Chapter 14

Earthworms: Diagnostic Indicators of Wastewater Derived Anthropogenic Organic Contaminants in Terrestrial Environments

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Analysis of earthworms for anthropogenic organic contaminants (AOCs) offers a potential diagnostic tool for assessing the presence and transfer of AOCs from wastewater sources into terrestrial environments and biota. Earthworms and soil samples were collected from one minimally affected agricultural field (soybean, Site 1) with no known history of biosolids or manure amendment and three agricultural locations amended with municipal biosolids. The biosolid-amended sites consisted of a soybean field amended for the first time with municipal biosolids (Site 2), a hay field with an extended history of amendment with municipal biosolids (Site 3), and a grassland pasture used for cattle grazing (Site 4) with an extended history of biosolids amendment with municipal biosolids from the same source as Site 3. Forty-two of the 72 AOCs monitored in

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this study were detected in quantifiable concentrations in one or more of the biosolids, soil, or earthworm samples. In most of these samples, the biogenic sterols had the highest concentration among measured AOCs, but the biosolids and biosolid-amended samples contained a variety of AOCs indicative of human use such as the disinfectant triclosan, detergent metabolites, and the synthetic fragrances galaxolide and tonalide. A number of AOCs were detected in the earthworm tissue samples, some of which bioaccumulated to concentrations greater than found in the soils from which were collected. Unexpectedly, some AOCs were detected in the soil and earthworms from the minimally impacted Site 1. However, the relative abundance of uniquely anthropogenic contaminants, such as personal care products, is much less in the earthworms from Site 1 compared to those from the biosolids amended sites. When possible, bioaccumulation factors (BAFs) on a dry mass basis were calculated for the AOCs detected in the earthworms. Triclosan and monoethoxy-nonylphenol detergent metabolites were measured to have BAFs as high as 41.0 and 21.7, respectively. Many of the AOCs measured in the earthworm tissue samples were below detectable concentrations in the corresponding soil samples. This study documents that some AOCs present in land-applied biosolids can be transferred to earthworms and that earthworms may serve as a diagnostic tool for assessing the presence of AOCs in terrestrial environments.

Background

Municipal wastewater treatment produces liquid and solid products, treated effluent and sewage sludge, respectively. Treated effluent is generally discharged into surface water, although increasingly, treated wastewater is used as a source of nonpotable water or for indirect drinking water reuse. Treated sewage sludge that meets regulatory standards for pathogen and metal content, can be classified as biosolid. Once classified as biosolid it may be land applied as an organic carbon- and nutrient-rich soil amendment or in land-reclamation projects (*1*).

Numerous organic contaminants, including pharmaceuticals, detergents, fragrances, antimicrobials, pesticides, and industrial products have been detected in wastewater end products (*2–4*) and are collectively referred to herein as anthropogenic organic contaminants (AOCs). Many AOCs are unaltered or incompletely removed in wastewater treatment plants (WWTPs), and subsequently have been identified in the environment, especially in surface waters receiving wastewater effluent (*2*, *5–7*). Many AOCs entering WWTPs have moderate to large log K_{OW} values and can be predicted to undergo hydrophobic partitioning into the organic-rich solids phase during treatment (*8*). This prediction is consistent with recent observations of concentrations of various AOCs, such as

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detergent metabolites, synthetic fragrances, disinfectants, and pharmaceuticals, in biosolids destined for land application (*4*, *9–13*).

Various studies have raised concerns about the potential impacts of the environmental presence of AOCs on humans and wildlife including reproductive impairment, immune deficiencies, and antibiotic resistance among pathogenic bacteria (*14–22*). Research has documented uptake of various AOCs by plants and animals (*23–26*), including humans (*27–29*). Most research investigating the effects or bioaccumulation of AOCs has focused on aquatic environments and organisms (*30–33*). There is a paucity of data on the movement of these compounds into terrestrial organisms.

The U.S. Environmental Protection Agency estimates that more than 8×10^6 dry tons of biosolids are produced in the United States annually (*34*); with more than 50% of the biosolids produced each year being land applied (*1*). In Europe an estimated 37% (2.39×10^6 dry tons) of the biosolids produced are land applied each year (*35*). Biosolids are predominantly applied on agricultural soil, but are also used for large-scale landscaping, home landscaping and gardens, remediation of abandoned mining sites, and revegetation projects (*36–38*).

In addition to human biosolids, thousands of tons of animal manure are generated annually from about 92 million swine, 109 million cattle, 292 million turkeys, and 7.5 billion chickens in the United States (*39*). Like biosolids, manure is generally applied as a source of nutrients to agricultural soil. Most concerns about the practice of land application of manure have focused on the quantity of nutrients (*40*). Recently, however, other constituents of manure such as veterinary pharmaceuticals (*41–43*) and natural and synthetic hormones (*44–46*) have raised many of the same concerns as those that exist regarding land application of biosolids.

The presence and distribution of AOCs in biosolids as well as in environments amended with biosolids have been well established (*4*, *47–50*). However, the range of sources and loadings to terrestrial and aquatic environments, exposure of humans and other organisms, and the effects of exposure are only beginning to be identified and understood.

Earthworms are common primary consumers of organic matter in soil and as such may be exposed to organic and inorganic contaminants contained in the organic fractions within soil. Earthworms can comprise as much as 60–80% of total soil biomass in some locations, and at least one species of earthworm can be found in most soils. Earthworms tend to migrate only for short distances and thus may be ideal sentinel terrestrial organisms for identifying AOCs in the food web (*51–53*). Earthworms can accumulate organic contaminants both by the passive equilibrium partitioning of contaminants present in the dissolved phase of soil and actively through ingestion (*54*, *55*). The relative importance of equilibrium partitioning and accumulation of organic compounds through the skin and uptake through the gut is compound dependent. Jager et al. (*55*) observed the relative importance of uptake through the gut increased with increasing hydrophobicity of the organic contaminant.

Earthworms are known to biomagnify inorganic and organic soil contaminants, including mercury, polycyclic aromatic hydrocarbons (PAHs), brominated fire retardants, and pesticides through soil contact or consumption

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(*54*, *56–62*). As primary consumers of contaminated soils, earthworms have been determined to introduce organic contaminants into the terrestrial food web. Harris et al. (*59*) determined that earthworms in orchard soils contaminated by historic application of DDT can bioaccumulate DDT and its metabolites. The earthworms from these orchard soils served as a primary source of nourishment for American Robin (*Turdus migratorius*) populations in proximity to the orchards and thus acted as the route of exposure to total DDT for the robins. In general, total DDT was determined to bioaccumulate in robins, as measured by total DDT concentrations in robin eggs. Earthworms may serve as a route of exposure to other terrestrial organisms besides birds, such as reptiles and small mammals (*63*).

Earthworms collected from WWTP percolating filter beds have been found to contain the anthropogenic endocrine disrupting compounds (EDCs) phthalate plasticizers, bisphenol-A, and 17β-estradiol (*64*). Moreover, these synthetic EDCs were found to elicit physical changes in the brain and brain physiology of European Starlings (*Sturnus vulgaris*) (*22*). These examples serve to illustrate the potential importance of earthworms to bioaccumulate and introduce a variety of organic contaminants into the terrestrial food web.

The research described herein assesses the potential transfer of AOCs from land applied biosolids into biota (earthworms) and the use of earthworms as diagnostic indicators of wastewater-derived AOCs in terrestrial environments. The sites selected for these field studies include agricultural soil receiving agronomic application rates of biosolids. Agronomic application of biosolids typically results in surface soil that contains 1 – 4% biosolids by mass. Furthermore, biosolids application method (broadcast application, post application tillage, subsurface injection, etc) and weathering may affect the availability of AOCs as a result of leaching, degradation, or changes in bioavailability (*49*, *65–67*).

Methods

Field Sites

Four field sites were used for this project (Table I). Three sites were agricultural fields (Sites 2-4) that were amended with biosolids within 31 days prior to sample collection. The final site (Site 1) was a minimally affected site with no known history of biosolids or manure amendment. All four sites were in use for commercial agronomic production at the time of this study. Site 1, which was located in the Midwestern United States, was a nonirrigated soybean field and was previously described in Kinney et al. (*3*). Soil and earthworm samples were collected from Site 1 on June 6, 2005. A second set of soil and earthworm samples were collected on September 29, 2005, and whereas the data are not presented here they are available elsewhere (*3*).

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Table I. Field Site Soil Characterization

Field Sites	Organic Carbon (%)[a]	Sand ≥ 2 mm (%)[b]	Sand < 2 mm (%)[c]	Silt (%)[c]	Clay (%)[c]	Earthworm Density (worms/hole)[d]
Site 1: Minimally Affected (soybean crop)	4.5	6.3	42.0	30.8	20.9	6.1 ± 3.4
Site 2: Midwest Biosolid-Amended (soybean crop)	1.9	2.0	60.1	23.1	14.8	12.3 ± 6.7
Site 3: Northwest Biosolid-Amended (hay crop)	2.7	3.1	38.2	26.0	32.7	14.2 ± 7.3
Site 4: Northwest Biosolid-Amended (pasture/grazing)	2.1	3.6	31.9	29.3	35.2	5.6 ± 4.3

[a]Estimated by loss on ignition
[b]Determined by sieve
[c]Determined by hydrometry
[d]Number of earthworms per hole (3.14×10^4 cm^3) ± 1 standard deviation

Site 2, one of the biosolids amended sites, was also located in the Midwestern United States and was a no-till, nonirrigated soybean field receiving biosolids as a fertilizer for the first time in spring 2005. The biosolidss were from a local WWTP that processed, on average, 3.98×10^7 liters per day of wastewater influent from residential, university, hospital and medical facility, industrial, and landfill leachate sources. The biosolids produced by this WWTP results from sludge that is processed through three anaerobic digestion steps at 130, 95 and 95°C, respectively. Prior to land application, the biosolids is pressed to decrease water content and stored on an outdoor pad for 3 to 6 months. The biosolids applied to Site 2 on April 18, 2005 had been stored for about 6 months then applied at a rate of 1.8 Mg/1000 m^2. Soil and earthworm samples were collected from Site 2 on May 19 (31 days post-application) and again September 21, 2005 (data not shown, (*3*)).

Sites 3 and 4 are located in the Northwest United States. Unlike Sites 1 and 2 that had no prior biosolids or manure application, Sites 3 and 4 had an extended history of biosolids application, and were specifically selected for this reason. Sites 3 and 4 received biosolids amendment on a regular schedule of 2 consecutive years of amendment followed by one year of no biosolids amendment. Site 3 was a no-till, nonirrigated hay field. The WWTP that produces the biosolids applied at Sites 3 and 4 employs secondary treatment, trickling filter and activated sludge, to prepare the biosolids. The sludge spends about 28 days in a digester at 36 °C. The biosolids is then transferred and stored in a facultative lagoon for 8 to 9 months prior to land application as a slurry (about 6% total solids). Application of biosolids to Site 3 was completed on July 18, 2006 at a rate of 1.0 Mg/1000 m^2 and soil and earthworm samples were collected on August 15, 2006 (28 days post-application). Site 3 was not amended in 2005.

Site 4 was amended with biosolids from the same WWTP as Site 3. Site 4 was a no-till, nonirrigated grassland pasture used for cattle grazing. Site 4 was amended with biosolids in 2005 and again in 2006. Biosolids application was completed on July 18, 2006 at a rate of 1.1 Mg/1000 m^2. Soil and earthworm samples were collected on August 16, 2006 (29 days post-application).

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Field Sampling

At each field site, earthworms were removed from 40-cm diameter circular holes to a depth of about 25 cm in a manner similar to that described by Salogovic et al. (*68*). The soil was removed using a pre-cleaned metal-blade spade and placed on a clean tarp (only used at a single field site). The spades used in this study were cleaned using soap and water followed by deionized (DI) water and isopropanol or methanol rinses. The soil was carefully sorted by hand while wearing nitrile gloves to remove all earthworms observed. To the extent possible, plant material was removed from the soil in the field, and any remaining plant material was removed in the laboratory prior to analysis. Undamaged worms were placed in a shipping container with air holes and loosely packed native soil. The samples were returned to the laboratory in an ice-filled cooler within 24 hours of collection. In the laboratory, the earthworms were cleaned using cool DI water and allowed to depurate on wet filter paper for 24 hours (*69*, *70*) to assure that AOCs detected in the earthworms originated from tissue and not gut contents. This was necessary to avoid overestimating AOC content and bioaccumulation factors (BAFs). After depuration, the worms were gently cleaned with cool DI water and dried then frozen for later extraction and analysis.

Soil subsamples for AOC analysis were collected once the earthworms were separated from the soil from each sampling hole. The soil placed on the tarp was homogenized by hand in the field. Any biosolids in the soil sample were therefore distributed throughout the homogenized soil prior to subsampling. Subsamples of the soil homogenate from each hole were placed into a glass bowl, thoroughly mixed, apportioned into trace-clean glass jars, and returned to the laboratory for soil texture, soil organic carbon, and triplicate AOC.

Samples of the biosolids applied to Sites 2 – 4 were collected at the time of field application or field sample collection and frozen for later AOC analysis. Biosolids applied to Site 2 was collected from the drying pad at the WWTP, and the biosolids applied to Sites 3 and 4 was sampled directly from the well-mixed WWTP retention pond while a tanker truck used to transport and surface apply the biosolids was being filled.

Solvent Extraction of Soil, Biosolids, and Earthworm Samples

Earthworm, soil, and biosolids source samples were prepared in triplicate for AOC quantification. Two different extraction, cleanup, and quantification methods were required to encompass the range of compounds determined in this study. Both methods are based on previously published pressurized liquid extraction (PLE; Dionex-100 & 200, Dionex Corp., Sunnyvale, Calif., USA) methods developed for AOC determination in soil and sediment samples (*71*, *72*).

The nonpolar AOCs were extracted from 1–2 g wet weight samples of homogenized soil, biosolids, or earthworm by PLE using mixtures of isopropanol and water (*72*). The sample was loaded into a 10-mL PLE cell and the void volume was filled with ashed Ottawa sand (400°C for 4 h). Prior to extraction method-performance surrogates were added to the top of the material to be extracted. Each sample was extracted twice; first at 120°C using 50:50

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isopropanol:water and second into a separate receiving vial at 200°C using 80:20 isopropanol:water. Both extractions were at 10300 kPa and consisted of three 5-min static cycles. The two resulting extracts for each sample were combined during solid-phase extraction (SPE) preconcentration and clean-up step using a modified polystyrene-divynlbenzene (PSDVB) phase SPE cartridge (1-g, 20-mL Oasis HLB Waters Corp., Milford, MA). Once loaded, the PSDVP cartridge was eluted with three 10-mL aliquots of 80:20 dichloromethane:diethyl ether through a Florisil SPE cartridge (1-g, 6-mL Iternational Sorbent Technologies, Mid Glamorgan, U.K.) that had about 4 g of sodium sulfate added to the top of the cartridge. The resulting eluent was brought to a final volume of about 1 mL by evaporation under a gentle stream of nitrogen. Prior to transferring the final extract to a 2-mL autosampler vial and quantitation, an internal standard mixture was added.

Pharmaceuticals in the samples were extracted by PLE using a 70:30 acetonitrile:water solvent mixture (*48*, *71*). About 10 g wet weight of soil or biosolids or 3–5 g wet weight of earthworms was loaded into a 10-mL PLE cell. Any void volume in the cell was filled with ashed Ottawa sand to maintain consistent extraction volumes. Prior to extraction a method performance surrogate was added to the sample. Each sample was extracted for five static cycles (10-min each) at 130°C and 10,300 kPa. One mL of the extract was filtered through a 0.20-µm syringe filter into a 2-mL autosampler vial. The acetonitrile was then evaporated off under a gentle stream of nitrogen. The sample was reconstituted to 1 mL using 100 µL internal standard solution and a balance of 10 mM aqueous ammonium formate buffer.

Chemical Analysis

Extracts from the two PLE methods were analyzed using separate instrumental methods. The largest subset of analytes, the nonpolar AOCs, were quantified by gas chromatography/mass spectrometry (GC/MS, Agilent Technologies Model 5973, Hewlett-Packard/Agilent, Palo Alto, CA) following a protocol described by Burkhardt et al. (*72*). The GC/MS was operated in the full-scan mode [from 45 to 550 mass/charge ratio (m/z)], using electron-impact ionization (70 electron volts) and external calibration (*72*). A detailed description of chromatographic conditions and ions monitored are described elsewhere (*72*).

The pharmaceuticals were analyzed by high-performance liquid chromatography coupled with electrospray ionization/quadrupole mass spectrometry (HPLC/ESI/MS, Hewlett-Packard/Agilent Model Series 1100 LC/MSD) operated in the positive ion mode using selected-ion monitoring to improve sensitivity and minimize chemical interferences. The chromatographic conditions, ions monitored, and other method parameters are described in detail by Cahill et al. (*73*).

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Table II. Concentrations of AOCs (ng/g) Detected in Biosolid, Soil, or Earthworm Samples[a]

Anthropogenic Organic Contaminants	Common Use/Source	Biosolid 1 Applied to Site 2	Biosolid 2 Applied to Sites 3 & 4	Site 1 Minimally Affected Soil	Site 1 Minimally Affected Earthworm	Site 2 Biosolid Amended Soil	Site 2 Biosolid Amended Earthworm	Site 3 Biosolid Amended Soil	Site 3 Biosolid Amended Earthworm	Site 4 Biosolid Amended Soil	Site 4 Biosolid Amended Earthworm
acetophenone	fragrance (PCP)	3450 (51)	ND	627 (12)	150 (37)	ND	110 (42)	ND	291 (35)	ND	135 (41)
d-limonene	fragrance (PCP)	1600 (14)	1977 (16)	393 (40)	ND	ND	ND	ND	ND	ND	32 (26)
galaxolide (HHCB)	fragrance (PCP)	427000 (19)	25900 (20)	633 (23)	61 (43)	1050 (16)	3340 (24)	33 (29)	ND	55 (44)	ND
indole	fragrance (PCP)	5 800 (22)	111900 (12)	ND	2320 (32)	285 (23)	1950 (29)	214 (25)	545 (29)	55 (37)	743 (28)
isoborneol	fragrance (PCP)	ND	ND	267 (25)	ND	ND	ND	ND	ND	ND	ND
isoquinoline	fragrance and flavor (PCP)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
tonalide (AHTN)	fragrance (PCP)	177000 (13)	6503 (34)	113 (38)	19 (73)	287 (26)	279 (22)	27 (18)	ND	34 (23)	ND
camphor	flavor and odorant (PCP)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
menthol	cigarettes, cough drops, and mouthwash (PCP)	ND	ND	177 (36)	< 42	ND	ND	ND	ND	ND	ND
4-cumylphenol	detergent metabolite (PCP)	ND	ND	37 (87)	ND	ND	140 (73)	ND	ND	ND	ND
4-n-octylphenol	detergent metabolite (PCP)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-tert-octylphenol	detergent metabolite (PCP)	ND	7623 (25)	ND	ND	ND	570 (57)	< 22.9	ND	< 22.9	ND
para-nonylphenol-total	detergent metabolite (PCP)	483000 (20)	315100 (18)	ND	ND	ND	5200 (37)	861 (26)	4938 (45)	944 (27)	3176 (14)
nonylphenol monoethoxy-total	detergent metabolite (PCP)	25300 (28)	11550 (24)	ND	ND	ND	ND	158 (37)	1647 (21)	75 (45)	1626 (16)
nonylphenol diethoxy-total	detergent metabolite (PCP)	760 (48)	12820 (27)	ND	ND	ND	ND	230 (32)	6432 (21)	ND	3542 (27)
octylphenol, monoethoxy	detergent metabolite (PCP)	5030 (21)	ND	ND	ND	ND	ND	ND	346 (52)	ND	ND
octylphenol, diethoxy	detergent metabolite (PCP)	ND	ND	ND	ND	74 (22)	ND	ND	ND	ND	ND
N,N-diethyltoluamide (DEET)	mosquito repellant (PCP)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
phenol	disinfectant (PCP), manuf. numerous products	6270 (18)	16810 (26)	5 970 (15)	ND	ND	ND	104 (35)	288 (48)	ND	145 (52)
triclosan	disinfectant (PCP)	10500 (17)	9046 (11)	833 (67)	ND	160 (38)	1740 (31)	99 (28)	3854 (31)	41 (36)	1679 (32)
benzophenone	fixative perfumes and soaps (PCP)	ND	1977 (29)	ND	< 31.8	ND	ND	ND	226 (46)	ND	123 (25)
3-beta-coprostanol	biogenic sterol	467000 (33)	1262000 (21)	ND	ND	1 910 (77)	ND	1834 (29)	1439 (18)	1702 (17)	798 (27)
cholesterol	biogenic sterol	86700 (24)	2858000 (18)	18 900 (59)	253000 (48)	7700 (51)	166000 (49)	4532 (41)	238768 (48)	4242 (39)	123990 (25)
beta-sitosterol	biogenic sterol	177000 (31)	302800 (15)	24 000 (17)	11600 (51)	4570 (37)	7030 (58)	613 (52)	18281 (48)	1731 (26)	8108 (43)
stigmastanol	biogenic sterol	77700 (29)	211100 (25)	4 900 (19)	ND	1300 (33)	ND	572 (22)	2882 (38)	552 (17)	851 (27)
anthracene	PAH	320 (24)	ND	ND	ND	ND	ND	ND	ND	ND	ND
benzo[a]pyrene	PAH	ND	386 (31)	ND	ND	ND	ND	< 24.6	ND	< 24.6	ND
naphthalene	PAH, moth repellant	610 (21)	ND	ND	ND	ND	ND	ND	ND	ND	ND
fluoranthene	PAH	950 (27)	1057 (32)	ND	ND	ND	ND	33 (28)	40 (70)	26 (36)	< 23.5
phenanthrene	PAH	1730 (27)	773 (19)	ND	ND	ND	ND	< 20.7	ND	ND	ND
pyrene	PAH	740 (24)	1092 (26)	ND	ND	ND	ND	24 (55)	33 (23)	< 20.8	< 20.8
1-methylnaphthalene	Alkyl-PAH	ND	ND	ND	< 27.8	ND	ND	ND	ND	ND	ND
2-methylnaphthalene	Alkyl-PAH	ND	ND	ND	< 27.8	ND	ND	ND	ND	ND	ND
2,6-dimethyl-napthalene	Alkyl-PAH	915 (44)	1579 (31)	ND	ND	ND	ND	ND	ND	ND	ND
bisphenol A	Fixative, polycarbonates	4600 (9)	1279 (13)	147 (17)	ND	ND	ND	< 31.6	512 (20)	ND	318 (22)
diethylhexyl phthalate	plasticizer	3330 (22)	444030 (16)	ND	ND	ND	ND	2251 (34)	288 (28)	529 (23)	154 (29)
diethyl phthalate	plasticizer	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
tetrabromo diphenylether	flame retardant	ND	1012 (9)	ND	ND	ND	ND	ND	ND	ND	ND
tri(2-chloroethyl) phosphate	plasticizer, flame retardant	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
tributylphosphate	antifoming agent and flame retardant	ND	ND	2 130 (17)	200 (27)	523 (21)	250 (23)	ND	ND	ND	ND
tri(dichlorisopropyl) phosphate	flame retardant	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
triphenyl phosphate	plasticizer	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
para-cresol	wood preservative	4970 (34)	29370 (12)	2 200 (8)	< 161	ND	270 (17)	< 161	396 (16)	ND	1185 (22)
1,4-dichlorobenzene	pesticide, moth repellant	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
atrazine	pesticide	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
bromacil	pesticide	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
carbazole	pesticide	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
chlorpyrifos	pesticide	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
diazinon	pesticide	ND	ND	ND	99 (26)	ND	ND	ND	ND	ND	ND
metolachlor	pesticide	ND	ND	320 (22)	ND	ND	720 (18)	ND	ND	ND	ND
prometon	pesticide	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-methyl-1H-indole (skatol)	Fecal indicator	5170 (23)	1878 (33)	ND	260 (20)	143 (41)	230 (27)	< 30.1	663 (36)	ND	482 (12)
anthraquinone	Manuf. Of dyetextiles, bird repellant	ND	ND	ND	ND	ND	ND	< 24.3	ND	ND	ND
isophorone	Industrial solvent	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
isopropylbenzene	Manuf. of phenol/acetone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
acetaminophen	Antipyretic (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
caffeine	Stimulant (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,7-dimethylxanthine	caffeine metabolite (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
carbamazapine	Antiepileptic (PH)	390 (16)	16 (11)	ND	ND	ND	ND	ND	ND	ND	ND
codeine	Analgesic (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
cotinine	Nicotine Metabolite (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
dehydronifedipine	Antianginal (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
diltiazem	Antihypertensive (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
diphenhydramine	Antihistamine (PH)	7000 (12)	227 (16)	ND	ND	< 1.4	ND	2 (27)	ND	3 (35)	ND
fluoxetine	Antidepressant (PH)	ND	30 (18)	ND	ND	ND	ND	ND	ND	ND	ND
miconazole	Antifungal (PH)	ND	110 (12)	ND	ND	ND	ND	3 (18)	ND	3 (22)	ND
salbutamol	Antiasthmatic (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
sulfamethoxazole	Antibiotic (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
thiabendazole	Anthelmintic/Pesticide (PH)	5000 (17)	ND	ND	ND	ND	ND	ND	ND	ND	ND
trimethoprim	Antibiotic (PH)	ND	ND	< 1.5	ND	ND	127 (5)	ND	90 (11)	ND	18 (9)
warfarin	Anticoagulant (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] Average from three replicate composite samples, individual compound concentrations in ng/g dry wt. ND = not detected. PCP = personal care product. PAH = polycyclic aromatic hydrocarbon. PH = pharmaceutical. Value in parentheses is percent relative standard deviation.

Quality Assurance and Quality Control

Several measures of quality assurance and quality control were used during the field and laboratory work. In the field, pre-ashed Ottawa sand was used as a field blank and was handled identically to the other samples to ensure that there were no artifacts resulting from the sampling and analytical procedures.

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At least one pre-ashed sand reagent spike and laboratory blank sample were analyzed with each set of extractions and quantifications. Method-performance surrogate compounds (GC/MS: decafluorobiphenyl, fluoanthene-d_{10}, bisphenol A-d_3; LC/MS: carbamazepine-d_{10}, ethyl nicotinate-d_4) were added to all samples, spikes, and blanks. Multiple ion monitoring for each compound and chromatographic retention time were compared to authenticate standards for compound verification. Internal standards (GC/MS:1,4-dichlorobenzene-d_4, naphthalene-d_8, acenapthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, perylene-d_{12}; LC/MS: nicotinamide-d_4) were added to each sample to correct for any differences in sample volume and as a time marker. *Eisenia foetida*, a commercially available earthworm, was selected as a reasonable surrogate matrix to validate method performance for the earthworms collected in the field. Earthworm matrix spike recoveries (n = 3) were determined for clean *Eisenia foetida* fortified with AOCs as reported elsewhere (*3*). The performance of the methods for sediment, soils, and biosolids has been previously assessed, including method spike and matrix spike recoveries as well as statistically determined method detection limits (MDLs, (*48*, *71*, *74*)). Trace quantities of cholesterol, galaxolide, phenanthrene, and tonalide were detected in one or more of the blank samples, but these compounds were at concentrations 1 to 4 orders of magnitude lower than that detected in any of the corresponding environmental samples, and therefore not excluded from the data. AOCs detectected in samples at concentrations lower than the MDL are preceded by a "<" followed by the existing MDL (Table II). All of the compounds detected below the MDLs qualify as positively identified compounds by meeting all reporting qualifications, namely chromatographic retention time and detection of quantitation and confirmation ions within acceptable ion ratio limits.

Results and Discussion

Biosolids

The biosolids that were land applied at the field sites used in this study had a similar number of AOCs detected; 28 and 27 AOCs were detected in Biosolids 1 and 2, respectively (Table II). Twenty-three of the AOCs were detected in common in the two biosolids. The fragrances galaxolide and tonalide were detected in high concentrations, between 6.5 and 427 μg/g, in both biosolids. Nonylphenol detergent metabolites were detected in the biosolids at over 315 μg/g. The four target biogenic sterols were detected at individual concentrations as high as 2.86 mg/g. A number of PAHs and pharmaceuticals were also present in the land applied biosolids.

For ease of comparison and discussion, the AOCs included in this study have been grouped into four general categories; personal care products (PCPs), biogenic sterols, pharmaceuticals (PHs), and others (e.g. PAHs and alkyl-PAHs, wood preservative, skatol, etc.). Table II can be used to identify which compounds compose each of these groups. Figure I shows the relative contribution of each group of AOCs to the overall AOC composition in the source biosolids. On a relative mass basis the AOCs in the biosolids as well as the soil and earthworms samples were dominated by the presence of biogenic sterols (Figure I), with the

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exception of the biosolids applied to Site 2. However, it is important to note that many other compounds and groups of compounds were detected in substantial concentrations as mentioned above (Table II).

Amended Soils

All of the field sites included in this study contained a variety of AOCs. Unexpectedly, Site 1, the minimally affected soil that has no known history of biosolids or manure amendment, contained detectable quantities of 17 of the target AOCs (Table II). Some of the AOCs detected at Site 1 are strictly anthropogenic compounds, such as galaxolide, tonalide, and triclosan, whereas others that have human uses or sources like d-limonine and the biogenic sterols also may originate from natural environmental sources. This may in part explain the presence of the AOCs in the soil at Site 1. The source of the strictly anthropogenic compounds at Site 1 is unknown, but may reflect runoff from fields upgradient from Site 1 or atmospheric transport and deposition (*75–78*), which also could affect the biosolid-amended fields. The array of AOC detections at Site 1 documents the difficulty in identifying a true control site given the ubiquitous nature of many AOCs.

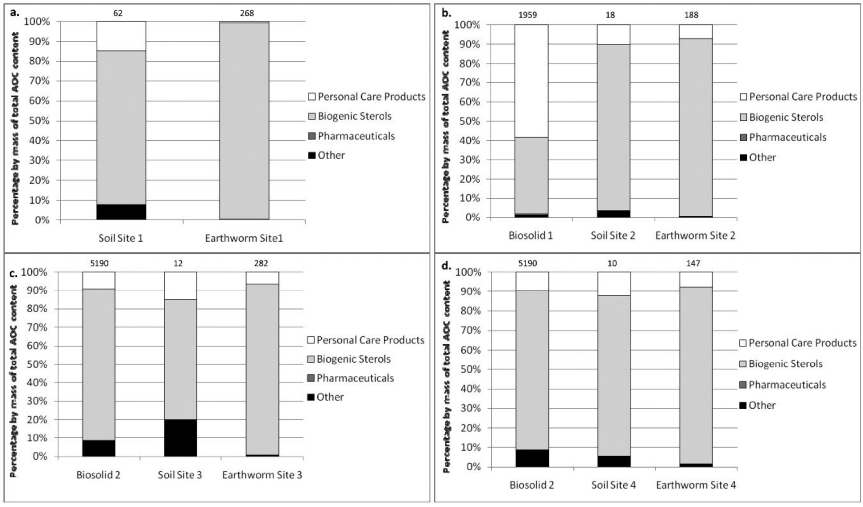

Figure I. Relative contribution of personal care products, biogenic sterols, pharmaceuticals, and other AOCs to the overall AOC composition of biosolid, soil, and earthworms from (a) Site 1 – nonirrigated soybean, (b) Site 2 – biosolid amended (1.8 Mg/1000 m²), no-till, nonirrigated soybean, (c) Site 3 – biosolid amended (1.0 Mg/1000 m²), no-till, nonirrigated hay, and (d) Site 4 – biosolid amended (1.1 Mg/1000 m²), no-till, nonirrigated grassland pasture. The total quantity of AOCs (μg/g) detected in each sample appears above each bar.

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The soil at Site 2 contained detectable quantities of 12 of the target AOCs, 10 of which were detected in the biosolids applied to the site. The presence of two AOCs in the soil at Site 2 not detected in Biosolids 1 indicates possible chemical heterogeneity in the product. On a mass basis, the AOCs detected in Site 2 are dominated by the biogenic sterols, but there are important contributions of synthetic fragrances and the disinfectant triclosan to the total AOCs at Site 2 (Figure I). The detergent metabolites detected at relatively high concentrations in the biosolids were not detected in the corresponding biosolid-amended soil samples (Table II). The soil samples were collected 31 days after biosolids amendment and the biosolids was not incorporated into the soil. Therefore, the detergent metabolites and perhaps some of the other AOCs were subject to photodegradation following application (*79*, *80*).

Soil samples from Site 3 contained detectable quantities of 24 of the target AOCs, all but one of which was present in Biosolid 2. Unlike Site 2, many of the detergent metabolites present in the biosolids were also detected in soil at Sites 3 and 4 (Table II). However, the biogenic sterols were still the dominant group of AOCs detected in the soil (Figure I). As noted previously, Sites 3 and 4 were amended with the same biosolids. All 17 of the AOCs detected at Site 4 were present in Biosolid 2. It is unknown why there is such a sizeable discrepancy in the number of AOCs detected at Sites 3 and 4. Five of the AOCs positively identified at Site 3 were below the MDL for the compound, and thus may have been present at concentrations below the MDL at Site 4. Two compounds, nonylphenol diethoxy-total and phenol, were detected in the soil from Site 3 at concentrations substantially above the MDL and not detected at Site 4. Although the biosolids applied to Sites 3 and 4 originates from the same source, the select differences in the AOCs detected at these sites and in the earthworms from each site may originate from chemical heterogeneity in the biosolids. The biosolids on the soil surface at both Sites 3 and 4 was observed to be heterogenously distribututed suggesting that the relatively small areas from which the samples were collected may be subject to heterogeneous quantities of biosolids. This may contribute to the observed differences. Despite the differences in the total number of AOCs detected at Sites 3 and 4, the relative composition of AOCs are similar between the two sites (Figure I).

Earthworms

Earthworms collected from all four field sites, including those from Site 1 with no known history of biosolids or manure amendment, had AOCs present in their tissue. The highest concentrations of AOCs in the earthworms at Site 1 were the biogenic sterols (Figure I), particular cholesterol, which may have originated from natural sources and is naturally present in earthworms. There were also some uniquely anthropogenic AOCs detected at low concentration in the earthworms from Site 1 such as galaxolide (61 ng/g), tonalide (19 ng/g), and tributyphosphate (200 ng/g). In total, 14 of the target AOCs were detected in earthworms from Site 1.

Fifteen of the AOCs included in this study were detected in earthworms from Site 2; many of which were not detected in the soil from Site 2 and in

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some instances AOCs that were below detectable concentrations in Biosolid 1. This may indicate the ability of earthworms to accumulate AOCs that were below detectable concentrations in the biosolids or that there is perhaps another anthropogenic source of these compounds to this site. Whereas the biogenic sterols cholesterol (166 μg/g) and beta-sitosterol (7030 ng/g) were the most dominant AOCs in the Site 2 earthworms, substantial quantities of nonylphenol detergent metabolites (5200 ng/g), galaxolide (3340 ng/g), and triclosan (1740 ng/g) were also detected (Table III).

Twenty AOCs were detected in the earthworms from Sites 3 and 4; 18 of which were detected in earthworms from both sites. This likely reflects the fact that both soils were amended with biosolids from the same source within a close timeframe. Both sites have an extended history of biosolids amendment, but only Site 4 was amended with biosolids the year prior to sample collection. This difference, however, does not appear to be reflected in the concentration of AOCs in the earthworms or the soil from each site (Table II). These observations likely indicate continuous weathering, degradation, volatilization, or leaching is occurring following biosolids application (*81–83*).

For many AOCs, the AOC composition profile of the earthworms is largely similar to the soil from which they were collected (Figure I). Moreover, the AOC profile in the soil and earthworms from Sites 2 – 4 generally reflect the composition of the biosolids used to amend the soil, especially at Sites 3 and 4. Moreover, the relative abundance of uniquely anthropogenic AOCs are substantially less in the earthworms from Site 1 indicating a lesser influence from anthropogenic AOC input. Therefore, some differences in AOC content of the source biosolids are reflected in the affected environment.

When possible bioaccumulation factors (BAFs, the ratio of mean AOC concentration in the earthworms to the AOC concentration in the corresponding soil) was calculated as a measure of AOC transfer into soil biota (Table III). Bioaccumulation factors were calculated instead of biota-to-soil accumulation factors because the individual species of earthworms collected from each site were not determined. Many of the AOCs had BAFs > 1, which indicates the potential for magnification in earthworms and perhaps transfer up the terrestrial food web (*22*, *59*, *64*). A few of the AOCs that are uniquely anthropogenic in source had consistently high BAFs including triclosan (10.9 – 41.0) and detergent metabolites (3.4 – 28.0).

Calculating BAFs in this study is complicated by the fact that many of the AOCs detected in the earthworms were below detection in the corresponding soil samples (Table II), which makes it impossible to calculate BAFs. Compounds for which this is the case are designated with a "> 1" in Table III. In all such instances where BAFs could not be calculated (> 1, Table III) due to an AOC not being detected in the soil sample, the BAF by definition must be > 1. In many such instances, such as total para-nonylphenols in Site 2, monoethoxy-octylphenol in Site 3, and para-cresol in Site 4, sizable quantities of the AOC were measured in the earthworm.

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Table III. Bioaccumulation Factors (BAFs)[a] of detected AOCs

Anthropogenic Organic Contaminants	Site 1 BAF	Site 2 BAF	Site 3 BAF	Site 4 BAF
acetophenone	0.2	> 1	> 1	> 1
d-limonene	0	--	--	> 1
galaxolide (HHCB)	0.1	3.2	0	0
indole	> 1	6.8	2.5	13.3
isoborneol	0	--	--	--
tonalide (AHTN)	0.2	1.0	0	0
menthol	NA	--	--	--
4-cumylphenol	0	> 1	--	--
4-tert-octylphenol	--	> 1	NA	NA
para-nonylphenol-total	--	> 1	5.7	3.4
nonylphenol monoethoxy-total	--	--	10.4	21.7
nonylphenol diethoxy-total	--	--	28.0	> 1
octylphenol, monoethoxy	--	--	> 1	--
octylphenol, diethoxy	--	0	--	--
phenol	0	--	2.8	> 1
triclosan	0	10.9	38.9	41.0
benzophenone	NA	--	> 1	> 1
3-beta-coprostanol	--	0	0.8	0.5
cholesterol	13.4	21.6	52.7	29.2
beta-sitosterol	0.5	1.5	29.8	4.7
stigmastanol	0	0	5.0	1.5
anthracene	--	--	--	--
benzo[*a*]pyrene	--	--	NA	NA
naphthalene	--	--	--	--
fluoranthene	--	--	1.2	NA
phenanthrene	--	--	NA	--
pyrene	--	--	1.4	NA
2,6-dimethyl-napthalene	--	--	--	--
bisphenol A	0	--	NA	> 1
diethylhexyl phthalate	--	--	0.1	0.3
tetrabromo diphenylether	--	--	--	--
tributylphosphate	0.1	0.5	--	--
para-cresol	NA	> 1	NA	> 1
diazinon	> 1	--	--	--
metolachlor	0	> 1	--	--
prometon	--	--	--	--
3-methyl-1*H*-indole (skatol)	> 1	1.6	NA	> 1
anthraquinone	--	--	NA	--
carbamazapine	--	--	--	--
diphenhydramine	--	NA	0	0
Miconazole	--	--	0	0
thiabendazole	--	--	--	--
trimethoprim	NA	> 1	> 1	> 1

[a] BAF is calculated as the ratio of the average concentration of AOC in the earthworm to the average concentration in the soil.

-- : BAF not calculated because compound was not detected in the soil or the earthworm.

NA: BAF was not available because the compound was detected below the MDL in the soil or earthworm.

> 1: Although the compound was detected in the earthworm, a BAF was not calculated because the compound was not detected in the corresponding soil sample. By definition the BAF must be > 1.

In addition, BAFs for many AOCs could not be calculated because the compound, although positively identified, was detected at concentrations below the MDL in the soil or corresponding earthworm sample, which is designated with an "NA" in Table III. Much like some of the compounds designated as ">1", there were some compounds, such as bisphenol-A at Site 3, designated as "NA" for which the concentration of the AOC is sizable in the earthworm but positively detected below the MDL in the corresponding soil.

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The highest concentration of AOCs in the samples were the biogenic sterols, which do not have known direct ecological or human health threats. However, biogenic sterols can be transformed into sex hormones in the environment, such as the microbial transformation of cholesterol to testosterone (*84*). Results of controlled laboratory exposure experiments, data not included, in which earthworms (*Eisenia foetida*) were exposed to differing quantities of biosolids indicate that the concentration of the biogenic sterols measured in the earthworms is directly related to the quantity of biosolids exposure.

Although this study was not designed to directly consider potential human or ecological health, some of the AOCs detected in earthworm tissue are known or suspected EDCs, including nonylphenol detergent metabolites and benzophenone (*85*). It has been demonstrated that some AOCs with estrogenic activity in earthworms at relevant environmental concentrations can adversely affect bird populations (*22*). In addition, the synthetic fragrances galaxolide and tonalide, which have been observed to accumulate in human tissue, are suspected to result in liver disorders (*86*). Triclosan, which was calculated to bioaccumulate in earthworms at all three biosolid-amended sites, is known to elicit an estrogenic response in fish eggs (*87*) and cause changes in expression of thyroid hormone receptor genes in tadpoles (*21*). It is unknown if the presence of triclosan in material consumed by earthworms will adversely affect earthworms or microorganisms in their gut.

The fact that many AOCs were detected at measurable concentrations in earthworm tissue despite not being detected in the corresponding soils illustrates the potential for earthworms to serve as a sentinel organism and diagnostic tool to detect the presence of AOCs in terrestrial environments. This is further supported by the ubiquitous nature of earthworms in soils globally and the fact that they do not tend to migrate over substantial distances (*52*, *53*).

Earthworms occupy a low trophic position in the terrestrial food web and therefore their ability to accumulate organic contaminants present in soils can facilitate the movement of these contaminants into higher trophic levels (*59*, *70*, *88*). Earthworms are known to be consumed by many bird species, representing up to 90% by weight of the diet of some species (*89*). In addition, species of mammals, reptiles, amphibians, fish, and other invertebrates feed upon earthworms (*88*). In fact, Markman et al. (*22*) demonstrated that male European starlings (*Sturnus vulgaris*) consuming a diet of mealworms containing a mixture of a select group of EDCs at concentrations consistent with those observed in earthworms (*Eisenia fetida*) collected from WWTP filterbeds (*64*) resulted in significant enlargement of the portion of the brain (HVC) controlling song production, increased song production and complexity, and a decrease in immune function. The presence of AOCs in earthworms in the biosolid-amended soils and the measured bioaccumulation of some AOCs suggests the presence of biosolids and the AOCs they contain are not immediately toxic to earthworms for exposure concentrations at the sample sites. However, it might be prudent to consider potential chronic effects of these substances on earthworm behavior, growth, and reproduction that might also indirectly affect soil fertility or terrestrial food webs (*70*, *90*, *91*).

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Conclusions

The results of this work demonstrate that some organic contaminants, many of which are distinctly anthropogenic, can be transferred from source materials, such as biosolids, to soil-dwelling earthworms. Whereas many researchers have reported the bioaccumulation of specific organic contaminants in a variety of earthworm species, particularly in laboratory controlled experiments (*54*, *57*, *61*, *66*, *70*, *92*, *93*), the results of this work demonstrate that earthworms in common agricultural soil environments amended with biosolids can accumulate AOCs. Moreover, by virtue of bioaccumulation earthworms may represent a more robust sample for the detection of soil contaminants than the soil itself. This phenomenon was observed in soil amended with biosolids for the first time only 31 days prior to earthworm collection, as well as in soil with multiple biosolids applications. Based on these findings some AOCs present in biosolids are bioavailable for uptake, and therefore future consideration of the effects of AOC bioaccumulation and exposure on earthworms is warranted. This finding suggests that through predation of earthworms, these compounds could be further dispersed beyond the point of application in terrestrial ecosystems.

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