Utilizing thin-film solid-phase extraction to assess the effect of organic carbon amendments on the bioavailability of DDT and dieldrin to earthworms


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Abstract
Improved approaches are needed to assess bioavailability of hydrophobic organic compounds in contaminated soils. Performance of thin-film solid-phase extraction (TF-SPE) using vials coated with ethylene vinyl acetate was compared to earthworm bioassay (Lumbricus terrestris). A DDT and dieldrin contaminated soil was amended with four organic carbon materials to assess the change in bioavailability. Addition of organic carbon significantly lowered bioavailability for all compounds except for 4,4'-DDT. Equilibrium concentrations of compounds in the polymer were correlated with uptake by earthworms after 48d exposure ($R^2 = 0.97; p < 0.001$), indicating TF-SPE provided an accurate uptake simulation. Bioavailability of residues in soil was compared with a spiked soil aged for 90d in laboratory. Dieldrin and DDX were respectively 18% and 11% less bioavailable in contaminated soil relative to spiked soil despite >40yr of aging. Results show that TF-SPE can be useful in examining potential risks associated with contaminated soils and to test effectiveness of remediation efforts.

1. Introduction

In assessing potential ecosystem exposure to hydrophobic pollutants, conservative estimates assume that 100% of soil-borne chemicals are available; however, bioavailability measurements are more appropriate than total concentration (Alexander, 2000; Di Toro et al., 1991). Bioavailability is frequently used to establish clean-up goals in historically-contaminated sites (Ehlers and Luthy, 2003). Although a variety of methods have been developed to assess bioavailability, no one method has been established as the most appropriate, therefore the acquisition of data for risk assessments remains difficult (Reichenberg and Mayer, 2006). Hydrophobic contaminant bioavailability is typically obtained by measuring contaminant concentrations in organisms dwelling within the contaminated medium (Lanno et al., 2004) and by using a chemical assay to extract the bioavailable portion from the contaminated medium (Mäempää et al., 2011; Tang et al., 1999). The latter usually involves mild solvent extractions, Tenax®-aided desorption, cyclodextrin extraction, and solid-phase micro-extractions (Hunter et al., 2011). To ensure that this chemical extraction is representative of bioavailability, measurements are then compared to organism concentrations. Some methods estimate bioavailability using fugacity (Mäempää et al., 2011; Reichenberg and Mayer, 2006; Wilcockson and Gobas, 2001) or the tendency of a molecule to escape from a physical phase (Mackay, 1979). Fugacity ($f_c$, Pa) of a chemical in soil is calculated as the ratio between the soil concentration ($C_s$, mol/m$^3$) and the sorptive capacity ($Z_s$, mol m$^{-3}$ Pa$^{-1}$) of the soil for a specific chemical ($f_c = C_s/Z_s$) (Golding et al., 2008). Methods used to
estimate bioavailability also rely on the equilibrium partitioning theory (Di Toro et al., 1991), which assumes that given sufficient time, equilibrium is reached between environmental phases. Equilibrium is controlled by differences in fugacities of each environmental phase, and if two or more phases are present, contaminants move between phases until fugacities in all phases are equal.

We evaluated the suitability of thin-film solid-phase extraction (TF-SPE) to assess the bioavailability of soil-bound pesticides to earthworms, which to our knowledge has never been evaluated before. TF-SPE assumes that the fugacity of a chemical in the soil \( (f_s) \) is equal to the fugacity of the chemical in the ethylene vinyl acetate (EVA) \( (f_{EVA}) \) (Eq. (1)).

\[
f_s = f_{EVA} = \frac{C_s}{Z_s} = \frac{C_{EVA}}{Z_{EVA}}
\]

Although useful in some instances, calculating fugacity may not be necessary as the measured concentration in the EVA film can be used as a surrogate for fugacity (Meloche et al., 2009). For example, in comparing two soils: 1 and 2, \( C_{EVA}/C_{L,1} \) equals \( Z_{EVA}/Z_1 \) and \( C_{EVA,2}/C_{L,2} \) equals \( Z_{EVA}/Z_2 \) and since \( Z_{EVA} \) is the same, the ratio of the chemical concentration in the EVA over that in the soil is inversely proportional to the sorptive capacity of the soil. Consequently, a higher ratio indicates a lower sorptive capacity or a higher bioavailability in the specific soil.

Soil was obtained from a former orchard that received multiple applications of DDT and dieldrin prior to the 1970s. The soil contained total DDT-related compound concentrations of 2–10 ppm, and dieldrin concentrations of 1–2 ppm, providing an excellent opportunity to test the performance of the TF-SPE method with aged pesticide residues. The first objective of the present study was to compare the TF-SPE method (Meloche et al., 2009) to an earthworm (Lumbricus terrestris) bioassay to predict bioavailability of dieldrin, 4,4'-DDT, 4,4'-DDE, and 4,4'-DDD in soil. The second objective was to test, utilizing TF-SPE and bioassay, the effect of four types of organic amendments on the bioavailability of contaminants to earthworms.

Thirdly, TF-SPE was used to assess differences in the bioavailability of aged dieldrin, 4,4'-DDT, and 4,4'-DDE residues (<40 yr) with the bioavailability of the same compounds which have been laboratory-aged for three months in an uncontaminated soil. Results are expected to further the development of rapid screening approaches for contaminated sites and to provide further information on the bioavailability of aged DDT and dieldrin residues in soils.

2. Materials and methods

2.1. Soil collection and organic carbon amendments

A surface soil sample (15-cm depth) of approximately 2 m² was collected in March 2011 from an abandoned orchard in Beltsville, Maryland, USA that had received routine DDT and dieldrin insecticide applications prior to the 1970s. Details of collection and processing are provided in supplementary materials (SM1). Briefly, the control soil sample was sieved (4 mm), well mixed, and stored at room temperature for 67 d prior to use. The soil series consisted of a gradation between Sassafras and Croom sandy loams and had an organic carbon (OC) content of 2.43% (n = 14).

Aliquots of the orchard soil sample (denoted as Control in the text and figures) were mixed with four organic amendments at a 5% rate by dry mass: dairy manure compost, aged four months; dairy manure compost, aged two years; Orgro®, a biosolids compost; and pine biochar pyrolyzed at 500 °C. The OC content of the mixed amended soils was: 4-mo. Compost (Soil 1), 3.34 ± 0.23% (n = 10); 2-yr. Compost (Soil 2), 3.31 ± 0.22% (n = 9); Biosolids Compost (Soil 3), 3.70 ± 0.46% (n = 10); and Biochar (Soil 4), 6.69 ± 0.83% (n = 10).

The purpose of using the two different composts was to observe the effects of material that were different in respect to the stability, OC content, and also macro and micro nutrients (data not shown).

2.2. Earthworm exposure

Earthworms (Lumbricus terrestris) (Wholesale Bait, Hamilton, OH USA) were introduced onto the pots containing control and amended soils. Details of collection and processing are provided in supplementary materials (SM2). Briefly, 12 earthworms were added to each of the 4-L polyethylene pots for each treatment. Mesh was put around the pots and on the bottom drain holes to prevent worms from escaping. Pots were held in a temperature- and light-controlled chamber at 15/10 °C day/night and 16-hr days; pots were watered to water holding capacity daily. Earthworms were exposed to the contaminated soil for 48 d, given that earlier data shows earthworms reach equilibrium with soil after 30 d (Edwards and Jeffs, 1974). After exposure, earthworms were removed from the soil, washed with deionized water, weighed, and stored non-depurated at –20 °C until processing. Survivability averaged 92 ± 9.6% in the control and 74 ± 10% in amended soils. Earthworm samples were freeze-dried, weighed, and ground prior to extraction.

2.3. Thin-film solid-phase extraction

TF-SPE experiments were carried out in parallel with earthworm exposure experiments, utilizing the same soil mixtures; coated vials were kept in the same temperature-controlled chamber. The polymer film and coated vials were prepared as described previously (Meloche et al., 2009). In brief, the inside surface of 20-ml glass vials (Fisher Scientific, Fair Lawn, NJ, USA) were coated with a thin film of polymer using 250 μL of a 6.21-g/L solution of EVA (ELVAX® 240 resin, DuPont, Wilmington, DE, USA) containing 28% vinyl acetate (density = 0.951 g/cm³) and 5 μL of a 2% solution of dichloromethyilsilane (CAS# 75-78-5, Chemtrec, Falls Church, VA, USA). The 0.55-μm thick polymer film was created by allowing the dichloromethane to evaporate from the vial. Coated vials were heated to 40 °C for one hour prior to use.

For control and each amended soil, 26 coated vials were prepared with soil and four remained empty as blanks. Thirty grams of the soil (unamended or amended) were added to each coated vial; deionized water was added to achieve 33% moisture content. Duplicate vials were capped and incubated in the growth chamber for 30 min, one, two, four, eight hours (h), one, two, four, eight, 15, 25, 35, and 45 days (d) and extracted; blanks were extracted at eight d and 45 d. Vials were sacrificed at different times to observe uptake of contaminants by the polymer film.

After each incubation period, soil and water were removed from the vials, which were rinsed with approximately 10 mL of deionized water until all soil residues were removed and only the polymer film remained. Vials were centrifuged for four minutes at 3000 rpm, allowing the remaining water in the vials to accumulate in the bottom. Water was removed using a syringe, 500 μL of hexane was added to extract pesticide residues from the polymer, and vials were transferred to a roller mixer and allowed to rotate for five minutes at 60 rpm. The extract was transferred to a 1-ml glass vial, and the extraction was repeated once. The final volume in the analysis vial was adjusted to 1 mL and 40 μL of a 500 ppm 13C-labeled 4,4'-DDT solution was added as an internal standard.

2.4. Assessment of long term and short term aging on availability

To evaluate bioavailability differences between pesticide residues naturally aged in the field with the same compounds in a
laboratory-aged soil, a sample from a nearby field with undetectable amounts of contaminants was spiked with 1570 µg of dieldrin; 5120 µg of 4,4′-DDT; 3210 µg of 4,4′-DDE; and 1420 µg of 4,4′-DDD to achieve similar concentrations to those found in the control (orchard) soil. The spiked soil was mixed for 15 h and aged in a closed container for three months at room temperature to allow the analytes to be absorbed into the soil matrix. The spiked soil was transferred to coated vials and incubated at same conditions as control soil for 15 and 30 min, one, two, four, and eight h, one, two, four, eight, 15, 20, 30, and 40 d, and then processed as above. Details of soil sample collection and preparation are provided in Supplementary materials (SM3).

2.6. Statistical analysis methods

Dunnet's test was used to evaluate differences in soil concentration and bioaccumulation factors (BAFs) for control and amended soils. Pearson's test was used to analyze correlation between BAFs and partition coefficients. All tests were performed using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA, USA).

3. Results and discussion

3.1. Hydrophobic contaminants in soil

Concentrations in control soil before earthworms were introduced were 4.76 ± 0.20, 3.44 ± 0.18, 0.90 ± 0.01, and 1.73 ± 0.06 µg/g (d.w.) for 4,4′-DDT, 4,4′-DDE, 4,4′-DDD, and dieldrin, respectively. Results were in the same range as results reported by Gaw et al. (2012), and one order of magnitude higher than White et al. (2007) results. Although contaminant concentration in amendments was below detection limit, concentration in some amended soils were different (p < 0.05) from the control for some compounds (Fig. 1 and Table S1). The amended soils were generally higher concentration than the control soils, but some were less. These differences likely represent the difficulty in getting a completely mixed bulk soil sample. However, identical exposure concentrations are not required to measure bioavailability, and all amended soils were within 1–2 µg/g of the control concentrations.

3.2. Bioaccumulation in earthworms

The bioaccumulation factor (BAF), defined as the ratio of chemical residue concentration in earthworms (Table S2) to concentration in soil, was calculated for control and amended soils on a dry weight and a lipid normalized basis (Figs. 2A, B). For the control soil, BAFs of 4,4′-DDE and 4,4′-DDD were significantly higher (p < 0.05) than 4,4′-DDT by approximately a factor of 4 despite the similarity in log Kow values of 6.39, 6.93,
and 6.33 for 4,4’-DDE, 4,4’-DDE, and 4,4’-DDD, respectively (Shen and Wania, 2005) (Fig. 2A). Although dieldrin is less hydrophobic (log $K_{ow} = 5.48$), its BAF was also larger than 4,4’-DDT by approximately a factor of 2. BAF results for the four compounds did not correlate with hydrophobicity (log $K_{ow}$) ($p > 0.05$), suggesting that other factors also influenced the BAFs. Biodegradation of 4,4’-DDT and the formation of the metabolites during the experiment could be contributing to higher observed bioavailability of the degradation products. However, this process would not explain higher BAFs for dieldrin as compared to 4,4’-DDT. BAFs measured in the current work were comparable to previous studies where earthworms were exposed to aged DDT residues (Gaw et al., 2012; Jafvert et al., 1997). Bioavailability of 4,4’-DDE and 4,4’-DDD were higher than 4,4’-DDT for mussels living in DDT contaminated sediments (Tomaszewski et al., 2008). In a 46-d study of purge-induced desorption from a DDT-contaminated sediment, 4,4’-DDT had a total percentage removal of only 22% compared to 75 and 58% for 4,4’-DDE, and 4,4’-DDD, indicating a large resistance to desorption for the parent compound (Jafvert et al., 1997). Results obtained by other groups with contaminated soil or sediment support results of the present study, suggesting the interactions between contaminants and environmental matrices are sometimes difficult to predict.

For amended soils, dry weight BAFs for 4,4’-DDE and 4,4’-DDD, were 17–37% and 42–80% lower ($p < 0.05$), respectively, than the control soil, suggesting that organic carbon addition reduced bioavailability to earthworms (Fig. 2A). For dieldrin, in comparison to the control, only Soil 2 produced a statistically smaller BAF, whereas Soil 4 produced a larger BAF ($p < 0.05$). For 4,4’-DDT, BAFs of Soils 2, 3, and 4 were also larger ($p < 0.05$) than in control soil, indicating an increase in bioavailability. Examination of lipid-normalized BAF values revealed trends similar to dry weight values. However the reductions in BAF values in amended soils relative to the control were more pronounced for 4,4’-DDE and 4,4’-DDD (Fig. 2B). For dieldrin, all amended soils were lower than the control except for soil 4. For 4,4’-DDT, only soil 1 had a statistically lower BAF value than the control with 2, 3, and 4 being either equal to or higher.

Organic amendments have been shown to increase sorption and reduce bioavailability of herbicides and polycyclic aromatic hydrocarbons (Gomez-Eyles et al., 2011; Spokas et al., 2009); therefore, increased 4,4’-DDT bioavailability in our study was unexpected. However, this result is not unique. Tomaszewski et al. (2008) treated contaminated sediments with activated carbon and observed a decreased 4,4’-DDE and 4,4’-DDD bioavailability while 4,4’-DDT bioavailability was slightly larger in comparison to control sediment, thereby supporting our findings. There is a clear difference in the interaction between 4,4’-DDE and 4,4’-DDD and the organic amendments when compared with 4,4’-DDT. 4,4’-DDT is held much more strongly in the soil matrix (Jafvert et al., 1997) and organic amendments may create an environment which allows 4,4’-DDT to be more bioavailable for a given period of time. After addition of an organic amendment, 4,4’-DDT may partition more slowly than its metabolites from the soil into the added organic matter from amendments, making it more available during the timeframe of our experiment. This effect would result in an apparent increase in its bioavailability. It may be that if our earthworm experiment were extended, or if the soil were given more time to stabilize with amendments before earthworms were inserted, we may have observed a decrease in 4,4’-DDT BAF compared to the control. A more in-depth experiment is required to clarify this issue. Results of the present study illustrate the complexity of interactions between aged soil residues, organic carbon amendments and the bioaccumulation of hydrophobic compounds by organisms.

### 3.3. Comparison of TF-SPE with earthworm bioavailability

TF-SPE was utilized to estimate the bioavailability of hydrophobic organic compounds using subsamples of the control and amended soils in polymer-coated vials. Time-dependent EVA concentrations from the TF-SPE experiment were fitted with mathematical models (Fig. 3; Table S3) and resulted in polymer phase equilibrium concentrations, which were utilized for further analysis. One-phase (Eq. (2)) and two-phase (Eq. (3)) nonlinear models were tested on measured solvent polymer concentration,
Fig. 3. Dieldrin, 4,4'-DDT, and 4,4'-DDE polymer concentrations in solvent C_{EVA} (µg/mL) versus incubation time t (h) for control and amended soils. Dieldrin, DDX, 4,4'-DDT, 4,4'-DDE, and 4,4'-DDE C_{EVA} (µg/mL) versus incubation time t (h) for the spiked soil. Lines are the model fits from either two-phase or one-phase models (Eq. (2), Eq. (3)).

C_{EVA} (µg/mL), versus incubation time, t (h), and the best fit was chosen by comparing the R^2 values obtained (Meloche et al., 2009).

\[ C_{EVA} = C_{EVA(eq)} \left(1 - e^{-kt}\right) \]  
\[ C_{EVA} = C_{EVA(eq)}^{fast} \left(1 - e^{-k_{fast}t}\right) + C_{EVA(eq)}^{slow} \left(1 - e^{-k_{slow}t}\right) \]

The two-phase model was the best fit for dieldrin (R^2 = 0.95–0.98), for 4,4'-DDE (R^2 = 0.98–1.00), and for 66% of the 4,4'-DDT datasets (R^2 = 0.95–0.99). The one-phase model was used for 34% of the datasets for 4,4'-DDT (R^2 = 0.89–0.96). For these datasets, equilibrium was not reached by the end of the experiment, and a two-phase transfer could not be distinguished. Polymer concentrations of 4,4'-DDD were below analytical detection limits and could not confidently be used to calculate equilibrium concentrations.

Model-calculated C_{EVA(eq)} were normalized to soil concentration to examine the effect of organic carbon amendments on the uptake of DDT and dieldrin by the polymer, or simulated bioavailability. For dieldrin, model results predicted that addition of organic amendments significantly reduced bioavailability (p < 0.01) relative to the control, except for biochar-amended soil (soil 4), which showed no change in bioavailability (Fig. 2C), agreeing with BAFs results in earthworms (Fig. 2A). Wang et al. (2012) reported that a biochar with 566 m^2 g^-1 of surface area was more effective in reducing bioavailability of the pesticide chlorantraniprole residue in soils than a biochar with 27 m^2 g^-1 of surface area. The surface area of the pine biochar used in the current study was 10 times lower at 2.77 m^2 g^-1 and was likely less efficient in adsorbing residues. Simulated bioavailability in amended soils were lower than control soil for 4,4'-DDE but higher for 4,4'-DDT, which was also observed in BAFs measured in earthworms.

The relationship between the equilibrium polymer concentration and the chemical residue concentration in earthworm was evaluated (Fig. 4A). Linear regression of the log of the concentrations was unsatisfactory; R^2 was equal to 0.53 and 0.61 for the lipid and dry weight normalized data, respectively. Thus, the TF-SPE method was unable to predict earthworm concentrations for all chemicals effectively. C_{EVA(eq)/soil} values for 4,4'-DDT were generally higher than lipid-normalized BAF values, while C_{EVA(eq)/soil} values for 4,4'-DDE were lower than lipid-normalized BAFs (Fig. 2). For the control soil, 4,4'-DDT C_{EVA(eq)/soil} values were 46% higher than lipid-normalized BAF values, suggesting that some degradation of 4,4'-DDT could have occurred in the earthworm assay (Davis and French, 1969; Gaw et al., 2012). Also, C_{EVA(eq)/soil} values for 4,4'-DDE were 19% lower than lipid-normalized BAF values, suggesting that the degradation of 4,4'-DDT in the earthworm assay

Fig. 4. Relationship between the log of equilibrium residue polymer concentration log C_{EVA(eq)} (µg/g) and the log of Lumbricus terrestris earthworm concentration on a lipid basis log C_{worm} (µg/g lipid) (white symbols) and on a dry weight basis log C_{worm} (µg/g) (black symbols) for (A) dieldrin, DDE, and DDT and for (B) dieldrin and DDX (5µg DDT, DDE) in control and amended soils. Dashed line is 1:1. Solid lines are the linear fit of the datasets: A – R^2 = 0.53 for lipid-based and R^2 = 0.61 for dry weight based; B – R^2 = 0.92 for lipid based and R^2 = 0.97 for dry-weight based.
generated approximately 19% of 4,4'-DDE and 27% other degradation products.

To address this finding, the linear regression of the log of the concentrations were recalculated based on the combined concentrations of 4,4'-DDT and 4,4'-DDE (DDX) (Fig. 4B). Correlation of the log concentrations was examined using concentrations based on dry weight (log $C_{\text{worm}}$ [µg/g]) = 0.90 log $EVA_{\text{eq}}$ [µg/g] – 1.20 ($R^2 = 0.97$; $p < 0.001$). Results suggest that the polymer film was 22 times more efficient in accumulating residues from soil than L. terrestris earthworms. Similar results were observed for organochlorines in clams (Maconoma balthica) (Melcho et al., 2009), where polymer was 7.5 times more sorptive than the lipids present in clams. However, a near 1:1 relationship was found when the concentrations were normalized on a lipid basis (log $C_{\text{worm}}$ [µg/g lipid]) = 0.89 log $EVA_{\text{eq}}$ [µg/g] – 0.10 ($R^2 = 0.92$; $p < 0.001$). This indicates that the equilibrium concentration in the polymer was similar to the concentration in the earthworm lipid, and suggests that the polymer and the L. terrestris lipid content have proportional sorptive capacities.

3.4. Historically versus artificially-aged soil residues

Organic pollutants in soils are expected to become less bioavailable available with time (Alexander, 2000 and references therein). The TF-SPE method was tested for sensitivity to differences in historical field-aged versus laboratory-aged residues. Polymer-coated vials were prepared with spiked soil that had been aged for three months in the laboratory, and uptake by the polymer was measured as in the earlier experiment. Results were compared with those from the historically contaminated control soil. The $EVA_{\text{eq}}$ uptake curves in the spiked soil indicated that the residues were much more available to undergo degradation during the course of the experiment as compared to the field-aged soil (Fig. 3). For spiked soil, polymer concentrations of 4,4'-DDT increased until approximately day 8, followed by a decline and an increase in the concentrations of 4,4'-DDD and 4,4'-DDE. Degradation of 4,4'-DDT to 4,4'-DDE or to 4,4'-DDE could have occurred via elimination or reductive dechlorination, respectively, inside the polymer coated vial (Brooks, 1974; Plimmer et al., 1968).

Calculation of $EVA_{\text{eq}}$ Values for the DDT compounds individually in the spiked soil was not possible because concentration of these compounds continued to change throughout the experiment. Therefore, the models (Eq. (3)) were applied using DDX (Σ DDT, DDE, DDD) concentrations. $EVA_{\text{eq}}$ [g/g] Residuals for field-aged residues and the spiked residues were 75.5 ± 3.8 and 110 ± 0, respectively, for dieldrin and 68.0 ± 2.7 and 85.4 ± 4.5, respectively, for DDX. Results equate to approximately 18% and 11% lower bioavailability of dieldrin and DDX, respectively, in the orchard soil compared to the spiked soil. However, ratio averages for DDX were not statistically different between treatments. From this experiment, it appears that the dieldrin and DDT residues that have been present in the orchard soil for more than 40 years are only moderately less bioavailable than a soil that has aged for only 3 months.

4. Conclusions

Because bioassays can be costly and time consuming, a chemical based methodology can be useful to assess the effectiveness of possible remediation actions. The present study illustrates that the TF-SPE methodology can be used to assess the bioavailability of soil-bound hydrophobic organic chemicals to earthworms. Measured soil-polymer equilibrium concentration ratios for DDT + DDE and dieldrin correlated strongly with measured earthworm bioaccumulation factors using the same soils. Our results demonstrate that the polymer coating was similar in sorptive capacity to lipids found in L. terrestris. While further experiments with additional earthworm species may be needed, results indicate this approach may be used as a screening tool to detect differences in bioavailability of hydrophobic compounds in soil.

Our results also indicate that bioavailability predictions based on a single chemical property like log $K_{\text{ow}}$ should be used with caution. It appears that differences in sorption characteristics of the different contaminants led to minimal reduction in bioavailability of 4,4'-DDT in treated soils within the timeframe of this experiment. Comparison of pesticide bioavailability in the orchard soil with a laboratory spiked soil suggests that aged residues were largely available for uptake by earthworms. Further long-term experiments are required to evaluate the effectiveness of organic carbon amendments at reducing bioavailability of contaminants in soil.

Disclaimer

Mention of specific products is for identification and does not imply endorsement by the US Department of Agriculture or the US Geological Survey to the exclusion of other suitable products or suppliers.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2013.11.008.

References


