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Nanomaterials in the Environment

EFFECT OF SOIL SORPTION AND AQUATIC NATURAL ORGANIC MATTER ON THE ANTIBACTERIAL ACTIVITY OF A FULLERENE WATER SUSPENSION

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Abstract—The present study investigated the association of a C_{60} water suspension (nC_{60}) with natural organic matter, present as a soil constituent or dissolved in the water column, and its effect on the antibacterial activity of nC_{60}. Sorption of nC_{60} to soil reduced its bioavailability and antibacterial activity, and the sorption capacity strongly depended on the organic content of the soil. Adsorption of aquatic dissolved humic substances onto nC_{60} and possible subsequent reactions also were found to eliminate nC_{60} toxicity at humic acid concentrations as low as 0.05 mg/L. These findings indicate that natural organic matter in the environment can mitigate significantly the potential impacts of nC_{60} on microbial activities that are important to ecosystem health.

Keywords—Fullerene water suspension Antibacterial Natural organic matter Soil Sorption

INTRODUCTION

Fullerenes, which were discovered in 1985, represent a third allotrope of carbon known for its caged structure and polyaromaticity [1]. These unique properties have made buckminsterfullerene (C_{60}) and its derivatives promising candidates for various applications including cancer therapeutics, drug delivery, computer sensors, and so on. [2–6]. As the nanotechnology industry develops, C_{60} is expected to be produced and consumed in large amounts [7], so there is little doubt that this nanomaterial increasingly will be found in the environment. Thus, it is imperative to understand how C_{60} may interact with abiotic and biological components of the ecosystem and assess the potential environmental impacts resulting from its widespread use and disposal.

Although pristine C_{60} is extremely insoluble in water, it can be suspended through several methods. In the present study, we call these stable C_{60} water suspensions nC_{60}. Because nC_{60} is considered the most environmentally relevant form of C_{60} when there is a spill of C_{60} powder or C_{60} solution in a solvent [8], several toxicological studies have focused on nC_{60}. These studies have shown that nC_{60} is toxic to bacteria, eukaryotic cell lines, water fleas, and fish [2,8–10]. Research also indicated that nC_{60} delayed zebrafish embryo and larva development and exerted teratogenic effects [11]. However, most previous studies were conducted in simple systems with well-defined aqueous media. Little is known about how natural organic matter (NOM), ubiquitous in soil or suspended in the water column, affects nC_{60} toxicity.

Recent research by Tong et al. [12] demonstrated that soil might eliminate the high toxicity of nC_{60} that has been observed in low-salt mineral media [8,9,13,14]. This indicates the need to consider nC_{60} interactions with common constituents in environmental matrices to obtain representative results of potential environmental impacts. In addition to toxicological tests, flow-through column studies using glass beads, clays, and natural soil have demonstrated the relatively limited mobility of nC_{60} [15–18]. Clay minerals have a strong propensity to associate with nC_{60} [18], corroborating the notion that nC_{60} is unlikely to disperse widely in a natural soil setting. However, only a limited amount of information currently is available regarding the bioavailability of C_{60} in the natural environment, which is commonly an important factor in controlling environmental impacts.

Previous work has demonstrated that NOM enhances the aqueous stability of carbon-based nanoparticles including nC_{60} and multiwalled carbon nanotubes [19,20]. Furthermore, it has been postulated that C_{60} partitioning into soil organic matter controls the solution-level bioavailability and thus reduces the toxicity of nC_{60} [12]. However, the extent to which nC_{60} toxicity decreases as a function NOM type and concentration has not been addressed in the literature.

The present study addresses the hypothesis that soil-associated or dissolved NOM attenuates nC_{60} toxicity by decreasing its bioavailability and/or modifying its surface chemistry. Specifically, the solution-level bioavailability and antibacterial activity of nC_{60} were examined in the presence of sorbents (powdered activated carbon [PAC] and soils) and low concentrations of dissolved humic substances to advance our understanding of the risks associated with environmental contamination by nC_{60}.

MATERIALS AND METHODS

Preparation of nC_{60}

The nC_{60} was prepared following a protocol described by Lyon et al. [13] with some modifications. The C_{60} (100 mg of 99.5% pure, SES Research, Houston, TX, USA, or Materials and Electrochemical Research, Tucson, AZ, USA) was dissolved in 4 L of tetrahydrofuran (certified spectra-analyzed, Fisher Scientific, Houston, TX, USA). The tetrahydrofuran was sparged with nitrogen for 10 min before and after C_{60} was added to prevent oxidation. The mixture was stirred overnight at room temperature in the dark. The solution was filtered through a 0.22-μm–pore size Osmonics nylon membrane (Fisher Scientific) to remove undissolved C_{60}. A 250-ml aliquot of the C_{60} tetrahydrofuran solution was stirred vigorously while

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adding an equal volume of Milli-Q® water (Millipore, Billerica, MA, USA) at a rate of 1 L/min. Tetrahydrofuran was evaporated using a Büchi Rotavapor (Büchi Labortechnik AG, Flawil, Switzerland) with a hot water bath, a refrigerated condenser, and a vacuum pump. One liter of the mixture was heated to 65°C to evaporate the tetrahydrofuran until a final volume of 300 ml was reached. Prior to concentration, the nC_{60} suspension was filtered through a 0.45-μm–pore size Osmonics nylon membrane filter to remove large particles. The nC_{60} was concentrated with a Büchi Rotavapor at 70°C to a final concentration of 10 to 15 mg/L nC_{60}. The concentrated suspension was filter-sterilized through a 0.22-μm–pore size cellulose syringe filter or a 0.22-μm–pore size mixed cellulose esters membrane vacuum filter (Fisher Scientific). The resulting suspension was stored in the dark at room temperature.

nC_{60} particles characterization

Particle size and zeta potential were determined using a noninvasive backscatter (NIBS) device (Zetasizer Nano, Malvern Instruments, UK). The NIBS detects light scattering at a 173° angle, which extends the range of sizes and concentrations of samples that can be measured. The mean diameters were weighted according to the number of particles in each size fraction. Zeta potential measurements were conducted in minimal Davis (MD) medium, which is described in the following section. The C_{60} particles also were analyzed by transmission electro microscopy ([TEM], resolution of 0.2 nm) performed with a JEOL 2100 high-resolution microscope operated at 120 kV (JEOL, Tokyo, Japan). The TEM samples were prepared by placing drops of nC_{60} suspension on 300 mesh copper grids (Ted Pella, Redding, CA, USA), which were placed on filter paper to remove excess water and then dried overnight.

The concentration of nC_{60} was determined by ultraviolet absorbance measurement using an Ultrospec 2100 *pro* spectrophotometer (GE Healthcare Life Sciences, Piscataway, NJ, USA) at 336 nm, as described by Lyon et al. [14]. One milliliter of 100 mM magnesium perchlorate and 2 ml of toluene were added to 2 ml of the nC_{60} suspension to extract nC_{60} from the aqueous phase. The vial was sealed and the mixture was stirred for 2 h. The vial then was placed in a −20°C freezer to freeze the water to aid removal of the toluene phase for analysis. A previous publication showed that this approach extracted 94 to 101% of the nC_{60} from the aqueous phase with toluene [8]. A standard curve was prepared by dissolving varying amounts of nC_{60} in toluene, and the absorbance of each test sample at 336 nm was compared to the standard curve.

Bacterial growth

The gram-negative bacterium *Escherichia coli* K12 (ATTC 25404) was chosen as the test organism in order for the results to be comparable with previous publications that used *E. coli* [13,14]. In addition, *E. coli* has been well studied and is easy to grow on the minimal mineral medium that is necessary for precluding nC_{60} coagulation and precipitation [14]. *Escherichia coli* K12 was maintained on Luria-Bertani plates and in Luria-Bertani broth. The MD medium was made according to the recipe described by Lyon et al. [13] in which the potassium phosphate concentration was reduced by 90% compared with Davis medium. Bacterial growth was quantified by measuring optical density at 600 nm (OD_{600}) using a Turner SP-830 spectrophotometer (Barnstead, Dubuque, IA, USA).

Sorbents

Powdered activated carbon, one of the most commonly used and well-studied sorbents, was used first to study how the sorption of nC_{60} influences antibacterial activity in a well-defined system. The PAC was purchased from Fisher Scientific (Pittsburgh, PA, USA). The average diameter of PAC was 80.6 μm according to the manufacturer. The surface area of PAC was determined by the Brunauer-Emmett-Teller method to be 754.4 m^2/g using a Quantachrome Autosorb-3B Surface Analyzer (Quantachrome Instruments, Boynton Beach, FL, USA). Pore size distribution was calculated by the Barrett-Joyner-Halenda method based on N_2 adsorption/desorption data [21]. The average pore size of PAC is 16.8 Å. Dry PAC was autoclaved at 120°C for 15 min before being mixed with the nC_{60} suspension.

Two kinds of soil were used in the experiments. The first, Lula sandy soil (R.S. Kerr Environmental Research Laboratory, Ada, OK, USA), consists of 92% sand, 6% clay, ~1.5% silt, and 0.27% organic carbon [17]. The Brunauer-Emmett-Teller surface area of Lula soil has been reported to be 1.24 m^2/g [17]. The other soil was from Amana Colonies, Iowa, USA, which is a silty loam to silty clay loam alluvium and contains 3.5% organic matter with a Brunauer-Emmett-Teller surface area of 34.1 m^2/g. Sand, one of the components of Lula soil, also was obtained from R.S. Kerr Environmental Research Laboratory.

Humic substances

Commercial humic acid (Sigma-Aldrich, St. Louis, MO, USA) was used in initial screening experiments. Number average and weight average molecular weights of Sigma-Aldrich HA (AHA) were determined by vapor pressure osmometry and reported to be 1,630 and 4,100 Da, respectively [22]. Because AHA is a complex material with many impurities that could confound the interpretation of the results, Suwannee River standard humic substances were used for subsequent experiments.

Suwannee River humic acid (SRHA; Standard II, International Humic Substances Society, Atlanta, GA, USA) and Suwannee River fulvic acid (SRFA; Standard II, International Humic Substances Society) were used as model aquatic NOM. The molecular weight of SRHA was reported by Hong and Elimelech [23] as 1,000 to 5,000 Da. The number average molecular weight of SRFA was determined by vapor pressure osmometry and reported to be 1,360 Da [22]. The SRHA and SRFA solutions were prepared by introducing 100 mg dry humic substance powder into 50 ml Milli-Q water and then stirring overnight. The solution was filtered through a 0.22-μm–pore size cellulose membrane filter and stored in the dark at 4°C.

Assessing antibacterial activity of nC_{60}

The minimum inhibitory concentration (MIC) of nC_{60} to *E. coli* was determined according to a standardized protocol [24]. *Escherichia coli* was grown in Luria-Bertani medium overnight. The next morning, bacteria were diluted to a final OD_{600} of 0.002 in MD medium containing different concentrations of nC_{60}. The bacteria were incubated with shaking at 37°C overnight. The lowest concentration of nC_{60} that inhibited growth, as determined visually by lack of turbidity, was selected as the MIC.

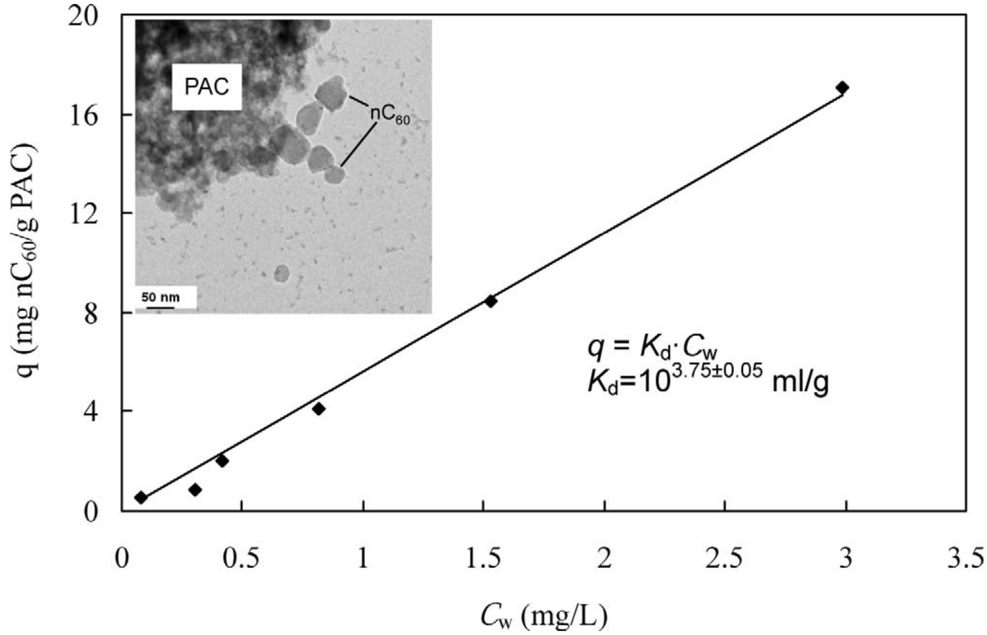

Fig. 1. Linear adsorption isotherm for nC_{60} with powder-activated carbon (PAC). Inserted transmission electro microscopy (TEM) micrograph shows nC_{60} adsorbed onto the outer surface of PAC.

Sorption of nC_{60} aggregates from aqueous solution to PAC

A sorption experiment was conducted to characterize the equilibrium partitioning of nC_{60} between water and PAC. The experiment was performed in triplicate, using different initial nC_{60} concentrations and a fixed PAC dose for each sample vial. The PAC (100 ± 0.1 mg) was mixed with 10 ml Milli-Q water to make a stock suspension of 10 mg/ml. For each 2-ml sample, 0.1 ml of the PAC stock suspension was added into a 5-ml vial. Then different amounts of nC_{60} (14.5 mg/L) were injected into the vials and corresponding volumes of Milli-Q water were added to achieve a final volume of 2 ml. The initial nC_{60} concentrations were 14.5, 7.25, 3.63, 1.81, 0.91, and 0.45 mg/L. The mixture was stirred for 48 h, filtered with 0.45-μm–pore size cellulose syringe filters, and analyzed for ultraviolet absorbance at 336 nm to determine the equilibrium aqueous phase nC_{60} concentration. The analysis of each sample was repeated three times. The average sorption losses of nC_{60} to membrane filters were determined in a preliminary study to be negligible, around 1%.

Effect of sorption on antibacterial activity of nC_{60}

To assess the effect of sorption on antibacterial activity of nC_{60}, a respirometer (Oxymax-ER, Columbus Instrument, Columbus, OH, USA) was used to monitor the heterotrophic activity of bacteria dosed with nC_{60} (positive control) or a mixture of sorbents and nC_{60}. *Escherichia coli* was grown overnight and then diluted into 50 ml of MD medium to a final OD_{600} of 0.002. Approximately 5 to 6 h later ($OD_{600} \sim 0.08$), while in exponential phase, bacteria were exposed simultaneously to nC_{60} (0.5 mg/L) and/or PAC. Another set of similar experiments was conducted with PAC that had been equilibrated with nC_{60} for 2 d prior to exposing to the bacteria. This modification was adopted to discern any sorption kinetics effect that might influence nC_{60} bioavailability and toxicity to bacteria. Soils of different organic content (geosorbents) were tested subsequently. They were equilibrated with nC_{60} for 2 d before the experiments.

Effect of aquatic NOM on nC_{60} antibacterial activity

The effect of aquatic NOM on heterotrophic activity was investigated using respirometry, following a procedure similar to that described above. Both humic acids, SRHA (5.4 mg/L) and AHA (10 mg/L), were mixed with nC_{60} for 2 d before exposure to the bacteria.

In addition, a cell growth inhibition assay was used to evaluate toxicity of nC_{60} in the presence of NOM. Varying levels of SRHA or SRFA and 1 mg/L nC_{60} were mixed in MD medium in a 24-well plate and equilibrated for 2 d before exposing to bacteria. *Escherichia coli* was grown in Luria-Bertani medium at 37°C overnight and was diluted in wells of the plate to a final OD_{600} of 0.002. The plate was incubated for 48 h, and growth of cells in each well was recorded. All samples were tested in duplicate.

RESULTS AND DISCUSSION

Sorption of nC_{60} by PAC and soils

The equilibrium partitioning data between nC_{60} and PAC were fitted with a linear isotherm (Fig. 1). At equilibrium, the solution-phase nC_{60} concentrations decreased by 58 to 77% from the initial values of 14.5, 7.25, 3.63, 1.81, 0.91, and 0.45 mg/L, indicating that PAC is an effective adsorbent for nC_{60}. A linear isotherm in the form of $q = K_d \cdot C_w$ was observed, where K_d denotes the partition coefficient, q ($mg \cdot g^{-1}$) is the mass of nC_{60} sorbed per unit mass of PAC at equilibrium, and C_w ($mg \cdot L^{-1}$) is the nC_{60} concentration in the solution phase at equilibrium. A K_d value of $10^{3.75 \pm 0.05}$ $ml \cdot g^{-1}$ was obtained, indicating PAC is an effective sorbent for nC_{60}. However, PAC has less affinity for nC_{60} than for naphthalene, a relatively soluble polynuclear hydrocarbon with a reported partition coefficient of $10^{5.17}$ [17]. We postulate that adsorption of nC_{60} to PAC mainly occurred on the outer surface of PAC because the average pore size of this PAC was 16.8 Å, which is much smaller than the average diameter of nC_{60} (108 nm from NIBS measurement). This also was visualized by TEM. As shown

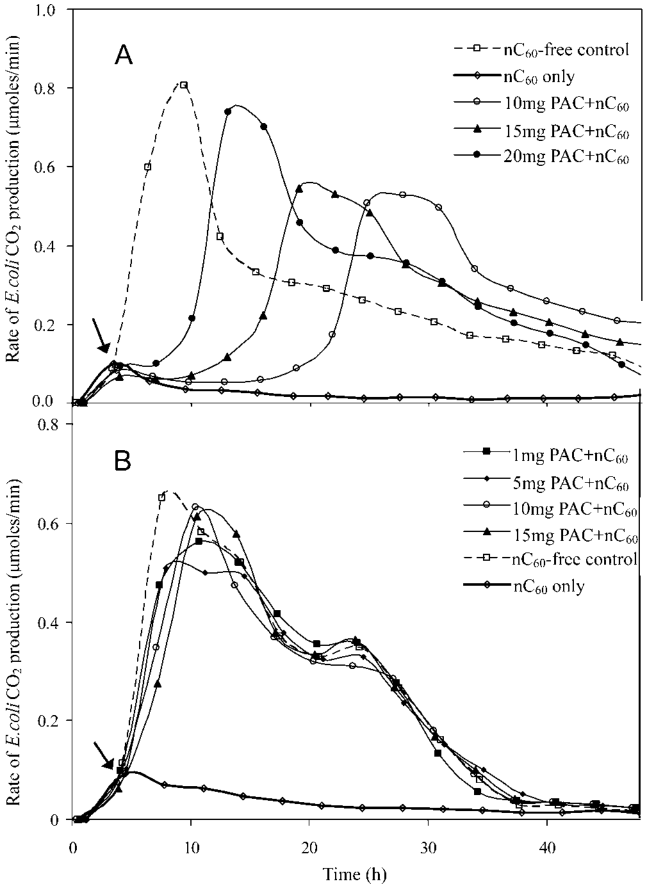

Fig. 2. Attenuation of nC_{60} toxicity to *Escherichia coli* by sorption onto powder-activated carbon (PAC). The addition of nC_{60} (0.5 mg/L, indicated by the arrow) significantly decreased the respiration rate of *E. coli* relative to nC_{60}-free control. The PAC mitigated this effect. Higher PAC amounts had a more pronounced attenuation effect than when PAC was mixed with nC_{60} at the time of exposure (**A**). This sorption kinetics effect was not observed when PAC and nC_{60} were equilibrated for 2 d prior to exposure (**B**).

in the insert in Figure 1, nC_{60} aggregates were found attached on the outer surface of the PAC particles.

Effect of sorbents on nC_{60} antimicrobial activity

Experiments were conducted to test the hypothesis that sorption of nC_{60} to potential geosorbents (e.g., soil constituents) or activated carbon would reduce its bioavailability (e.g., hinder direct contact with bacteria) and attenuate its antibacterial activity. A respirometer was used to monitor the heterotrophic activity (measured as CO_2 produced) of *E. coli* exposed to nC_{60} alone or in the presence of various sorbents (PAC, soil, and sand). Considering that nC_{60} particles agglomerate and even precipitate in the presence of high salt and protein concentrations [14,25], MD medium was chosen for bacteria culture throughout the antibacterial test.

Figure 2 shows that the addition of nC_{60} (0.5 mg/L, indicated by arrow) significantly decreased the respiration rate of *E. coli* relative to an nC_{60}-free control. This bactericidal effect was mitigated by PAC. More than 90% of nC_{60} was removed from the solution in all samples containing different amounts of PAC when sorption reached equilibrium (data not shown). The residual nC_{60} concentrations, 0.001 to 0.05 mg/L, were much lower than the MIC for *E. coli*, which previously was determined to be between 0.1 and 0.5 mg/L using a lower

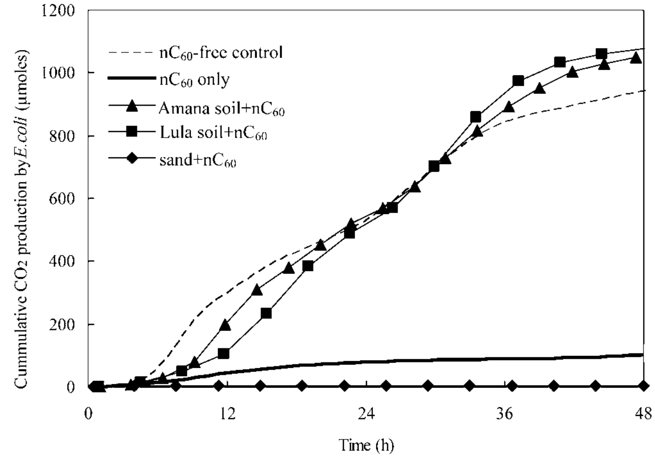

Fig. 3. Lula soil (0.27% organic matter, Ada, OK, USA) and soil from Amana (3.5% organic matter, Amana Colonies, IA, USA) both reduced the antibacterial effect of nC_{60} significantly at the concentration of 100 mg/50 ml minimal Davis (MD) medium. Sand with very low concentration of organic matter (less than 0.01%) was not effective in attenuating nC_{60} toxicity at the same concentration.

bacterial concentration ($OD_{600} = 0.002$) than in this experiment ($OD_{600} = 0.3$). The fact that the higher OD_{600} values require more nC_{60} to inactivate the bacteria [14] reinforces the notion that these residual nC_{60} concentrations were much lower than needed to inhibit bacterial growth. When PAC was mixed with nC_{60} at the time of exposure, higher dosage of PAC resulted in more pronounced attenuation in the antibacterial activity of nC_{60} (Fig. 2A). However, the beneficial effect of adding more PAC was not observed when PAC and nC_{60} were equilibrated for 2 d prior to exposure (Fig. 2B). These observations indicate that all PAC doses tested were able to remove nC_{60} to below the concentration needed for growth inhibition, but longer nC_{60}-PAC contact time was required for lower PAC doses. It should be noted that a negative control containing PAC only showed no adverse effects on *E. coli* respiration rate (data not shown).

Two types of soils, a high organic content soil from Amana, Iowa, and a sandy soil from Lula, Oklahoma, and the sand component of the Lula soil also were tested in the present study. Negative controls using the soils or the sand without nC_{60} did not show any adverse effects on *E. coli* respiration rate (data not shown). Both soils were found to reduce the toxicity of nC_{60} to *E. coli*, assessed by respirometry (Fig. 3). In contrast, sand with very low organic carbon content (<0.01%) was not effective in attenuating nC_{60} toxicity. In a separate sorption experiment using the same soil content as in the CO_2 production measurement (100 mg/50 ml of MD medium), the residual nC_{60} concentration (initial concentration 0.5 mg/L in MD medium) was 0.16 to 0.26 mg/L after sorption by the Lula soil, and 0.03 to 0.04 mg/L after sorption by Amana soil. It is noted that, although the residual nC_{60} concentration after sorption by Lula soil falls within the range of previously reported MIC, the bacterial cell concentration used in the respirometry experiment was much higher than that used to evaluate the MIC ($OD_{600} = 0.002$) and thus required a higher nC_{60} concentration for inhibition [14], which permitted growth. The higher sorption capacity of the Amana soil is probably the result of larger surface area (34.1 vs. 1.24 m^2/g for the Lula soil), and higher concentration of organic matter (3.5 vs. 0.27% in Lula soil). The unchanged toxicity of nC_{60} in the presence of sand probably is due to the low organic carbon content of the sand and consequently low sorption capacity for nC_{60}. This suggests that the microbial community in soils with low concentration of organic matter and small surface area would be more susceptible to the presence of nC_{60} than organic soils with larger surface area.

Effect of dissolved NOM on nC_{60} antimicrobial activity

To investigate the impact of dissolved NOM on nC_{60} toxicity, CO_2 production by *E. coli* in the presence of nC_{60} and SRHA (5.4 mg/L) or AHA (10 mg/L) were measured. No adverse effects were observed in negative controls with SRHA and AHA alone (data not shown). As shown in Figure 4, both SRHA and AHA significantly mitigated the antibacterial effect of nC_{60}. All three suspensions exhibit similar toxicities initially, but the humic acid–spiked systems recover more rapidly than the one spiked with nC_{60} alone. The nC_{60} toxicity in the presence of NOM also was evaluated by assessing *E. coli* growth in a 24-well plate (Table 1). *Escherichia coli* growth was completely inhibited by 1 mg/L of nC_{60}. However, SRHA at concentrations as low as 0.05 mg/L enabled growth, indicating mitigation of nC_{60} toxicity. No mitigating effect was observed at lower SRHA concentrations. Similar results were obtained with SRFA, although the minimum concentration that mitigated antibacterial activity was slightly higher (0.1 mg/L), indicating that SRFA was less effective than SRHA in attenuating nC_{60} toxicity. Typical humic acid concentrations in natural waters are much higher than the threshold toxicity-mitigating concentrations observed in our experiments. This underscores that dissolved NOM in natural waters is likely to mitigate significantly the nC_{60} toxicity.

Two hypotheses, which are not mutually exclusive, are proposed to explain how NOM attenuates the antibacterial effects of nC_{60}: Adsorption of NOM on nC_{60} surface interferes with direct contact of nC_{60} with bacterial cells, and NOM may react with nC_{60}, promote its disaggregation, or change its surface chemistry and consequently antibacterial activity. Both effects could be occurring, so the attenuation of the toxicity of nC_{60} could be a combined result. Note that disaggregation alone, without NOM coating or changes in surface properties, likely would increase toxicity because of the higher surface area offered by smaller nC_{60} particles [13]. Adsorption of NOM on nC_{60} was evident from the zeta potential measurement. The zeta potential of nC_{60} particles changed from -27 ± 0.68 mV to -30 ± 0.77 mV ($n = 3$ replicates) as soon as the negatively charged SRHA was added into the suspension. This is a statistically significant decrease ($p < 0.05$) that suggests that the adsorption of SRHA onto nC_{60} occurred immediately. Suwannee River humic acid has been shown to associate strongly with nC_{60} in previous publications [19].

The minimum concentration of SRHA needed to completely coat the surface of a 1-mg/L nC_{60} particle suspension was estimated assuming that: C_{60} and nC_{60} are both rigid spherical particles with diameter of 1 nm [26] and 108 nm (number-averaged particle diameter measured by NIBS), respectively; the hydrodynamic diameter of SRHA ranges from 1.5 to 3.5 nm [27], with a molecular weight of 5,000 Da [23]; and the adsorbed NOM forms a monolayer.

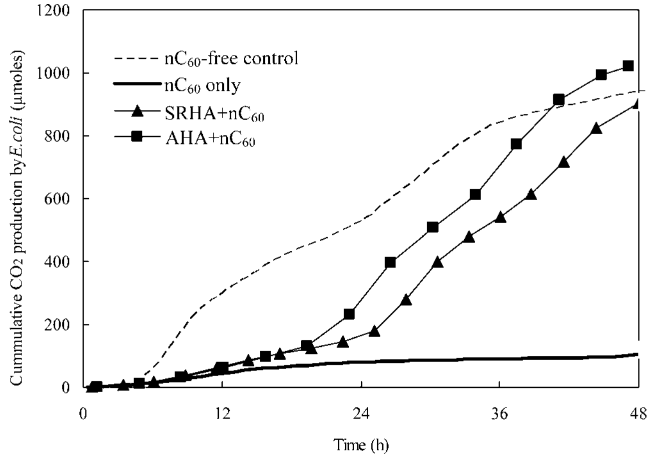

Fig. 4. Suwannee River humic acid ([SRHA], 5.4 mg/L) and Aldrich humic acid ([AHA], 10 mg/L) attenuated the toxicity of nC_{60} to bacteria.

The number concentration of nC_{60} is calculated as

$$\begin{aligned} nC_{60}\ \text{number concentration} &= (nC_{60}\ \text{concentration} \times C_{60}\ \text{molecular volume}) \\ &\quad \div (nC_{60}\ \text{aggregate volume} \times 64\% \\ &\quad \times C_{60}\ \text{molecular weight}) \\ &= \frac{1\ \text{mg/L} \times \frac{4}{3} \times \pi \times (0.5\ \text{nm})^3}{\frac{4}{3} \times \pi \times (54\ \text{nm})^3 \times 64\% \times 720\ \text{Da}} \\ &= 1.037 \times 10^{15}\ \text{aggregates}\ L^{-1} \end{aligned}$$

Although nC_{60} is known to have a crystalline structure, a random packing density for rigid spheres, 64% is assumed for C_{60} packing in an nC_{60} particle for conservative estimation of the nC_{60} number concentration; a highest nC_{60} number concentration requires more SRHA for complete surface coverage (http://mathworld.wolfram.com/SpherePacking.html).

Table 1. Growth of *Escherichia coli* in minimal Davis (MD) medium with varying natural organic matter addition and 1 mg/L nC_{60}. (+) denotes bacterial growth and (−) denotes no growth. SRHA = Suwannee River humic acids; SRFA = Suwannee River fulvic acids

Treatment	Natural organic matter	*E. coli* growth
MD alone	None	+
MD + 1 mg/L nC_{60}	None	−
MD + 1 mg/L nC_{60}	0.02 mg/L SRHA	−
MD + 1 mg/L nC_{60}	0.05 mg/L SRHA	+
MD + 1 mg/L nC_{60}	0.1 mg/L SRHA	+
MD + 1 mg/L nC_{60}	0.5 mg/L SRHA	+
MD + 1 mg/L nC_{60}	1 mg/L SRHA	+
MD + 1 mg/L nC_{60}	0.02 mg/L SRFA	−
MD + 1 mg/L nC_{60}	0.05 mg/L SRFA	−
MD + 1 mg/L nC_{60}	0.1 mg/L SRFA	+
MD + 1 mg/L nC_{60}	0.5 mg/L SRFA	+
MD + 1 mg/L nC_{60}	1 mg/L SRFA	+

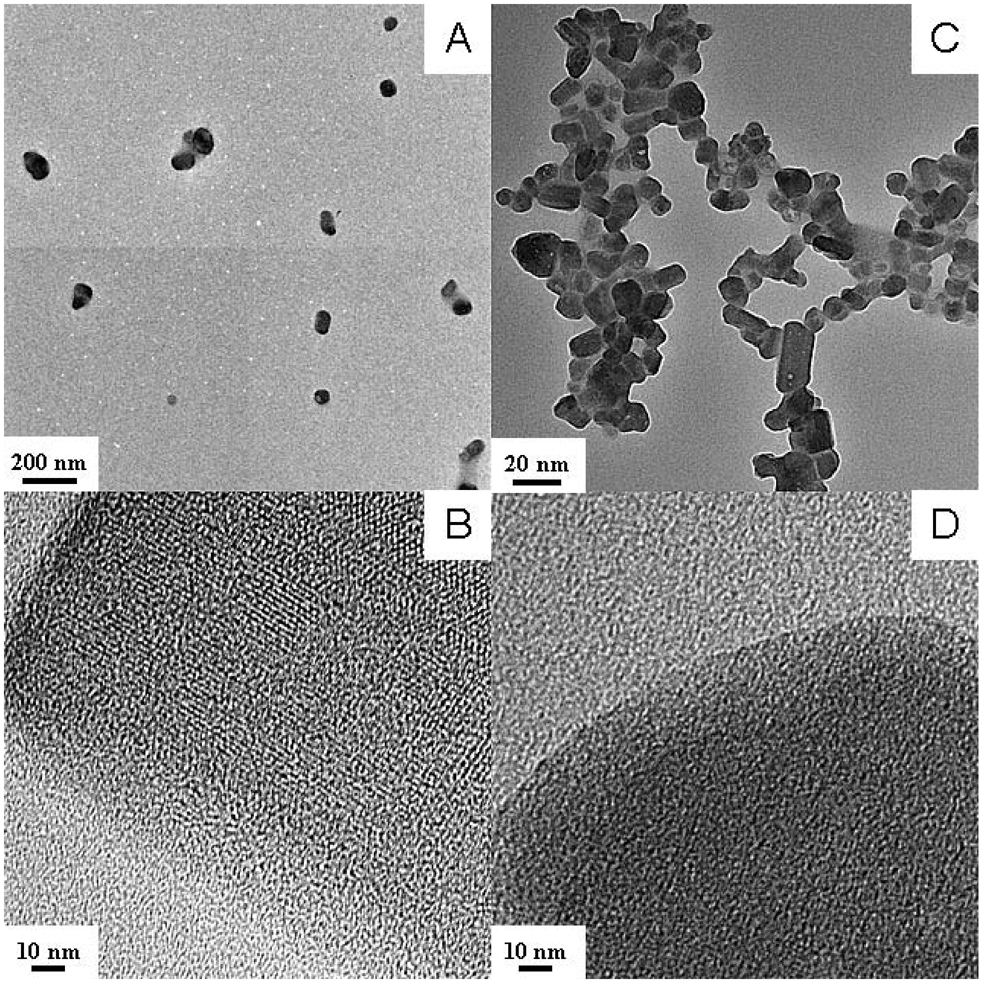

Fig. 5. Transmission electromicroscopy (TEM) micrographs of nC_{60} before and after addition of Suwannee River humic acid (0.05 mg/L). (**A**) nC_{60} particles without humic acid. (**B**) Magnified part of nC_{60} particle in image A, showing crystalline structure. (**C**) nC_{60} particles after addition of humic acid. (**D**) Magnified part of nC_{60} particle in image C, showing loss of crystallinity.

For SRHA with a hydrodynamic diameter of 1.5 nm, the concentration needed to form a monolayer coating on all nC_{60} particles in a 1-mg/L suspension is

$$= nC_{60} \text{ number concentration} \times \frac{nC_{60} \text{ particle surface area}}{\text{SRHA particle cross section area}} \times \text{SRHA molecular weight}$$

$$= 1.037 \times 10^{15} \text{ aggregates L}^{-1} \times \frac{4 \times \pi \times (54 \text{ nm})^2}{\pi \times (0.25 \text{ nm})^2} \times 5{,}000 \text{ Da}$$

$$= 0.18 \text{ mg/L}$$

If the hydrodynamic diameter of the SRHA increases to 3 nm, the concentration of SRHA needed for a monolayer coating is only 0.03 mg/L. This range of estimated SRHA concentrations needed for a monolayer coating of nC_{60} (0.03–0.18 mg/L) is consistent with the low concentrations observed to mitigate nC_{60} antibacterial activity in the 24-well plate experiment.

High-resolution TEM images of nC_{60} before and after NOM addition (Fig. 5) show evidence of changes in nC_{60} particle surface. In the absence of NOM, the crystalline structure of nC_{60} clearly is visible in Figure 5B for all particles inspected. After adding 0.05 mg/L SRHA, nC_{60} loses crystalline structure in some parts of the particle (Fig. 5D, large areas without clearly identifiable crystal lattice). This was observed in many nC_{60} particles. Apparently, in addition to coating the nC_{60} surface, which hinders direct contact with cells, NOM also may have altered the structure of nC_{60} causing its disaggregation [28]. The disaggregation also was verified by the observed change in average particle size from 82 to 35 nm after the addition of humic acid. It is unclear whether this interaction is a redox reaction or simple disaggregation of nC_{60}. The mechanism of such structural changes is the subject of further investigation.

CONCLUSION

The antibacterial activity of nC_{60} can be mitigated by the presence of NOM as a soil constituent or dissolved in the water column. Sorption to soil might decrease the bioavailability of nC_{60} and thus its toxicity to bacteria, and this mitigating effect likely increases with the organic content and surface area of the soil. Aqueous organic matter also may mitigate nC_{60} toxicity, by coating nC_{60}, hindering direct contact of with cells, and possibly altering nC_{60} surface chemistry through an undetermined mechanism. This notion is supported by zeta potential measurements, high-resolution TEM observations, and theoretical coating calculations. Overall, the present study implies that the impacts of nC_{60} to indigenous microbial communities that are important to ecosystem health can be mitigated significantly by NOM, and suggests the need for further research to elucidate the mechanisms by which NOM reduces the toxicity of nC_{60} nanoparticles.

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