total} in the field-contaminated and spiked soils were measured after exhaustive solvent extraction (Bao et al., 2013) and are given in Table S4. The bouyoucos hydrometer method (Ashworth et al., 2001) was used to characterize the soil textures. The organic carbon (OC) contents of soil A, B, and C are 7.48, 34.5, and 5.31%, respectively, which were determined on a Flashea 1112 series N/C Analyzer (Thermo Electron).

2.2. Isotope dilution measurement

The principle of IDM for estimating bioaccessibility was given in Delgado-Moreno and Gan (2013) and Jia et al. (2014). Briefly, IDM operates on the presumption that the phase distribution of native HOC and the externally added isotope-labeled analogue (*HOC) for the accessible phase reach an apparent equilibrium after a short-term equilibration. In a soil-water system, the distribution of HOC in the different phases can be expressed as:

$$C_{Total} = D \times C_w + C_a + C_{na} \tag{1}$$

where C is the concentration of HOC in each phase, and the subscripts w, a, and na represent water, accessible, and non-accessible phases, respectively. C_{Total} (μg/kg) is the total concentration of HOCs in the whole system, which is derived after exhaustive solvent extraction, and D (L/kg) is the ratio of the water volume to the soil mass. Therefore, the accessible concentration C_e (μg/kg) in soil can be expressed as:

$$C_e = D \times C_w + C_a \tag{2}$$

When a small amount of isotope-labeled analogue *HOC is added into the soil-water system, it will distribute itself between the aqueous and accessible phases in the same way as the native HOC. After an apparent equilibrium is reached:

$$\frac{{}^*C_w}{{}^*C_a} = \frac{C_w}{C_a} \tag{3}$$

the sum of $^*C_w$ and $^*C_a$ is the added concentration of *HOC ($^*C_0$), or:

$$^*C_a = {}^*C_0 - D \times {}^*C_w \tag{4}$$

Combining Eqs (3) and (4), C_a is given below:

$$C_a = (C_w/{}^*C_w) \times ({}^*C_0 - D \times {}^*C_w) \tag{5}$$

Combing Eqs (2) and (5), the accessible concentration C_e is given as:

$$C_e = (C_w/{}^*C_w) \times {}^*C_0 \tag{6}$$

The accessible fraction of HOCs (E) is the ratio of C_e over C_{Total}, or

$$E = \frac{C_e}{C_{Total}} \qquad (7)$$

A preliminary experiment was used to test the prerequisite. Briefly, aliquots (2 g, dry weight) of the three soils were placed in glass centrifuge tubes, and 20 mL of sodium azide (200 mg/L) solution was added to suppress the microbial activity. The samples were then mixed on a shaker at 120 rpm for 24 h, followed by addition of 100 μL of the stock solution containing each stable isotope-labeled HOC at 1.0 mg/L. To understand the time dependence of desorption of native HOCs and sorption of isotope-labeled analogues, the soil-water slurry was returned to the shaker and triplicate samples were removed for analysis after 1, 3, 6, 12, 24, 48, 96, or 192 h of equilibration. The samples were centrifuged at 3000 rpm for 30 min to collect the supernatant. The concentrations of HOCs and *HOCs in supernatant were analyzed on GC-MS/MS, as described below and also in the SI.

2.3. Comparison against other bioavailability measurement methods

The terrestrial invertebrate *Eisenia fetida* was used for bioaccumulation assays as a direct measurement of bioavailability (Morrison et al., 2000). The earthworms (Uncle Jim's Worm Farm, Spring Grove, PA) were cultured in dark plastic containers at room temperature in the laboratory for two weeks before exposure. Five earthworms were exposed to 200 g of each soil at room temperature for 28 d. Triplicates were included for each soil. After exposure, the earthworms were rinsed with deionized water and placed on wet filter paper for 24 h to clear their gut. They were then freeze-dried and pulverized with liquid nitrogen. The earthworm lipid content was determined gravimetrically by sonication extraction using a 1:1 dichloromethane/acetone solvent mixture and evaporation under N_2 to dryness. The extraction and analysis of HOCs in earthworm tissue are given in SI.

Multiple chemically-based methods were carried out to validate the performance of IDM. The performance of IDM was compared to the 24-h Tenax desorption test, which has been frequently used for estimating bioaccessibility of HOCs (Cornelissen et al., 2001; Harwood et al., 2012). Briefly, 1.0 g of soil was placed in a 50-mL glass centrifuge tube, and 0.1 g of Tenax beads and 10 mL of 200 mg/L sodium azide solution were then added. The tubes were mixed at 200 rpm for 24 h. After centrifugation at 3000 rpm for 20 min, Tenax beads were collected by filtering the supernatant through a glass fiber filter paper. Tenax beads were then rinsed with deionized water, air-dried, extracted by sonication, and analyzed for HOCs (SI).

The 24-h desorbable fraction F_{24h} (Fig. S3) was calculated from the ratio of the amount of HOCs desorbed from the soil (C_{Tenax}) over C_{Total} (Cornelissen et al., 2001).

$$F_{24h} = \frac{C_{Tenax}}{C_{Total}} \qquad (8)$$

The IDM was further compared to passive samplers, i.e., SPME and PE, which have been frequently used for detecting the freely dissolved concentration (C_{free}) of HOCs in sediment or soil pore-water. The SPME method was carried out using PDMS fiber as a passive sampler (Bao et al., 2013). Briefly, a 1-cm PDMS fiber was placed into a 7 mL vial, followed by addition of 5.0 g of the soil and 5 mL of sodium azide solution (200 mg/L). The sample vials were then equilibrated on a horizontal shaker for 28 d at room temperature. Sampling with PE sampler was performed by mixing 20 g of soil, 20 mL of sodium azide solution and a 1×2 cm PE strip for 28 d at room temperature on a horizontal shaker. The time interval

was deemed sufficient for SPME and PE to attain equilibrium for DDTs and PCBs (Bao et al., 2013; Hale et al., 2009). At the end of mixing, PDMS fibers and PE strips were removed from the soil slurry samples, cleaned with wet wipes, extracted, and analyzed for HOCs (SI).

The C_{free} was calculated by dividing the concentration in the fiber or film (C_P) over the passive sampler-water partition coefficients K_P (Styrishave et al., 2008).

$$C_{free} = \frac{C_P}{K_P} \qquad (9)$$

2.4. Instrumental analysis

All sample extracts were analyzed on a Varian 3800 GC equipped with a Varian triple quadrupole mass spectrometer (GC-MS/MS; Varian, Sunnyvale, CA). The samples were injected at 250 °C in the splitless mode, and the separation was achieved with a DB-5MS Ultra Inert capillary column (60 m × 0.25 mm × 0.25 μm, Agilent, Wilmington, DE). The initial column temperature was set at 80 °C for 1 min, increased to 210 °C at 15 °C/min, further increased to 300 °C at 5 °C/min, and held at 300 °C for 10 min. High purity helium (99.9999%) was used as the carrier gas at a flow rate of 1.0 mL/min. The triple-quadrupole mass spectrometer was operated in the electron ionization (EI) mode at 70 eV with multiple reaction monitoring (MRM), and the monitored ions for each compound are listed in Table S2. The transfer line, ionization source, and manifold temperatures were 300, 250, and 40 °C, respectively. Argon (99.999%) at the pressure of 1.0 mTorr was used as the collision gas.

2.5. Quality assurance and quality control

All measurements were conducted in triplicates, and the results were expressed as mean ± standard deviation. External surrogates were used in all samples to determine extraction efficiency, and internal standards were added before analysis to correct for any instrument drift. Clean soil and earthworm were used as blank controls and spiked with a known amount of HOCs to measure the method recovery for solid samples, while deionized water was used to determine the recovery in aqueous samples. The recoveries for chemicals in aqueous samples ranged from $75.2 \pm 7.6\%$ to $88.3 \pm 5.3\%$, and the recoveries for soil and tissue samples ranged from $85.3\% \pm 6.1\%$ to $110.5 \pm 6.4\%$. The limits of instrumental detection of 9 HOCs were determined by injecting the valid lowest acceptable calibration standard and setting at a value of three times of the background noise, which were 0.05−0.1 ng/mL for the different HOCs. The instrumental calibration was constructed with 9 point calibration standards at concentrations ranging from 0.5 to 1000 ng/mL, and the regression coefficient (r) of the calibration curve was >0.99. Difference between treatments was determined by one-way analysis of variance (ANOVA) using SPSS 16.0 (SPSS, Chicago, IL). Student *t*-test, linear regression, and Pearson's correlation were also performed using SPSS 16.0.

3. Results and discussion

3.1. Selection of equilibration time interval

The symmetrical exchange between a labeled reference and the native (non-labeled) analyte in the accessible phase is a prerequisite for IDM (Mechlinska et al., 2010). The principle of isotope exchange was previously applied for measuring metal bioavailability (Huang et al., 2011; Sterckeman et al., 2009), where various

equilibration time intervals have been employed (Young et al., 2000). To determine the time interval needed for reaching an apparent equilibrium, the soil samples were equilibrated for different lengths of time after the addition of *HOCs.

The derived E values for PCB 70, p,p'-DDE, and p,p'-DDD increased with mixing time initially, and reached a steady state at 48 h (Fig. 1). Besides, the difference between 24 and 48 h was sharply smaller than the time intervals prior to 24 h (Fig. 1). There was no significant differences among E values between time intervals longer than 48 h and those derived at 48 h (PCB 70, $p = 0.46-0.83$; p,p'-DDE, $p = 0.17-0.41$; p,p'-DDD, $p = 0.31-0.76$). The derived E values for other HOCs also similarly reached a steady state after 48 h of mixing, and no significant increase was observed thereafter (Fig. S1). The observation suggested that 48 h of mixing
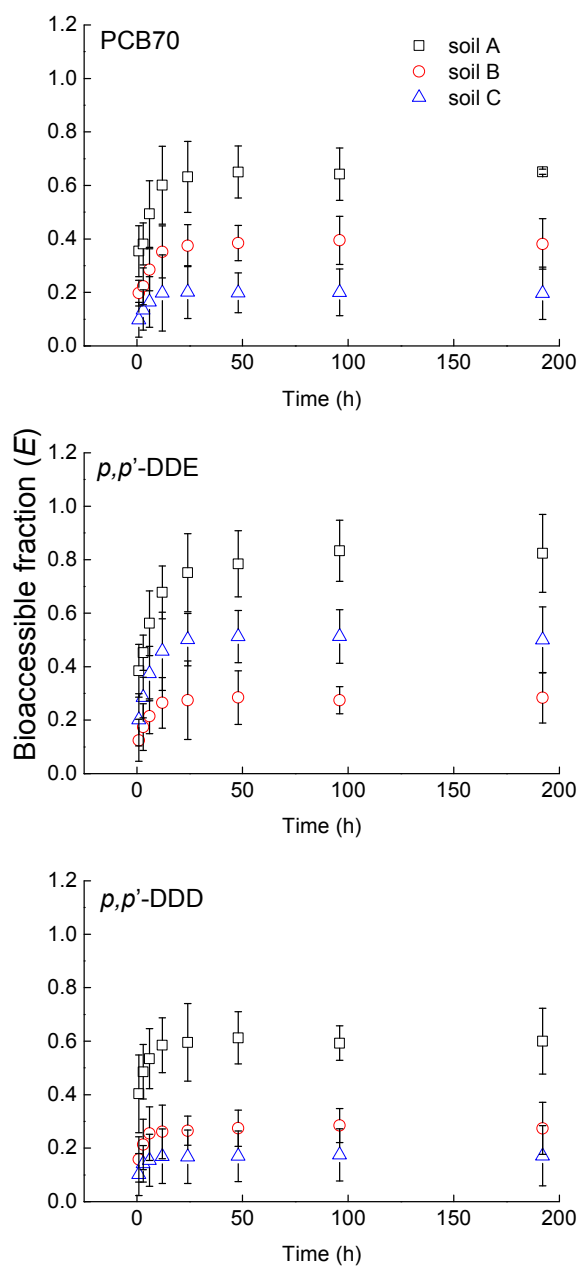

Fig. 1. Effects of equilibration time intervals on the accessible fractions (E) of PCB 70, p,p'-DDE, and p,p'-DDD in the three soils as determined by isotope dilution method. Error bars represent standard error (n = 3).

under the experimental conditions appeared to be adequate for attaining apparent distribution equilibrium between the native HOCs and the externally introduced *HOCs. This finding was in agreement with previous studies using sediments and with other HOCs (Delgado-Moreno and Gan, 2013; Jia et al., 2014). Therefore, IDM may be used for assessing the accessible concentration of HOCs in soil with an equilibration time as short as 48 h, lending a great advantage to the applicability of this method in comparison with most other methods.

3.2. Accessibility of HOCs estimated by IDM

The accessible concentration C_e of DDTs and PCBs in the three soils varied greatly (Table 1). The relative accessibility E, which was calculated as the ratio of C_e over C_{Total} for each HOC, eliminated the influence of different total concentrations and thus expressed the contaminant accessibility as a consequence of effects of variables such as soil properties and aging. The derived E values were all smaller than 1 in the three soils, clearly indicating that a significant fraction of the native HOCs was sequestered in the "glassy" regions of organic matter or inner micropores of soil aggregates (Alexander, 2000). The derived E values of DDTs and PCBs in soil B were consistently smaller than those in soil A, which may be attributed to the much higher OC content in soil B (34.5%) as compared to that in soil A (7.48%). For example, the E values of PCB 70, p,p'-DDE, and p,p'-DDT in soil B were 0.39, 0.27 and 0.34, respectively, which were much smaller than those in soil A (0.62, 0.82 and 0.55 for PCB 70, p,p'-DDE, and p,p'-DDT, respectively). Different E values of DDTs and PCBs were also observed in soil A and soil C, which suggested that other factors, such as contact time or aging and clay content, may also have affected the accessibility of HOCs in soil. The soil C used in this study contained a much greater fraction of clay (72%) than the other two soil. Clay acts as an important sorbent for many HOCs and has the potential to contribute greatly to their phase distribution and further, bioavailability (Biswas et al., 2015).

3.3. Validation by bioaccumulation and other bioavailability methods

Due to their different principles and intended measurement endpoints, direct comparisons between different methods are often not straightforward. Therefore, prediction of concentrations in biota was employed in this study to compare the different methods. Concentrations of DDTs and PCBs in earthworm were predicted from the chemically-derived bioavailability measurements and bioconcentration factor (BCF) values by assuming equilibrium partition (Gomez-Eyles et al., 2011). For SPME and PE passive samplers, prediction of tissue concentrations was carried out by simply using C_{free} in place of the aqueous concentration C_w in the following equation:

$$C_{worm} = C_w \times BCF \tag{10}$$

For Tenax and IDM measurements, the C_w values were obtained by dividing the organic carbon-normalized accessible concentration by the organic carbon-water partition coefficients (K_{OC}), which was subsequently used in Eq. (10) to predict C_{worm}. The specific BCF and K_{OC} values may be found in SI (Table S3).

The performance of IDM was evaluated by plotting the predicted tissue concentrations against the experimentally measured tissue concentrations in earthworm *Eisenia fetida* exposed to the same soils (Fig. 2). A significant linear relationship ($p < 0.05$) was obtained, with R^2 of 0.68. In particular, the slope of the linear regression (0.95 ± 0.09) was statistically equal to 1, indicating that IDM was capable of accurately predicting the tissue concentration

Table 1
Accessible concentrations (Ce, µg/kg) and fractions (E) of PCBs and DDTs determined by the isotope dilution method (IDM) in the three soils (n = 3). Data are presented as mean ± standard deviation.

	Soil A		Soil B		Soil C	
	C_e	E	C_e	E	C_e	E
PCB 52	60.5 ± 8.23	0.75 ± 0.24	247 ± 15.3	0.25 ± 0.13	884 ± 35.1	0.21 ± 0.10
PCB 70	178 ± 15.2	0.62 ± 0.13	591 ± 20.6	0.39 ± 0.14	858 ± 42.1	0.20 ± 0.07
PCB 153	179 ± 17.8	0.80 ± 0.17	207 ± 11.5	0.20 ± 0.11	2374 ± 137	0.63 ± 0.14
o,p'-DDE	19.8 ± 4.91	0.32 ± 0.12	108 ± 18.3	0.19 ± 0.07	541 ± 11.9	0.63 ± 0.21
p,p'-DDE	559 ± 15.3	0.82 ± 0.24	1380 ± 38.9	0.27 ± 0.14	829 ± 18.7	0.50 ± 0.12
o,p'-DDD	84.7 ± 7.21	0.48 ± 0.17	344 ± 15.7	0.47 ± 0.15	501 ± 15.7	0.77 ± 0.28
p,p'-DDD	209 ± 13.1	0.56 ± 0.14	558 ± 23.1	0.27 ± 0.09	212 ± 8.72	0.17 ± 0.10
o,p'-DDT	127 ± 11.7	0.63 ± 0.23	75.4 ± 9.24	0.32 ± 0.18	23.6 ± 5.67	0.21 ± 0.09
p,p'-DDT	366 ± 20.1	0.55 ± 0.09	103 ± 8.41	0.34 ± 0.17	18.7 ± 6.70	0.55 ± 0.14

of these HOCs in soil-dwelling organisms such as earthworm. Even though the correlation was statistically significant, the data appeared to be scattered. A possible reason may be that the DDT derivatives and PCB congeners represent different HOCs from chemical classes (Harwood et al., 2012). For example, when the correlation was only analyzed for PCB 52, the linear relationship was infinitely improved ($R^2 = 0.98$, $p < 0.01$). Nevertheless, the slope (0.95) and the intercept (0.01) of the correlation between IDM and the experimentally-derived bioaccumulation clearly indicated that the accessible concentration C_e as determined by IDM may be used to predict HOC bioavailability across different chemical classes and soils for soil-dwelling invertebrates such as earthworm.

The results of IDM were further compared again accessibility given by Tenax desorption for 24 h that has been previously tested on various HOCs in soil or sediment (Cornelissen et al., 2001; Harwood et al., 2012; Ten Hulscher et al., 2003). In the current study, the derived F_{24h} values of DDTs and PCBs in soil B were consistently smaller than those in soil A (Fig. S3). For example, the F_{24h} values of PCB 52, p,p'-DDE, and p,p'-DDT in soil B were 0.12, 0.28 and 0.23, respectively, which were much smaller than those in soil A (0.39, 0.93 and 0.72 for PCB 70, p,p'-DDE, and p,p'-DDT, respectively). Highly significant linear correlation ($R^2 = 0.91$, $p < 0.05$) was observed between the tissue concentrations predicted by IDM and those by 24-h Tenax extraction (Fig. 3). In addition, the slope (0.89 ± 0.07) of the linear relationship was statistically identical to 1, suggesting that IDM provided similar estimation of bioavailability to Tenax desorption, likely because both methods target the readily desorption fraction for measurement. This observation again affirmed that accessibility-based methods such as IDM and Tenax extraction better mimic bioavailability of

HOCs to soil-dwelling invertebrates such as earthworms that are exposed to contaminants through both dermal contact and ingestion of soil particles.

The results of IDM were further compared again measurements by passive samplers. The C_{free} of DDTs and PCBs estimated by the PE sampler ranged from 0.54 (soil B, o,p'-DDT) to 191.85 ng/L (soil C, PCB52), and those by SPME from 3.14 (soil B, PCB52) to 249.80 ng/L (soil C, PCB52) (Fig. S2). The predicted concentrations in the earthworm lipid phase by these passive samplers are plotted against the predicted earthworm tissue levels by IDM in Fig. 4. Significant linear correlations were observed between the predictions given by IDM and those by the passive samplers ($p < 0.05$). However, the slopes of the linear relationships were 1.22 ± 0.11 for PE and 1.49 ± 0.11 for SPME, respectively. These slopes were significantly greater than 1 ($p < 0.05$), suggesting that compared to passive samplers such as PE and SPME, IDM predicted a greater bioaccumulation of HOCs into the earthworm. The lower bioaccumulation predicted by passive samplers may be attributed to the fact that C_{free} derived by PE or SPME may only account for dermal exposure (Di Toro et al., 1991; Mayer and Reichenberg, 2006), while ingestion through the intestine and other digestive tract compartments may have also contributed appreciably to the final tissue accumulation (Jager et al., 2003; Lu et al., 2004). Earthworms live in direct contact with soil porewater and particles, and they may accumulate HOCs simultaneously through their intestine and skin via alimentary and dermal uptake (Krauss et al., 2000; Vijver et al., 2003). It has been indicated that worm gut ingestion becomes the dominant exposure route for strongly hydrophobic compounds, especially when log K_{ow} is > 6 (Jager et al., 2003). In previous studies, enhanced desorption and diffusion of
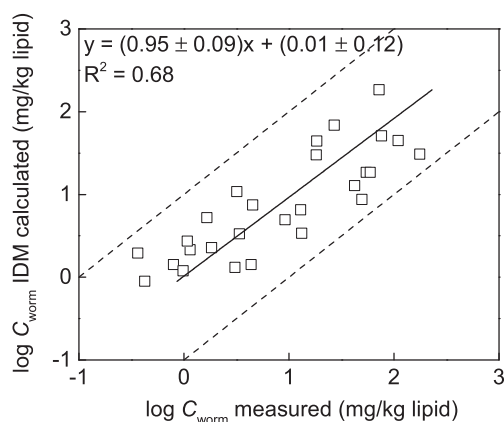
Fig. 2. Linear regression between contaminant concentrations in earthworms predicted by IDM and concentrations measured in earthworm.
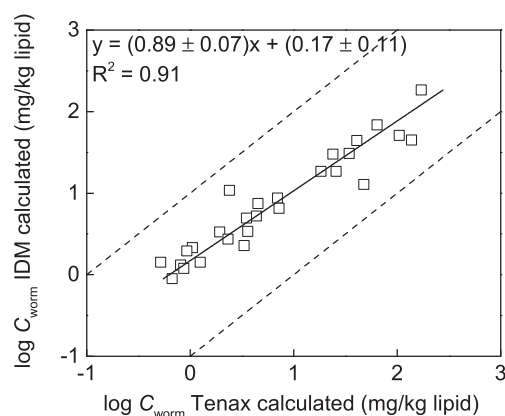
y = (0.95 ± 0.09)x + (0.01 ± 0.12)
$R^2 = 0.68$

Fig. 3. Linear regression between contaminant concentrations in earthworms predicted by IDM and 24-h Tenax desorption.

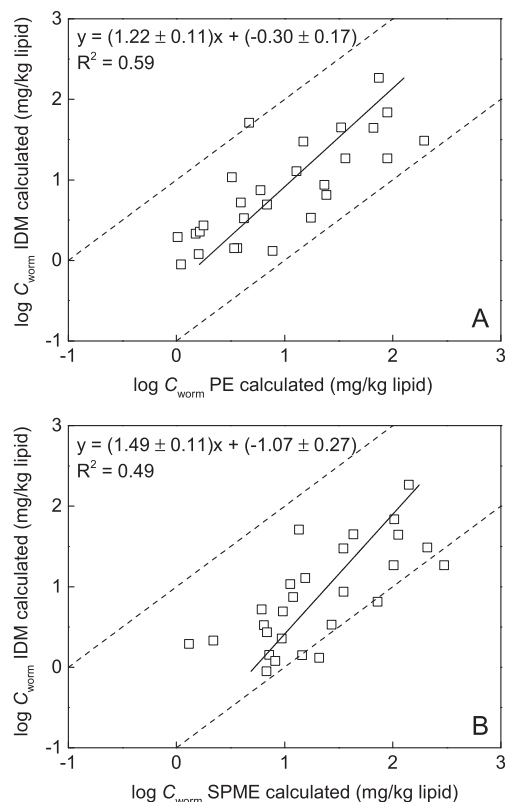y = (0.89 ± 0.07)x + (0.17 ± 0.11)
$R^2 = 0.91$

Fig. 4. Linear regressions between contaminants concentrations in earthworms predicted by (A) IDM and polyethylene passive sampler (PE) and (B) IDM and solid phase microextraction (SPME).

together, IDM offers the desired versatility for quickly assessing bioavailability of HOCs in contaminated soils.

4. Conclusions

In the current study, the potential application of IDM for measuring bioavailability of DDTs and PCBs in soil samples was explored. Compared with other methods, a relatively short duration and simple sample handling steps are involved in IDM, which contributes to the feasibility of applying IDM to evaluation of soil-borne HOCs. Linear regression analysis between IDM predicted results and experimentally-derived bioaccumulation in earthworm showed that IDM was capable of accurately predicting HOC bioavailability to soil-dwelling organisms. Applications of IDM may be made for improving risk assessment of contaminated soil sites, and evaluating progress and effectiveness of various remediation practices. Future studies may explore additional application scenarios for IDM, such as bioavailability measurement for other HOCs and other non-target organisms, influence of environmental factors on bioavailability, and risk assessment of oral and inhalation bioavailability of particle-associated HOCs.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.01.108.

particle-bound HOCs in the gut fluids were observed (Ahrens et al., 2001; Mayer et al., 2007). Therefore, the actual bioaccumulation of HOCs in earthworm was likely greater than that estimated by passive diffusion, suggesting that the accessibility-oriented methods such as IDM or Tenax extraction may provide more accurate estimates for HOC bioavailability to soil-dwelling organisms such as earthworm. Other studies have also investigated relationship between the C_{free} and bioaccessible fraction. For example, a correlation was previously reported between SPME-derived concentrations and the reversible desorption for bifenthrin and permethrin, and the slope of the linear regression also deviated from 1 (Harwood et al., 2012).

In practice, the application of IDM involves only a few easy and convenient steps, i.e., addition of stable isotope-labeled analogues, mixing the sample for a short time interval (48 h), and phase separation using either centrifugation or filtration. These steps are similar to protocols used in sample preparation of most research and commercial laboratories. In addition, stable isotope-labeled compounds are routinely used as internal standards or recovery surrogates in analysis. Therefore, the use of IDM for bioavailability measurement is highly feasible, and likely requires only minor modifications to existing protocol. Compared to IDM, a lengthy series of extractions is usually employed for Tenax desorption test, which is laborious- and time-consuming (Cornelissen et al., 1997; You et al., 2011). In light of this disadvantage, a single time-interval desorption (6 h or 24 h) has been proposed in the application of Tenax-assisted extraction (Cornelissen et al., 2001; Harwood et al., 2012). However, the results have been found to depend closely on the properties of sample matrix and HOCs (Cui et al., 2013). Additionally, the amount of Tenax beads should be sufficiently high to maintain the desorption rate of HOCs (Nutile et al., 2017). Taken

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