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Comparison of Three Extraction Methods for 17β-Estradiol in Sand, Bentonite, and Organic-Rich Silt Loam

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Extraction is an important procedure for samples that contain soil because other compounds in soil may affect analysis of estrogens. This study was conducted to evaluate three different extraction methods for 17β-estradiol in soil. Sand, bentonite, and organic-rich silt loam were spiked with 1 mg kg^{-1} of 17β-estradiol as a model compound of estrogens. 17β-estradiol and its metabolites, estrone and estriol, were extracted using (i) a modified Bligh and Dyer extraction, (ii) a pressurized fluid extraction, and (iii) a diethyl ether extraction, and measured by liquid chromatography tandem mass spectrometry. There were significant differences in the extraction efficiency for 17β-estradiol among the extraction methods and the soils: the efficiencies ranged from 10% to 97%. Overall, the diethyl ether extraction method had the largest efficiency of 17β-estradiol with 45% and 57% for bentonite and silt loam, respectively. Transformation of 17β-estradiol to estrone and estriol in the different extraction methods was less than 3.6% during the extraction procedures. This study underlined the importance of sample preparation for estrogen analysis in soil samples.

Key Words: 17β-estradiol; Estrone; Estriol; Extraction efficiency; Liquid chromatography; Tandem mass spectrometry.

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INTRODUCTION

Endocrine disrupting chemicals (EDCs) are defined as chemicals that can induce adverse health effects by disrupting an organism's endocrine system or normal development in vivo.[1] The United States Geological Survey reported that 95 different organic wastewater contaminants, including EDCs (i.e., 17β-estradiol, estrone, and estriol), were detected in water from 139 streams in 30 U.S. states during 1999 and 2000.[2] Finlay-Moore, Hartel, and Cabrera[3] also reported that concentrations of 17β-estradiol reached 150–2300 ng L^{-1} in the runoff from a field in which manure had been applied, indicating that a great number of people and wildlife can be impacted by exposure to EDCs through soil-water systems. Although the human health risk associated with environmental exposure to EDCs is not clear, the compounds have been shown to induce estrogenic responses in fish at extremely low concentrations of less than 1 ng L^{-1}.[4]

With respect to analysis, the need for a sensitive, comprehensive, rapid, and accurate detection method for estrogens has been emphasized in environmental studies because contaminants are present at extremely low concentrations and coexist with compounds that can interfere with the quantitative analysis, e.g., water-soluble organic materials and Ca^{2+} and Mg^{2+} ions.[5–7] Therefore, many sample preparation steps are required in order to concentrate samples and to avoid interference with other compounds before analysis. At the sample preparation stage, extracting estrogens from soil samples is difficult because estrogens have low water solubility (0.8–13.3 mg L^{-1}) and these are moderately hydrophobic compounds (log K_{ow} 2.6–4.0).[8–9] Estrogens are strongly sorbed to soil and desorption is limited. In previous studies, Colucci, Bork, and Topp[10] reported that less than 40% of ^{14}C-17β-estradiol was extracted from soils after 12 h of treatment. However, mineralization of 17β-estradiol in soils occurred slowly in their study (only 15% of 17β-estradiol was released as ^{14}C-CO_2 in three months), and they concluded that the rest of 17β-estradiol remained in soils as a nonextractable form of 17β-estradiol or its metabolites.

The most common extraction methods for target contaminants in environmental samples are solid-lipid extractions (i.e., Bligh and Dyer extraction[11] and pressurized liquid extraction), solid-phase extraction, Soxhlet extraction, (ultra) sonication, microwave extraction, and supercritical fluid extraction. In solid-lipid extractions, a solvent dissolves a target compound with a similar polarity and moves it from a complex matrix to the solvent, or from the solvent to another solvent, during multistep extraction. This method was first reported by Folch et al.[12] who used a solvent mixture of chloroform and methanol and then purified the extracts with aqueous KCl solution. Bligh and Dyer[11] modified the Folch et al.[12] method to improve extraction and purification efficiencies for total lipid and develop a one-phase, initial, and rapid method. Subsequently, many modified Bligh and Dyer methods are currently used as

a standard solid-lipid extraction method.[13–14] Pressurized liquid extraction, however, has also been successfully applied to the extraction for soil, sludge, and other waste samples.[15–16] Casey et al.[17] recently used a pressurized liquid extraction method to extract 17β-estradiol in soil.

The main objective of this study was to compare the commonly used solid-lipid extraction methods—(i) a modified Bligh and Dyer extraction, (ii) a pressurized liquid extraction, and (iii) a diethyl ether extraction—for the extraction efficiency of 17β-estradiol in soil. We hypothesized that diethyl ether would increase the extraction efficiency of estrogens because diethyl ether (log K_{ow} 3.2) has a similar polarity of estrogens. In this study, 17β-estradiol was used as a model compound of estrogens.

MATERIALS AND METHODS

Sand (Rex International Co., Longview, TX) and pure Southern Bentonite (American Colloidal Co., Chicago, IL) were heated at 450°C for 3 h to remove organic matter. LaDelle silt loam (fine-silty, mixed, superactive, frigid, cumulic Hapludoll) with 9.2% organic matter was dried and passed through a 2-mm sieve. To avoid microbial effects, all the soils were fumigated twice with chloroform for 24 h in the dark[18] and heated at 60°C for 24 h to remove all chloroform vapors before starting experiments.

17β-estradiol, estrone, and estriol were purchased from Sigma-Aldrich (St. Louis, MO). A stock solution of 5 mg L^{-1} 17β-estradiol was made with methanol. The stock solution (200 μL) was spiked into 10 g of each soil sample to make a concentration of 1 mg kg^{-1} of 17β-estradiol. The mass of 17β-estradiol, 10 μg, is equivalent to the mass of 17β-estradiol in approximately 6.7 g of hog manure.[9] Two replicates were made. All glassware was washed with deionized water and oven-dried for 4 h at 450°C to remove any organic contaminants.

Modified Bligh and Dyer Extraction Method

We used a single-phase chloroform and methanol buffer system designed for the total lipid extraction method.[13–14] Ten mL of chloroform, 20 mL of methanol, and 8 mL of phosphate buffer (50 mM, pH 7.4) were added to a 10-g soil sample and allowed to equilibrate for 3 h. Extraction of the single phase was collected by centrifugation at 1000 g for 20 min and by decanting into another test tube. Five mL of chloroform was used to wash the pelleted solids, which were then vortexed for 5 min and recentrifuged. The supernatant was again decanted and added to the first chloroform extract. An additional 5 mL of water was added to the extract and centrifuged at 1000 g for 20 min to separate the aqueous phase from the organic phases. The bottom layer for the organic phase, 10 mL, was pipetted into a new test tube and dried under a stream of nitrogen gas at 37°C.

Pressurized Liquid Extraction

Samples for the pressurized fluid extraction method were prepared based on a standard EPA method 3545[14] using Dionex Accelerated Solvent Extraction 200 (Sunnyvale, CA). An 11-mL stainless steel extraction cell was used for 10 g soil, and the solvent mixture acetone-hexane (1:1, v/v) was used for extraction. Sample running conditions were selected as follows: oven temperature was 100°C; system pressure was 1800 psi; static time was 5 min after a 5-min preheat equilibration; flush volume was 60% of the cell volume, followed by a 180-sec nitrogen purge at 150 psi. The extracted solution was dried under a stream of nitrogen gas at 37°C.

Diethyl Ether Extraction Method

A 10-g soil sample was added to a test tube with 25 mL of diethyl ether.[19] The soil sample was vortexed for 5 min and centrifuged at 1000 g, 1500 rpm, for 20 min. Aliquots of the organic layer, 10 mL, were collected and filtered through glass wool packed in the bottom of a pipette. The extracted solution was dried under a stream of nitrogen gas at 37°C.

Analysis of 17β-Estradiol, Estrone, and Estriol

For the analysis of 17β-estradiol and its metabolites, all the extracted and dried samples were redissolved in 1 mL of a 1:1 (v/v) mixture of methanol and a mobile phase (30% water: 70% acetonitrile, v/v). High-performance liquid chromatography (Hewlett-Packard 1100 system) coupled with electrospray ionization and tandem mass spectrometry (PE Sciex API 365, Concord, ON, Canada) was used to measure 17β-estradiol and its metabolites, i.e., estrone and estriol. Sample separation of estrogens was performed with a 30 $\times$ 4.1 mm, 3 μm LUNA $C_{18(2)}$ column (Phenomenex Co., Torrance, CA). Flow rate of mobile phase was 50 μL min^{-1} with a gradient of 20–80% acetonitrile in water containing 0.1% ammonium hydroxide (pH 10). The column separation time was less than 2 min. A chromatogram of a standard mixture of estrogens is shown in Figure 1. 17β-estradiol ($C_{18}H_{24}O_2$), estrone ($C_{18}H_{22}O_2$), and estriol ($C_{18}H_{24}O_3$) were detected according to their molecular ions (Q1) at *m/z* 271, *m/z* 269, and *m/z* 287, respectively, and characteristic fragment ions (Q3) at *m/z* 183, *m/z* 145, and *m/z* 171, respectively (Fig. 2). It was conducted on negative electrospray ionization mode with the condition of -4200 V spray voltage and capillary temperature 425°C. The limits of quantification based on the ratio of signal to noise (S/N $\geq$ 10/1) for the compounds were 0.025, 0.010, and 0.025 mg L^{-1} for 17β-estradiol, estrone, and estriol, respectively. Good linearity of the standard calibration curves was obtained for concentrations from 0.025 to 2.0 mg L^{-1} for 17β-estradiol (R^2, 0.996), estrone (R^2, 0.999), and estriol (R^2, 0.999).

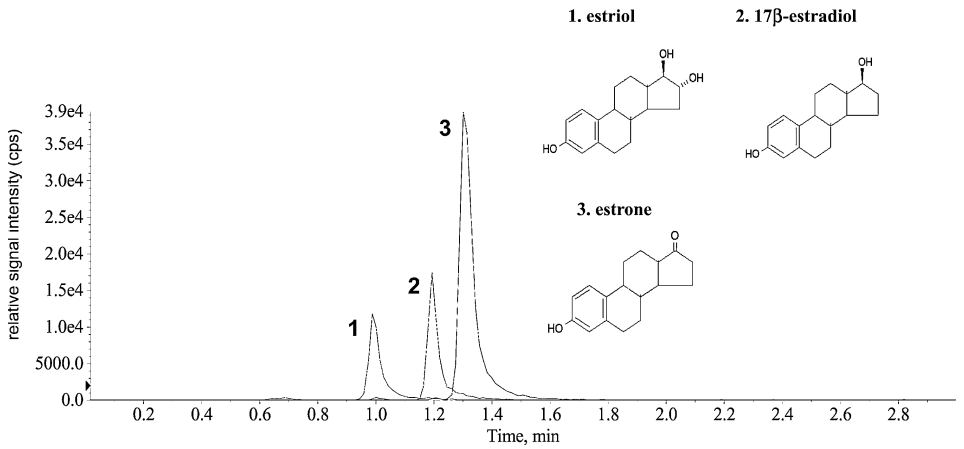

Figure 1: The structures of 17β-estradiol, estrone, and estriol, and a chromatogram of a standard estrogen mixture. We used 200 μL of a 5 mg L^{-1} standard solution of estrogens.

RESULTS

Figure 3 shows the extraction efficiencies of three different extraction methods for 17β-estradiol in soils. The extraction efficiencies ranged from 10% to 97%. For sand, the diethyl ether extraction method (92%) was as good as the pressurized fluid extraction method (97%) and was better than the modified Bligh and Dyer extraction method (78%). The diethyl ether extraction method had the largest efficiency of 17β-estradiol in bentonite and silt loam with 45% and 57%, respectively. The mass recovery in this study is consistent with previous studies that showed high sorption affinity.[10,17,20,21] In the study of Colucci, Bork, and Topp,[10] the extractable ^{14}C-17β-estradiol (1 mg kg^{-1}) rapidly decreased, and the nonextractable 17β-estradiol in loam and silt loam was 70% and 56%, respectively, following three days of incubation. They also reported that 17β-estradiol was transformed to estrone in a few hours and suggested that analysis of estrogens in environmental samples should include estrone.

One possible explanation that the diethyl ether extraction method had the largest efficiency in Figure 3 involves the similar polarities of diethyl ether and estrogens. We believe that 17β-estradiol was more selectively extracted by diethyl ether than by other solvents. Again, estrogens are moderately hydrophobic compounds and have a polarity similar to diethyl ether compared to the solvents in the Bligh and Dyer extraction method and the pressurized fluid extraction method. However, the presence of water-soluble organic materials in the environment, such as fulvic and humic acids, is one of the reasons why estrogens can be overestimated when nonspecific methods of detection are used to detect the compounds.[5] Therefore, it is strongly recommended that analysis of estrogens in environmental samples should be based on a mass spectrometry

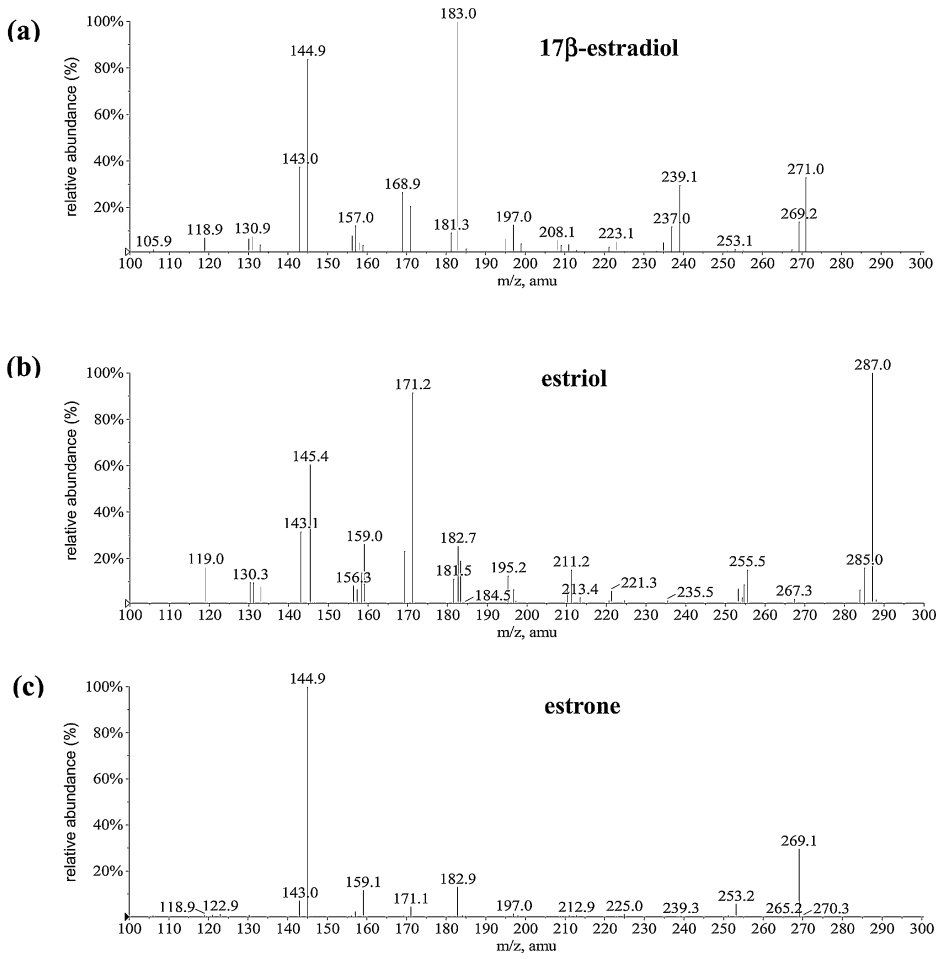

Figure 2: Product-ion-scan mass spectra of (*a*) 17β-estradiol, (*b*) estriol, and (*c*) estrone.

system, such as LC-MS/MS.[5,22] When nonspecific methods (e.g., spectroscopy) are used, diethyl ether can reduce interference with the hydrophilic water-soluble organic materials in soil and thus minimize their overestimation.

Table 1 shows the relative concentrations of estrone and estriol in this study. The total of the metabolites in the three extraction methods was less than 3.6% of the initial concentration of 17β-estradiol. Thus, transformation of 17β-estradiol to estrone or estriol did not significantly affect the extraction efficiency of the three methods. The rest of the 17β-estradiol must have remained in the soils and was not extracted by any of the three extraction methods. Aging is thought to be an explanation for the nonextractable 17β-estradiol in Table 1, and passive processes of aging include a number of intra-soil processes: sorption onto soil particles, diffusion into spatially remote areas such as soil micropores,

Table 1: Extraction efficiency for estrone and estriol in sand, bentonite, and silt loam and the total amount of nonextractable 17β-estradiol in the soils ($n = 2$). The initial concentration of 17β-estradiol in the soils was 1 mg kg^{-1}

Extraction method	Soil type	17β-estradiol metabolites (%)				Total of 17β-estradiol metabolites (%)	The amount of nonextractable 17β-estradiol (%)[§]
		Estrone	RSD[†]	Estriol	RSD		
Modified Bligh and Dyer	Sand	0.7	±0.04	2.5	±0.01	3.2	19
	Bentonite	0.5	±0.00	1.4	±0.02	1.9	58
	Silt loam	0.2	±0.01	ND[‡]	ND	0.2	81
Pressurized fluid extraction	Sand	0.8	±0.02	0.7	±0.02	1.5	2
	Bentonite	0.1	±0.05	ND	ND	0.1	90
	Silt loam	0.2	±0.04	ND	ND	0.2	86
Diethyl ether	Sand	0.6	±0.02	3.0	±0.42	3.6	4
	Bentonite	0.4	±0.18	3.0	±0.41	3.4	52
	Silt loam	0.5	±0.03	ND	ND	0.5	43

[†]Relative standard deviation (RSD) was estimated as (the standard deviation/mean 17β-estradiol concentration in each extraction method) $\times$100.
ND, not detected.
[§]Total of nonextractable 17β-estradiol was estimated as 1-(the sum of concentrations of 17β-estradiol, estrone, and estriol in each extraction method) $\times$ 100.

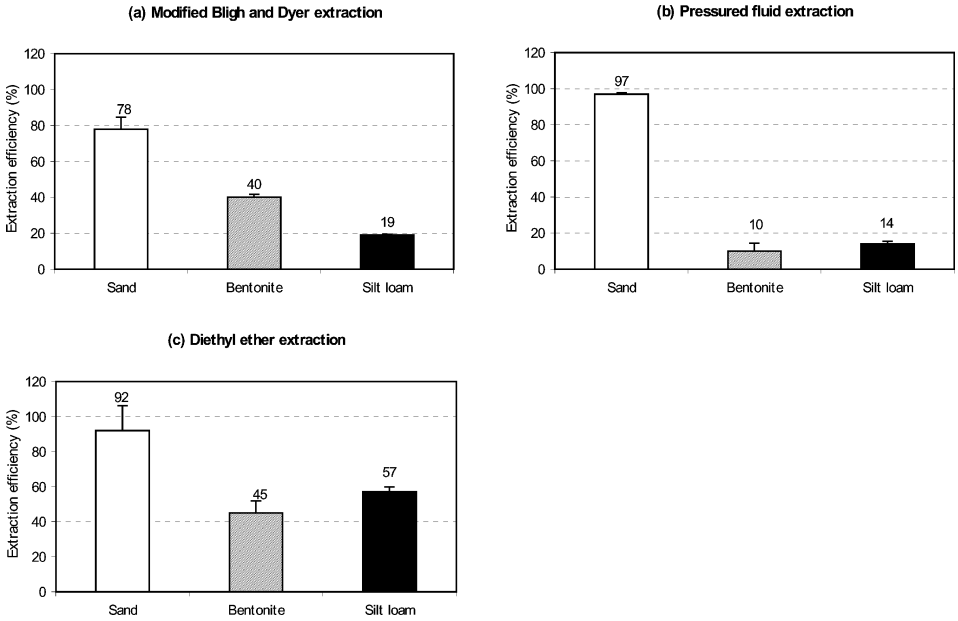

Figure 3: Extraction efficiency (%) for 17β-estradiol from sand, bentonite, and LaDelle silt loam spiked with 1 mg kg^{-1} 17β-estradiol.

and entrapment within soil organic matter.[23] Casey et al.[17] reported that the high sorption affinity of 17β-estradiol appeared to be associated with the surface area and/or the cation exchange capacity of soils. Further study including the aging effect will be necessary to establish a standard extraction method of estrogens in soil samples.

This study shows the importance of sample preparation and extraction methods for estrogen analysis in soil, although the spiked concentration of 17β-estradiol was higher than in real-world samples and, thus, our results may not be representative of all soils. We also used disturbed soils (e.g., dried, sieved, heat-treated, autoclaved) in this study to mainly focus on comparing the solvent effect on extraction efficiency. However, we believe that this study provides important preliminary data with respect to the analysis of estrogens in soil and water systems.

CONCLUSION

There is a great need to develop and evaluate a standardized analysis method for estrogens in soil/water samples in order to systematically determine the fate and transport of estrogens. We evaluated three different methods for their extraction efficiencies for 17β-estradiol in soil samples. The three methods showed significantly different extraction efficiencies in various soils, implying the importance of extraction procedures at the sample preparation stage for

quantitative analysis of estrogens in environmental study. Overall, we showed the possibility of increasing the efficiency of estrogens in soil samples with diethyl ether extraction.

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REFERENCES

1. Ashby, J.; Houthoff, E.; Kennedy, S.K.; Stevens, J.; Bars, R.; Jekat, F.W.; Campbell, P.; Van Miller, J.; Carpanini, F.M.; Randall, G.L.P. The challenge posed by endocrine-disrupting chemicals. Environ. Health Perspect. **1997**, *105*, 164–169.

2. Kolpin, D.W.; Furlong, E.F.; Meyer, M.T.; Thurman, E.M.; Zaugg, S.D.; Barber, L.B.; Buxton, H.T. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. Environ. Sci. Technol. **2002**, *36*, 1202–1211.

3. Finlay-Moore, O.; Hartel, P.G.; Cabrera, M.L. 2000. 17β-estradiol and testosterone in soil and runoff from grasslands amended with broiler litter. J. Environ. Qual. **2000**, *29*, 604–661.

4. Purdom, C.E.; Hardiman, P.A.; Bye, V.J.; Eno, N.C.; Tyler, C.R.; Sumpter, J.P. Estrogenic effects of effluents from sewage treatment works. Chem. Ecol. **1994**, *8*, 275–285.

5. Erickson, B.E. Analyzing the ignored environmental contaminants. Environ. Sci. Technol. **2002**, *36*, 140–145.

6. Eckel, W.P. Comment on "Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance." Environ. Sci. Technol. **2002**, *36*, 4003.

7. López de Alda, M.J.; Diaz-Cruz, S.; Petrovic, M.; Barceló, D. Liquid chromatography–(tandem) mass spectrometry of selected emerging pollutants (steroid sex hormones, drugs and alkylphenolic surfactants) in the aquatic environment. J. Chromatogr. A. **2003**, *1000*, 503–526.

8. Lai, K.M.; Johnson, K.L.; Scrimshaw, M.D.; Lester, J.N. Binding of waterborne steroid estrogens to solid phases in river and estuarine systems. Environ. Sci. Technol. **2000**, *34*, 3890–3894.

9. Hanselman, T.A.; Graetz, D.A; Wilkie, A.C. Manure-borne estrogens as potential environmental contaminants: A review. Environ. Sci. Technol. **2003**, *37*, 5471–5478.

10. Colucci, S.M.; Bork, H.; Topp, E. Persistence of estrogenic hormones in agricultural soils: I. 17β-estradiol and estrone. J. Environ. Qual. **2001**, *30*, 2070–2076.

11. Bligh, E.G.; Dyer, W.J. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **1959**, *37*, 911–917.

12. Folch, J.; Lees, M.; Stanley, G.H.S. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. **1957**, 226, 497–509.

13. White, D.C.; Bobbie, R.J; Heron, J.S; King, J.D; Morrison, S.J. Biochemical measurements of microbial mass and activity from environmental samples. In *Native Aquatic*

Bacteria: Enumeration, Activity, and Ecology (ASTM STP 695); Costerton, J.W., Colwell, R.R., Eds.; Am. Soc. Test. Mat.: Philadelphia, PA, 1979; 69–81.

14. Peacock, A.D.; Mullen, M.D.; Ringelberg, D.B.; Tyler, D.D.; Hedrick, D.B.; Gale, P.M; White, D.C. Soil microbial community responses to dairy manure or ammonium nitrate applications, Soil Biol. Biochem. **2001**, *33*, 1011–1019.

15. U.S. Environmental Protection Agency (EPA). 1996. Method 3545. In EPA (ed.) EPA SW-846 Methods (http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3545.pdf).

16. Popp, P.; Keil, P.; Möder, M.; Paschke, A.; Thuss, U. Application of accelerated solvent extraction followed by gas chromatography, high-performance liquid chromatography and gas chromatography–mass spectrometry for the determination of polycyclic aromatic hydrocarbons, chlorinated pesticides and polychlorinated dibenzo-*p*-dioxins and dibenzofurans. J. Chromatogr. **1997**, *774*, 203–211.

17. Casey, F.X.M.; Larsen, G.L.; Hakk, H.; Šimůnek, J. Fate and transport of 17β-estradiol in soil-water systems. Environ. Sci. Technol. **2003**, *37*, 2400–2409.

18. Vance, E.D.; Brookes, D.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. Soil Bio. Biochem. **1987**, *19*, 703–707.

19. Raman, D.R.; Layton, A.C.; Moody, L.B.; Easter, J.P.; Sayler, G.S.; Burns, R.T.; Mullen, M.D. Degradation of estrogens in dairy waste solids: Effects of acidification and temperature. Transactions of ASAE **2002**, *44*, 1881–1888.

20. Lee, L.S.; Strock, T.J.; Sarmah, A.K.; Rao, P.S.C. Sorption and dissipation of testosterone, estrogens, and their primary transformation products in soils and sediment. Environ. Sci. Technol. **2003**, *37*, 4098–4105.

21. Das, B.S.; Lee, L.S.; Rao, P.S.C.; Hultgren, R.P. Sorption and degradation of steroid hormones in soils during transport: Column studies and model evaluation. Environ. Sci. Technol. **2004**, *38*, 1460–1470.

22. Petrovic, M.; Eljarrat, E.; López de Alda, M.J.; Barceló, D. Recent advances in the mass spectrometric analysis related to endocrine disrupting compounds in aquatic environmental samples. J. Chromatogr. A. **2002**, *978*, 23–51.

23. Gevao, B.; Semple, K.T.; Jones, K.C. Bound pesticide residues in soil: A review. Environ. Poll. **2000**, *108*, 3–14.