Bioaccumulation of $^{14}C_{60}$ by the Earthworm *Eisenia fetida*

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Carbon fullerenes, including buckminsterfullerene (C_{60}), are increasingly available for numerous applications, thus increasing the likelihood of environmental release. This calls for information about their bioavailability and bioaccumulation potential. In this study, ^{14}C-labeled C_{60} and ^{14}C-phenanthrene (positive control) were added separately to soils of varying composition and organic carbon content (OC), and their bioaccumulation in the earthworm *Eisenia fetida* was compared. Biota-sediment accumulation factors (BSAF) were measured after 24 h depuration in soils with high C_{60} dosages (60, 100, and 300 mg-C_{60} kg^{-1} dry soil), which exceed the soil sorption capacity, as well as in soils with a low C_{60} dose (0.25 mg kg^{-1}) conducive to a high fraction of sorbed molecular C_{60}. The BSAF value for the low-dose soil (0.427) was 1 order of magnitude lower than for less hydrophobic phenanthrene (7.93), inconsistent with the equilibrium partition theory that suggests that BSAF should be constant and independent of the K_{OW} value of the chemical. Apparently, the large molecular size of C_{60} hinders uptake and bioaccumulation. Lower BSAF values (0.065−0.13) were measured for high-dose soils, indicating that C_{60} bioaccumulates more readily when a higher fraction of molecular C_{60} (rather than larger precipitates) is available. For the high-dose tests (heterogeneous C_{60} system), soil OC content did not significantly affect the extent of C_{60} bioaccumulation after 28 d of incubation, although higher OC content resulted in faster initial bioaccumulation. For low-dose soils, C_{60} BSAF decreased with increasing soil OC, as commonly reported for hydrophobic chemicals due to partitioning into soil OC. There was no detectable transformation of $^{14}C_{60}$ in either soil or worm tissue. Overall, the relatively low extent but rapid bioaccumulation of C_{60} in *E. fetida* suggests the need for further studies on the potential for trophic transfer and biomagnification.

Introduction

Carbon fullerenes, particularly buckminsterfullerene (C_{60}), represent a class of engineered carbon nanomaterials with unique photochemical and electronic properties. Many fullerenes, including C_{60}, are being considered for applications in numerous products and processes such as cancer therapeutics, drug delivery, and computer sensors (*1*−*5*). Furthermore, fullerene nanomaterials are becoming increasingly available and affordable. For example, Frontier Carbon is on track to produce up to 1500 ton/year of fullerenes and carbon nanotubes (*6*). The growing use of fullerene materials increases the likelihood of accidental or inadvertent release to natural systems, which calls for a better understanding of its behavior, fate, and impact in natural systems. Such understanding is critical to accurately inform the ecologically responsible use and disposal of fullerenes and support related risk assessment efforts.

Currently, little is known about the bioavailability of fullerenes (including C_{60}) in the environment, and C_{60} bioaccumulation potential has not been quantified. Bioavailability is defined here as the extent to which ecological receptors are exposed to hydrophobic organic contaminants (HOCs) in soil and sediment. Bioavailability is critical to the apparent toxicity of HOCs and controls their bioaccumulation and biomagnification potential (*7*). Normally, bioavailability depends on matrix properties (e.g., soil type and organic carbon content (OC), pore size distribution, mixing) and HOC properties (e.g., hydrophobicity, volatility, solubility). As C_{60} is nearly insoluble in water (estimated solubility from 1.3×10^{-11} to 7.96×10^{-9} g/L) and only soluble in certain organic solvents (*8*−*10*), most biological studies have focused on surface-charged, nanoscale C_{60} aggregates (nC_{60}) (*11, 12*).

Previous studies have shown that nC_{60} associates with soil OC (*13, 14*) and that dissolved natural organic matter (NOM) decreases the deposition rate of nC_{60} to solid surfaces due to steric repulsion (*15*), thus enhancing its potential mobility in aqueous systems. The heteroaggregation of nC_{60} with organic colloidal particles may also influence its bioavailability in the environment (*16*). Although the interactions between pristine molecular C_{60} and soil OC have received limited attention, if any, soil OC is known to affect the bioavailability of other polycyclic compounds that partition into the soil organic phase. For example, pyrene bioavailability decreases dramatically with increasing soil OC, consistent with equilibrium partitioning expectations (*17*).

Earthworms, including *Eisenia fetida*, are the prey of both vertebrates and invertebrates (*18*) and can act as an entry point for HOCs in soil into terrestrial food webs (*19, 20*). *E. fetida* can assimilate dissolved contaminants through skin contact as well as by soil ingestion (*21, 22*). Many studies have shown bioaccumulation of HOCs such as polynuclear aromatic hydrocarbons (PAHs) in the fatty tissue of earthworms (*23*−*25*). Thus, C_{60}, which is also a large, hydrophobic, polycyclic molecule, might similarly accumulate in earthworm fatty tissues. This raises the possibility of subsequent transfer to upper trophic levels occupied by insects, birds, and rodents. Furthermore, bioaccumulation pathways in earthworms may also have relevant implications for potential bioaccumulation in higher order species including humans.

Current knowledge of earthworm uptake and bioaccumulation of nanomaterials is limited. Carbon nanotubes were reported to bioaccumulate with relatively low propensity in *E. fetida* (Biota-sediment accumulation factors, BSAF = 0.006−0.02) (*17*), possibly due to strong sorption to soil OC

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TABLE 1. Physicochemical Characteristics of the Tested Soils

soil properties	Grenada-Loring[a]	Ft. Drum[b]	Lula[c]
pH	6.72	6.80	7.4
% fines (silt and clay)	97.0	91.5	55
% sand	3.0	8.5	45
cation exchange capacity (mEq/100 g)	10.8	27.0	6.2
organic carbon content, %	0.7	5.16	0.27

[a] Data from ref *51*. [b] Data from ref *52*. [c] Analyses performed by A&L Plains Agricultural Laboratory, Inc., Lubbock, TX.

(which can decrease partitioning into fatty tissue) and the relatively large size of the nanotubes (which may physically hinder uptake processes). The potential for C_{60} bioaccumulation in terrestrial systems has not been addressed in the literature, primarily due to the unavailability of accurate quantification methods for complex, carbon-rich matrices such as soil and biological tissues (*17*). Scintillation counting of ^{14}C-labeled C_{60} enable such measurements, and $^{14}C_{60}$ has been previously synthesized to assess the uptake of ^{14}C-labeled nC_{60} by human keratinocytes and rat tissue (*26, 27*). However, $^{14}C_{60}$ is not commercially available, and its synthesis is challenging, which represents an obstacle to quantifying its bioaccumulation propensity.

To our knowledge, this is the first study to assess the bioaccumulation of "pristine" (i.e., untransformed) C_{60} from soil into a terrestrial organism. The rate and extent of $^{14}C_{60}$ bioaccumulation were quantified using the earthworm *E. fetida* as a model organism. Bioaccumulation of ^{14}C-phenathrene was used as a positive control and baseline for comparison. The effects of soil OC content and C_{60} concentration in soil were also investigated.

Methods

Materials. C_{60} (99.5% pure) was purchased from SES Research (Houston, TX). Radiolabeled (^{14}C) C_{60} (18.75 ± 2.02 mCi $mmol^{-1}$ or 500 mg L^{-1} C_{60} in toluene at >99% purity) was custom synthesized by the Research Triangle Institute (Research Triangle Park, NC). Toluene (ACS grade) and phenanthrene (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO). ^{14}C-Phenanthrene (55 mCi $mmol^{-1}$ or 324 mg L^{-1}) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO).

Organisms. *E. fetida* was purchased from The Worm Farm (Durham, CA). Worms were maintained in fiberglass bins with Premier Sphagnum peat moss hydrated to achieve a moisture content of about 35% dry weight. The pH was neutralized with $CaCO_3$ (Fisher Scientific, Pittsburgh, PA). Earthworms were fed every other day with Magic Worm Food (Magic Products Inc., Amherst Junction, WI) containing 12% crude protein, 1.0% crude fat, and 6.0% crude fiber. Prior to exposure to C_{60}, a potassium chloride (KCl) toxicity test was conducted to check the health of the worms (*28*). Only worm cultures that produced KCl LC_{50} values ≥ 8000 mg kg^{-1} were chosen for the experiment. Sexually mature earthworms (i.e., clitellated), in a range of 0.3−0.6 g, were selected for C_{60} experiments.

Soils. Three different soils were selected to investigate the effect of OC on C_{60} bioaccumulation: (1) silty loam Grenada-Loring field soil (GL soil, 0.7% OC) collected from the Brown Loam Experimental Station (Learned, MS), (2) soil collected from Ft. Drum, NY (FD soil, 5.2% OC), and (3) Lula sandy soil (R. S. Kerr Environmental Research Laboratory, Ada, OK) (Lula soil, 0.3% OC). All soils were air dried, processed through a hammer mill, and sieved (1.4 mm mesh size) before use. The chemical and physical properties of these soils are listed in Table 1.

Soil Amendment with C_{60} and Aging. Soils were amended with C_{60} at concentrations of 0.25, 60, 100, and 300 mg kg^{-1} dry soil (8747, 5382, 8613, and 29860 dpm g^{-1} dry soil, respectively, measured with a biological oxidizer) by adding toluene solutions containing labeled and unlabeled C_{60} (0.43, 30, 50, and 150 mL of toluene solution per kg of soil, respectively). Toluene was then allowed to evaporate for 7 d, during which the amended soil was thoroughly mixed three or four times daily. The residual toluene concentration at the time of exposure was determined by a commercial analytical laboratory (Pace Analytical, Lenexa, KS) to be below the detection limit (<5 μg/kg) and thus inconsequential for the bioaccumulation experiments. Avoidance tests (unpublished data) showed that the worms did not differentiate between unamended control soils and soils exposed to toluene alone.

The lowest C_{60} concentration, 0.25 mg kg^{-1}, was estimated to be below the soil sorption capacity (Supporting Information), allowing bioaccumulation measurements for primarily molecularly available, sorbed C_{60} in the soil. The higher C_{60} concentrations exceeded the soil sorption capacity, which simulates contamination after accidental releases of C_{60} powder such as during transportation, likely resulting in the presence of both aggregated/precipitated C_{60} and sorbed molecular C_{60} in the soil.

To investigate the possibility of C_{60} transformation, C_{60} was applied to GL and FD soils at 100 mg kg^{-1} dry soil (8441 DPM g^{-1}) and aged for 30 d. A mixture of C_{60} and $^{14}C_{60}$ dissolved in toluene was sprayed evenly onto 50 g of each soil. After toluene evaporated (7 d evaporation time), the soils were tumbled, mixed, and then stored open to the air at 22 °C. Triplicate soil samples (1 g each) were taken after 1 month of incubation. The samples were shaken and sequentially extracted for 24 h (each) with 10 mL of water, methanol, and toluene. Extracts were then centrifuged at 10000 rpm for 30 min, and supernatants were filtered through 0.22 μm cellulose syringe filters to remove suspended soil particles. Triplicate aliquots of 1 mL of water and methanol samples were analyzed using a liquid scintillation counter (LSC) (LS 6500, Beckman Coulter, Brea, CA) to confirm the absence of ^{14}C in these hydrophilic fractions, while the supernatant of the toluene extract (containing ^{14}C) was analyzed by high-performance liquid chromatography (Waters 4695, Milford, MA) coupled with radiochromatographic detection (IN/US Systems, Inc., Tampa, FL) (HPLC-RC). The HPLC is also equipped with a photodiode array detector (Waters, 996, Milford, MA), which was used for nonradiolabeled C_{60} analysis. Separation was accomplished with a Delta Pak C18 column (150 mm × 3.9 mm i.d., 300 Å, Waters, Milford, MA) at a constant flow rate of 1 mL/min with a mobile phase of 100% toluene. $^{14}C_{60}$ had a retention time of 1.51 min at these settings (Figure 1). Serial extractions of moist soils and analysis of the extracts were also conducted after bioaccumulation tests. The water−soil weight ratios were determined by weighing the moist soils and drying the soils at 60 °C to constant weight. The total radioactivity of the soils was determined by combustion in a biological oxidizer (OX-600, R. J. Harvey Instrument, Tappan, NY) followed by measurement of trapped $^{14}CO_2$ by LSC.

Uptake Experiments. C_{60} uptake by earthworms from three different soils was assessed according to standard procedures described by Environment Canada (*28*) with modifications. Uptake patterns of ^{14}C-phenanthrene, a representative PAH, were also investigated for comparison. Nonradiolabeled phenanthrene was dissolved in ethanol to make a 1 g L^{-1} solution. ^{14}C-Phenanthrene was added to the phenanthrene solution and mixed thoroughly. The mixed solution was applied dropwise to GL soil to a final concentration of 50 mg kg^{-1}, with a radioactivity of 5818 dpm g^{-1}. The ethanol was then allowed to evaporate overnight. All

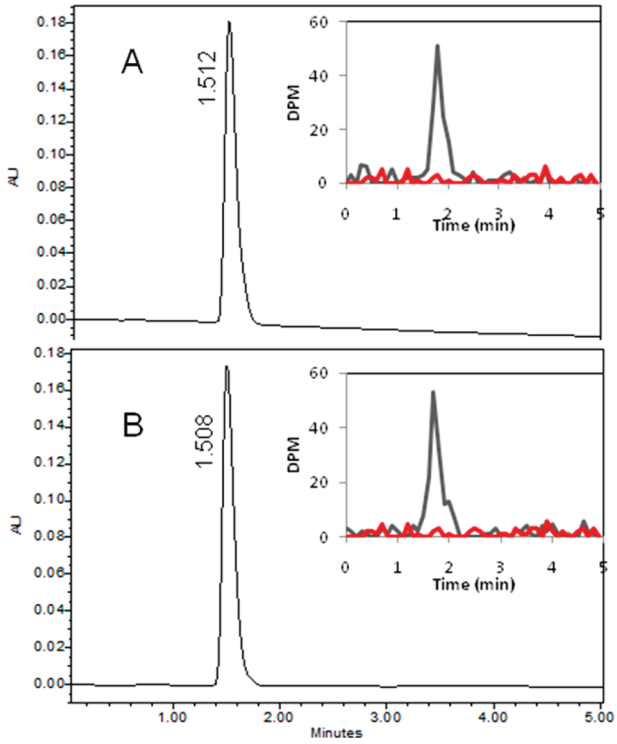

FIGURE 1. HPLC UV chromatograms for total C_{60} initially (A) and after aging in soil for 30 days (B). The corresponding radiochromatograms for $^{14}C_{60}$ are shown as inserts. Red lines represent the radiochromatogram baseline. The consistency of a single peak indicates the initial purity of C_{60} and the lack of transformation.

test soils were tumbled overnight after the solvents evaporated. At least three 0.5 g random samples of each soil were analyzed for total radioactivity to assess uniform mixing.

Prior to the experiment, earthworms were depurated for 24 h on filter paper hydrated with deionized (DI) water. The depurated worms were then exposed to C_{60}-laden soils (hydrated overnight with DI water to 70−80% water holding capacity) for 1, 3, 7, 14, and 28 d, respectively, with 10 earthworms per 200 g of soil (dry weight). Triplicate treatments were run for each data point. After exposure, the earthworms were washed, depurated on hydrated filter paper for 24 h, freeze dried, weighed, and analyzed for bioaccumulated radioactivity by combustion in the biological oxidizer with LSC. In order to investigate the potential biotransformation of C_{60} assimilated by worms, one worm of each sample was sacrificed, freeze dried, crushed into powder, and sequentially extracted by water, methanol, and toluene prior to HPLC-RC analysis as described above.

Elimination Experiments. C_{60} elimination from worms was also investigated. After exposure for 14 d in C_{60}-laden FD soils (100 and 300 mg-C_{60} kg^{-1} dry soil), worms were depurated for 24 h, transferred to C_{60}-free (unamended) FD soil, and analyzed for radioactivity after 1, 2, or 7 d.

Data Analysis. Bioaccumulation data were fit to a first-order accumulation rate model (*29*) using nonlinear regression with Prism (GraphPad Software, Inc., La Jolla, CA)

$$C_{org} = \frac{(k_s C_{s,0})}{k_e - \lambda}(e^{-\lambda t} - e^{-k_e t}) \quad (1)$$

where C_{org} is the ^{14}C concentration in the organism (mg g^{-1} of organism dry weight), $C_{s,0}$ is the initial concentration in soil (mg g^{-1} of soil dry weight, approximately equal to the total applied amount, Supporting Information), k_s is the uptake rate constant (g of dry soil g^{-1} of dry organism d^{-1}), k_e is the elimination rate constant (d^{-1}), t is the duration (d), and λ is the rate constant for the decreasing bioavailability of the compound (d^{-1}) (*29*).

Because the uptake of C_{60} or phenanthrene by *E. fetida* was expected to occur mostly through soil ingestion, the biota-sediment accumulation factor (BSAF) was used to quantify bioaccumulation. BSAF was defined as the ratio of the concentration of a compound in an organism to that in the sediment (*30*). The BSAFs were calculated at selected exposure times as the ratio of the C_{60} or phenanthrene concentration in the earthworms (C_{org}, mg g^{-1} dry weight biomass) to that in the soils (C_s, mg g^{-1} dry soil)

$$BSAF_t = \frac{C_{org}}{C_s} \quad (2)$$

Whether differences in BSAF values between different treatments were statistically significant was determined using Student t tests and ANOVA at the 95% confidence level. Goodness of fit to the model (eq 1) was assessed using the chi-square test ($\alpha = 0.05$) in Excel.

Results and Discussion

Aging of C_{60} in Natural Soil. Nanomaterials may undergo biological, chemical, or physical transformations in soils; thus, characterizing the material form to which ecological receptors are exposed is necessary for risk assessment. Unfortunately, current analytical capabilities are insufficient to fully characterize the state of C_{60} transformation, aggregation, or agglomeration in complex matrices such as soil or organism tissue. Nevertheless, converging lines of evidence suggest that C_{60} was not transformed nor formed aqueous aggregates (nC_{60}) after being aged in different soils. First, ^{14}C was detected only in the toluene extract of the amended soils but not in the water or methanol extracts (0.01 mg L^{-1} or 30 DPM mL^{-1} detection limit) (Tables S1 and S2, Supporting Information). Since surface-charged nC_{60} cannot be readily extracted by toluene without oxidizing agents (*31*) (which were not used in this study) and no ^{14}C was found in water or methanol extracts, nC_{60} formation and C_{60} transformation to hydrophilic products (if any) are assumed to have been negligible. The single ^{14}C peak detected by HPLC-RC analysis corroborates the initial purity of $^{14}C_{60}$ in soil and the lack of transformation over the exposure period (Figure 1). A similar, single radiochromatographic peak in worm extracts in toluene and no detectable radioactivity in water or methanol extracts (data not shown) also indicate no transformation within worm tissue upon accumulation. Apparently, C_{60} was not metabolized under the conditions studied, possibly due to its structural stability, insolubility, and/or relatively large molecular size (~0.7 nm in diameter (*32*)).

Complete recovery of the added $^{14}C_{60}$ was obtained by toluene extraction of the dry soil (Table S1, Supporting Information), which confirms the dominant presence of untransformed C_{60} and indicates the absence of irreversibly bound residue. Interestingly, lower $^{14}C_{60}$ recoveries were obtained by toluene extraction of moist soils (Table S2, Supporting Information), possibly because the soil particles were not fully dispersed in the toluene phase and water-saturated soil pores were not fully accessible to toluene. Hydrophobic C_{60} has a tendency to be sorbed or coated with soil OC (*14, 15*), and pore water represents a diffusion barrier for the partitioning of C_{60} from soil OC into the toluene phase. In this case, full ^{14}C recovery was only achieved by soil combustion in the biological oxidizer (Table S2, Supporting Information).

C_{60} Bioaccumulation. Earthworm bioaccumulation patterns under high-dose conditions were compared for three different soils with varying OC. Note that C_{60} was likely present in these experiments as both aggregated/precipitated powder

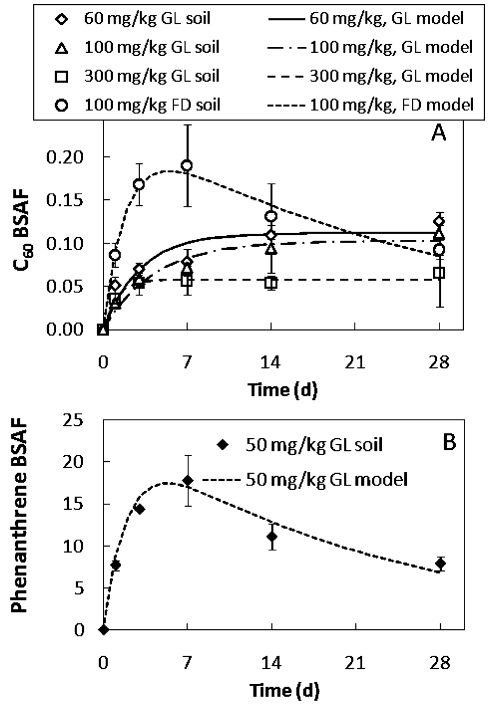

FIGURE 2. Biota-sediment accumulation factor (BSAF) for (a) C_{60} and (b) phenanthrene in *E. fetida* as a function of exposure time for different treatments. The relatively high C_{60} concentrations in these experiments likely resulted in its presence in both aggregated/precipitated powder and absorbed molecular forms. Data curves are simulations using eq 1 with parameters (and R^2 values) listed in Table 2. Error bars represent ± one standard deviation.

and molecular C_{60} sorbed by soil OC (Supporting Information calculations). Thus, BSAF values estimated in these experiments correspond to a heterogeneous C_{60} system, possibly representative of extreme events such as accidental spill of C_{60} powder during transport. Bioaccumulation of carbon nanotubes (*17*) and hydrocarbon after oil spills (*33*) has also been studied in similar multiphase systems where pure, sorbed, and possibly dissolved phases are present.

BSAF for earthworms in the high-OC FD soil containing 100 mg-C_{60} kg^{-1} dry soil increased initially to a peak of 0.190 ± 0.047 after 7 d and then decreased to 0.093 ± 0.011 over the remainder of the 28 d exposure period (Figure 2A). A similar bioaccumulation pattern was observed for phenanthrene in GL soil (Figure 2B), where the BSAF peaked at 17.75 ± 3.02 on day 7 and decreased to 7.93 ± 0.86 during the remaining time. Dissolved NOM is known to adsorb onto the surface of carbon nanoparticles via van der Waals forces and $\pi-\pi$ stacking (*34*), which helps disperse and stabilize fullerene in water (*35, 36*). Thus, the early BSAF peak (Figure 2A) might be due to temporarily enhanced C_{60} availability in pore water with dissolved NOM (*15, 36*), and the subsequent decrease in BSAF may be attributed to sorption of C_{60} by

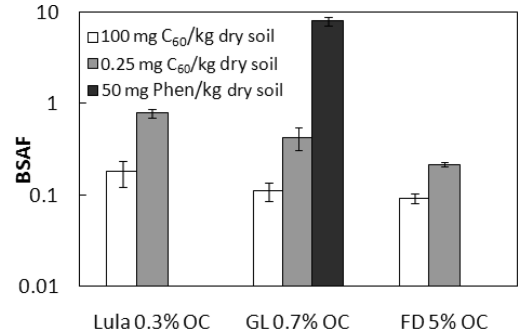

FIGURE 3. Biota-sediment accumulation factors (BSAF) for different treatments after 28 d exposure. Soil OC content did not significantly affect BSAF for high-dose (heterogeneous C_{60}) systems ($p > 0.05$), whereas BSAF decreased with increasing soil OC in low-dose (0.25 mg kg^{-1}) systems. Phenanthrene (Phen) had significantly higher ($p < 0.05$) BSAF, despite its lower hydrophobicity. The soil concentration of phenathrene was lower than that of C_{60} to avoid acute toxicity. Error bars represent ± one standard deviation.

soil-bound organic matter (*15*), which is conducive to decreased bioavailability. Whether temporary adsorption of C_{60} onto the gut wall (and subsequent excretion) also contributed to the observed peak was not determined.

C_{60} bioaccumulation in the two soils with low OC content followed a different pattern, increasing asymptotically over the incubation period to values similar to (statistically undistinguishable) that of FD soil (0.111 ± 0.025 for GL and 0.179 ± 0.057 for Lula soils) (Figure 3). Therefore, for these high-dose experiments (i.e., heterogeneous C_{60} systems), soil OC content had no significant effect on the extent of C_{60} bioaccumulation in earthworms after 28 d of exposure, although higher OC resulted in faster initial bioaccumulation (Figure 2A). The biokinetic model (eq 1) fit the data well (R^2 = 0.68−0.87), passing the chi-square test at the 95% confidence level, with $\lambda = 0.037$ d^{-1} for FD soil and $\lambda = 0$ for GL soil (Table 2). This corroborates that C_{60} bioavailability in high-OC FD soil decreased over time after a transitory peak while it did not decrease in low-OC GL soil.

Interestingly, C_{60} BSAF values decreased as C_{60} concentrations increased in high-dose GL soils (Figure 2A), stabilizing at 0.126 ± 0.005 for 60 mg kg^{-1}, 0.111 ± 0.025 for 100 mg kg^{-1}, and 0.065 ± 0.039 for 300 mg kg^{-1}. The same trend was observed for FD soil, with final BSAF values of 0.093 ± 0.011 at 100 mg kg^{-1} and 0.047 ± 0.007 at 300 mg kg^{-1} (Figure S1, Supporting Information). Apparently, as the C_{60} concentration in soil increases beyond sorption capacity, so does the fraction of C_{60} precipitates that are more difficult to pass through the gut wall and/or easier to eliminate after ingestion. However, the possibility for a higher elimination rate due to the toxic effect at high concentrations cannot be excluded (*37, 38*).

^{14}C elimination from worms exposed to FD soil amended with 100 and 300 mg kg^{-1} of C_{60} showed a rapid initial rate (≤24 h after initial depuration), indicative of gut clearing (Figure S2, Supporting Information). Note that the amount

TABLE 2. Biokinetic Model Parameters (± one standard error) for C_{60} and Phenanthrene (phen) Bioaccumulation in *E. fetida*[a]

treatment	k_s (d^{-1})	k_e (d^{-1})	λ (d^{-1})	R^2
GL soil, C_{60}, 60 mg kg^{-1}	0.036 ± 0.006	0.33 ± 0.07	0	0.86
GL soil, C_{60}, 100 mg kg^{-1}	0.024 ± 0.005	0.23 ± 0.06	0	0.85
GL soil, C_{60}, 300 mg kg^{-1}	0.057 ± 0.031	0.98 ± 0.56	0	0.68
FD soil, C_{60}, 100 mg kg^{-1}	0.12 ± 0.02	0.52 ± 0.13	0.037 ± 0.006	0.87
FD soil, phen, 50 mg kg^{-1}	10.04 ± 3.51	0.07 ± 0.01	0.36 ± 0.05	0.81

[a] Data correspond to a high-dose system where C_{60} was likely present in the soil in both aggregate/precipitate powder and absorbed molecular forms.

of ingested soil remaining after 24 h depuration should be relatively small. Hartenstein *et al.* reported that the gut loading of *E. fetida* is about 0.63 ± 0.022 (dry weight egesta per dry weight worm), and less than 5% of the gut load remains in the worm after 24 h depuration (*39*). Accordingly, theoretical calculations (Supporting Information) indicate that the measured radioactivity in worm tissue was contributed primarily by $^{14}C_{60}$ that had been absorbed (or adsorbed to the gut surface) rather than by soil-associated C_{60}.

Additional bioaccumulation experiments were conducted with a lower soil concentration of C_{60} (0.25 mg kg^{-1}), at which C_{60} was much more likely available in molecular form (sorbed by soil constituents including OC). Higher BSAF values (0.22−0.79) were measured compared to high-dose experiments (Figure 3), possibly because sorbed/molecular C_{60} is more available for bioaccumulation than the larger precipitates that form when the soil sorption capacity is exceeded. BSAF decreased with increasing OC content (i.e., 0.786 ± 0.078 for Lula soil (0.3% OC), 0.427 ± 0.121 for GL soil (0.7% OC), and 0.217 ± 0.012 for FD soil (5% OC)), and this trend was statistically significant ($p < 0.05$) (Figure 3). This is attributed to the lower bioavailability due to greater partitioning into soil with higher OC content. Similar trends have been observed in studies with PAHs (*17, 40*).

The BSAF for phenanthrene (added at 50 mg kg^{-1} in GL soil) on day 28 was 7.93 ± 0.86, which is significantly higher than that of C_{60} in both the high- and the low-dose scenarios (Figure 3). With a higher log K_{OW} of 6.67 (*10*), C_{60} yielded a much lower BSAF than phenanthrene (log K_{OW} = 4.48 (*41*)). This seems to be inconsistent with equilibrium partition theory, which suggests that BSAF should be constant for a given system (inherently assuming similar chemical potential for the organic phases in biological and sediment compartments) and thus independent of the K_{OW} value of the HOC (*42*). The lower C_{60} BSAF may be due to its larger molecular size (which hinders cellular uptake) (*25*) and/or stronger binding to soil OC. The organic-carbon-normalized partition coefficient (K_{OC}) of C_{60} was recently determined as $10^{6.2}$−$10^{7.1}$ (*43*), which is significantly higher than that for phenanthrene ($K_{OC} = 10^{4.1}$) (*44*) and contributes to its lower bioavailability. Precipitation of larger aggregates also likely hindered C_{60} bioaccumulation. To date, no studies have shown significant toxicity of pristine C_{60} to earthworms or microbes (*14, 45*), and the relatively low BSAF observed here is consistent with low-toxicity observations.

When considering the bioaccumulation factor (BAF), which represents the partitioning of a compound between an organism and the water phase (freely dissolved), the BAF of C_{60} at 0.25 mg kg^{-1} in GL soil was calculated as $10^{4.58}$ L kg^{-1}, much higher than that of phenanthrene at 50 mg kg^{-1} in GL soil, which was calculated as $10^{2.84}$ L kg^{-1} (Calculations, Supporting Information). These numbers agree with the values reported by Arnot and Gobas for the organic compounds with similar K_{OW} (*30*) and reflect higher BAF with higher K_{OW} as predicted by theory (*46*).

While C_{60} is relatively stable in the environment (*47*) and no biochemical or physical transformations were observed here, it could form stable aqueous suspensions upon association with dissolved NOM (*35*). The aqueous C_{60} nanoparticles or nC_{60} may be oxidized under certain environmental conditions (e.g., sunlight or UVA irradiation) (*48, 49*) or harsher conditions during water or wastewater treatment (e.g., irradiation with high UV intensity and/or ozonation) (*31, 50*), thus becoming more hydrophilic. Such transformations would change the mobility and bioavailability of C_{60}, resulting in different bioaccumulation potential(s) than found in this work.

Overall, this study clearly demonstrates that C_{60} bioaccumulates in *E. fetida*, indicating a potential risk of exposure to higher order organisms through food web transfer. This underscores the need for further studies on C_{60}, among other engineered nanomaterials, regarding trophic transfer, biomagnification potential, and associated sublethal effects.

Acknowledgments

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Supporting Information Available

Calculations on soil sorption capacities, contribution to ^{14}C content in worms from unexcreted soil, and bioaccumulation factors as well as additional data for $^{14}C_{60}$ bioaccumulation and elimination from worms and mass balances. This material is available free of charge via the Internet at http://pubs.acs.org.

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