Environmental fate of endocrine-disrupting chemicals: Association with biosolids-derived dissolved organic matter
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Environmental Fate of Endocrine-Disrupting Chemicals: Association with Biosolids-Derived Dissolved Organic Matter

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ABSTRACT

Endocrine-disrupting compounds interrupt the normal reproductive systems of organisms. Natural and synthetic endocrine-disrupting compounds have been found in aquatic systems throughout the world where they have been shown to interfere significantly with reproduction of certain fish species and amphibians at very low concentrations. Endocrine-disrupting compounds commonly occur in municipal wastewater and biosolids that are used as agricultural soil amendments. While the environmental fate of land-applied endocrine-disrupting compounds is uncertain, they may accumulate in amended soils because their association with soil components may retard their degradation. In addition, because endocrine-disrupting compounds preferentially associate with dissolved organic matter, they may be transported through soils to a greater degree than studies of pure compounds suggest. More complete knowledge of the factors that contribute to the persistence and migration of endocrine-disrupting compounds in soil is critical to improve the management of land-applied biosolids and wastewater irrigation and to develop robust predictive models for risk assessment.

In this project, we focused on the role that dissolved organic matter and organic colloids play in the fate of three model endocrine-disrupting compounds: 17α-ethinylestradiol, bisphenol-A, and 4-nonylphenol. We have also investigated 17β-estradiol and estrone, two hormones commonly found in animal waste that is land applied. We sought to identify and characterize the components of dissolved and colloidal organic components in soils and biosolids that may bind with endocrine-disrupting compounds. We investigated the adsorption of the compounds directly to soil materials as well as their transport in intact soil columns. We also explored the potential for the different components of dissolved organic matter to be adsorbed by soil materials.
Summary Sheet

Publication Summary

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Contribution of Collaboration

In this project, the PIs and their student collaborated in a number of important ways.

(1) We consulted with one another by email exchanges.

(2) We met in person to discuss project objectives and progress at two scientific meetings: the EMCON conference in Iowa City, Iowa, in August 2014, and the Soil Science Society of America Annual Meetings in Minneapolis, Minnesota, in November 2015.

(3) In summer of 2016, ISU graduate student Fritzie Rivas, traveled to Israel and worked alongside Dr. Chefetz’s graduate student Maya Engel to learn the procedures for adsorptive fractionation of dissolved organic matter components. As of the writing of the final report, Ms. Rivas has completed a comparable experiment with fractionated Iowa organic matter and Iowa soils. Her experiment will form one chapter of her PhD thesis, which should be completed in summer 2017. The Israeli collaborators will be co-authors on a forthcoming publication dealing with this topic.
Environmental Fate of Endocrine-Disrupting Chemicals:
Association with Biosolids-Derived Dissolved Organic Matter

Project Achievements

This section of the report describes significant achievements of the scientific work we conducted in the project. It is divided into four sections: (1) adsorption of BPA, EE2, and 4-NP by alluvial soils in Iowa, (2) transport of endocrine disrupting chemicals in soil, (3) adsorptive fractionation of dissolved organic matter, and (4) sorption equilibrium and kinetics of 17β-estradiol and estrone with soil and environmental colloids.

Adsorption of BPA, EE2, and 4-NP by Alluvial Soils in Iowa

Bisphenol-A (BPA), 17α-ethinylestradiol (EE2), and 4-nonylphenol (4-NP) are endocrine disrupting chemicals that may interfere with the reproductive systems of aquatic species. To better understand the potential for movement of these compounds from soil to aquatic systems, in the present study, we determined the degree of their adsorption by two Iowa alluvial soils with varying organic matter and clay concentrations [Zook (clay) and Hanlon (sandy loam)]. The soil samples were equilibrated against a range of EDC concentrations in solution under conditions that minimized microbial activity and photodegradation, and the difference in the initial and the equilibrium concentrations represented the amount of the EDC that had been adsorbed by the soil.

The data fit a combined Langmuir-Freundlich model, also referred to as Sips’ isotherm, and three adsorption parameters – \( Q_{\text{max}} \) (adsorption capacity), \( K \) (affinity constant), and \( n \) (index of heterogeneity) – could be determined. Over the range of EDCs and equilibrium concentrations, the model fit was good, with \( R^2 = 0.99 \), except for the EE2-Hanlon system with \( R^2 = 0.95 \). Among the soil – EDC interactions, the \( n \) values varied from less than 1 to greater than 1, suggesting considerable heterogeneity of adsorption energies, although in the BPA-Hanlon system, \( n = 0.96 \) indicated relatively homogenous adsorption sites.

For bisphenol A (BPA) sorption, modeling indicated that the sandy Hanlon soil would have a maximum adsorbed concentration similar to that of the clayey Zook soil, although the affinity index, \( K \), was somewhat lower than that for Zook soil. In both soils, the heterogeneity of adsorption energies was high, as indicated by the \( n \) values. At the low equilibrium concentrations for BPA
that are normally found in the environment, there was little desorption of the compound from either soil.

Adsorption of the polar (but still hydrophobic and nonionic) EE2 differed between the two soils. In the case of the sandy Hanlon soil, adsorption was very strong at low concentrations and then leveled off at higher concentrations. In contrast, the EE2 adsorption to the high-clay Zook soil indicated weak adsorption initially, but adsorption did increase with higher EE2 concentrations. Modeling indicated that the Zook soil would have the higher maximum adsorption capacity and the higher affinity for EE2. The value of the heterogeneity index, \( n \), was greater than 1, suggesting that at low concentrations EE2 was being retained in relatively hydrophobic nanoscale pockets of clay microaggregates, although adsorption to clay and organic matter was likely at higher equilibrium concentrations. Desorption of EE2 was again minimal at low, environmentally relevant concentrations.

Both soils strongly adsorbed 4NP, much more so than with EE2 or BPA. The high-clay Zook soil had the stronger affinity for 4NP as well as the higher predicted adsorption capacity. Among the three compounds, 4-NP is the least polar and BPA is the most polar, and this property framed the continuum of interactions between the compounds and the soils. The value of the affinity index, \( K \), for 4-NP in both the Hanlon and Zook soils was many times larger than that of EE2 and BPA, suggesting that hydrophobic interaction with the soil components was an important adsorption mechanism. Between the two soils, the Zook soil had much greater adsorption affinity and capacity for EE2 and 4-NP than the Hanlon soil, although with BPA the differences in affinity and capacity were not significant. Hydrophobic interactions were likely to occur with both soil organic matter as well as with the low-charge clay minerals that dominated in the Zook soil. While soil organic matter and clay concentration played a large role in adsorption, the chemical properties of the compounds, reflected in their water solubility and octanol-water partition coefficients, were also useful predictors of adsorption behavior.

**Transport of Endocrine Disrupting Chemicals in Soil**

Column transport experiments were conducted with intact soil cores. A total of 42 intact cores was collected in Iowa from Zook and Hanlon soils (21 from each) in 2015. Saturated hydraulic conductivity was measured for each core, and a subset of cores was selected with similar hydraulic properties for continuing measurements.
Transport experiments were then conducted on the hydraulically grouped columns using bromide (Br\(^{-}\)) as a conservative tracer and ethinylestradiol (EE2) and bisphenol-a (BPA) as the chemicals of interest. The transport experiments were repeated in the presence of colloidal material fractionated from biosolids. Transport experiments were conducted with two different flow velocities in order to compare the effects of physical and chemical non-equilibrium. With two different soils, two different flow rates, and the presence or absence of colloidal materials there were a total of eight column experiments conducted. Soil samples were collected from sections of the columns to determine where chemicals remained on the solid phase.

BPA and EE2 were both delayed in their transport through soil compared to the non-interacting tracer, Br\(^{-}\). BPA was delayed less and was transported in larger amounts than EE2. BPA showed some effects of interaction with soil during transport, but far less than EE2 exhibited. Colloidal material from biosolids enhanced the transport of both BPA and EE2, with the effect on EE2 being more pronounced, likely due to the higher affinity of EE2 for sorption to both soil organic matter and to the biosolids colloidal material.

Hanlon and Zook soils have significantly different textures; Hanlon is a sandy soil and Zook is a clayey soil. From the perspective of chemical transport, the two soils differed primarily in their structural characteristics, with Hanlon being almost devoid of structure and Zook being strongly aggregated. This difference resulted in Hanlon acting as a filter for colloids while the macropores of Zook allowed some to pass through. Retention of colloids was exhibited by both soils, though it was very pronounced in the Hanlon soil cores. Varying the flow rates during column transport experiments resulted in breakthrough curves that were different. Slower flow rates produced chemical breakthrough curves that exhibited more sorption and degradation, while faster flow rates showed less interaction of EE2 and BPA with soil materials.

**Adsorptive Fractionation of Dissolved Organic Matter**

Dissolved organic matter (DOM) is a complex, heterogeneous mixture of water-soluble natural organic compounds. It is operationally described as the organic matter fraction that is smaller than a specified size, usually 0.45 \(\mu\)m. In general, DOM is composed of macromolecules such as enzymes, amino–sugar complexes, polyphenols, and humic substances, as well as low-molecular-weight compounds such as free amino acids and sugars. In agriculture, DOM is introduced into the soils through natural sources such as plant litter, soil humus, microbial biomass.
and root excretion. In addition, it is contributed by exogenous sources, such as irrigation with treated wastewater and organic amendments to soils such as biosolids.

One of the major processes affecting the fate of DOM in soils is adsorption to mineral and organic soil components. In this project, the nature of adsorptive fractionation of DOM to soil was demonstrated by evaluating the changes in DOM composition and properties before and after interactions with soil. DOM originating from two different sources (composted biosolids and organic-rich river water) was found to exhibit different adsorptive affinities to a high-clay, low organic matter mineral soil according to the content of aromatic and carboxylic moieties. In both instances, the HoA (hydrophobic acid fraction) was the dominant DOM fraction adsorbed by the soil, resulting in enhanced hydrophobicity of soil mineral surfaces and enrichment of the aqueous phase with hydrophilic components. A variety of analytical results corroborated this conclusion, including:

- The SUVA$_{254}$ value, a proxy for the aromatic content of DOM in the nonadsorbed fraction was significantly reduced due to the interactions with the soil, suggesting preferential adsorption of aromatic-rich DOM moieties.
- The E$_2$/E$_3$ values, which are related to molecular size, for the non-bound DOM were significantly higher than those measured for the DOM before adsorption, implying that larger DOM components were adsorbed, thus increasing the fraction of smaller-molecular-size DOM components in the supernatant.
- Changes in the C/H ratio of the nonadsorbed fraction was smaller than that of the original, bulk DOM, suggesting preferential adsorption of aromatic-rich DOM moieties. The higher higher affinity of DOM aromatic components for the soil at low DOM concentrations is likely due to direct interactions of DOM with mineral surfaces. The collected data strongly suggest that the DOM was fractionated due to its adsorption by mineral soil.

The non-bound DOM was fractionated into five fractions: hydrophobic acid (HoA), hydrophobic neutral (HoN), hydrophilic acid (HiA), hydrophilic base (HiB) and hydrophilic neutral (HiN). For both DOM samples, a lower content of HoA in the supernatant (i.e., non-bound DOM) was obtained following interactions with the soil as compared to the level of this fraction in the initial bulk DOM solution. The observed trend suggested that HoA was preferentially adsorbed by Al and Fe oxides in the soil. Our data are in agreement with other workers who have reported that binding of the hydrophobic DOM fraction to soils and oxides is stronger than that of
the hydrophilic fractions, and that at increasing concentrations, the hydrophobic DOM fractions are capable of replacing indigenous hydrophilic DOM components.

Although the HoA fraction of DOM was the dominant fraction adsorbed by the soil, assessment of the fractional distribution of DOM after adsorption demonstrated variability in the quantitative distribution of the adsorbed fractions. This emphasizes the impact of the DOM chemical and structural properties on its adsorptive fractionation.

**Sorption Equilibrium and Kinetics of 17β-estradiol and Estrone with Soil and Environmental Colloids**

Estrogenic compounds occur in land-applied animal manure and pose a biological risk if they enter aquatic ecosystems through agricultural drainage or shallow groundwater, but the mechanisms and factors controlling their dissipation in soils have not been fully investigated. Estrogens are nonpolar and strongly adsorbed by soil organic matter, limiting their miscible transport, but presenting another possible transport vector, co-transport with colloids which may facilitate the transport of estrogens through soil and ultimately to surface water. The sorption and degradation kinetics of 17 β-estradiol (E2) and estrone (E1) represent important dissipation pathways of these two endocrine-disrupting chemicals.

As a first step in considering colloid mediated transport mechanisms, the objective of this study was to determine the intensity and kinetics of adsorption and the kinetics of degradation of 17β-estradiol (E2) and estrone (E1) with three Iowa soils, colloidal components of those soils, and colloidal components of swine manure. By using batch incubations, adsorption isotherms were measured for E2 and E1 binding to these just mentioned materials. Additionally, time series incubations (0.3 to 66 h) were conducted to determine the kinetics of both adsorption and degradation of E2 and E1 with each material. Adsorption isotherms showed linear relationships and measured partition coefficients (Kd values) ranged from 15 L kg⁻¹ to 34 L kg⁻¹ for the soil materials. The log Koc values (organic carbon normalized Kd) for the soils were similar to values reported by others and ranged from 2.94 to 3.32. Kd values for colloidal material ranged from 72 L kg⁻¹ to 213 L kg⁻¹. The log Koc values for colloidal materials ranged from 2.79 to 3.33.

Results from the time series incubations for both E2 and E1 indicate two sorption mechanisms or phases that contrasted in rate. Both sorption phases can be approximated as pseudo first-order processes, although the initial rapid phase may be more accurately described as
instantaneous. Moreover, degradation of E2 can be approximated as a first-order process with rate coefficients varying from 0.006 to 0.06 h\(^{-1}\) and with a mean of 0.026 h\(^{-1}\) (sd = 0.016) in the tested materials. The calculated half-life of E2 had a mean of 47.0 h (sd = 31.4 h) with soils and 39.8 h (sd = 37.2 h) with soil colloids.
Changes to Original Research Plan

Once the project was underway, we made some changes in our plans to allow a broader perspective on the issues of the characterization, adsorption, and mobility of endocrine disrupting chemicals (EDCs) in association with organic matter in soils. First, we conducted a project to assess the seasonal occurrence of EDCs in the waste streams of two municipal wastewater treatment plant and in two Iowa rivers. Second, we explored the equilibrium adsorption and adsorption kinetics of two EDCs that were not on our original list to study, i.e., 17-β estradiol and estrone. These hormones are commonly found in livestock manure which is land-applied in Iowa. Finally, we used the adsorptive fractionation approach to explore the adsorption of dissolved organic matter by an important new environmental contaminant, carbon nanotubules.

Not all aspects of our original research plan were completed by the end of the project, but we expect to complete remaining experimental work in the coming six months. Ms. Fritzie Rivas’s PhD dissertation will be completed in the summer of 2017. It will be organized in a manuscript format so that additional publications originating from this grant can be realized quickly.
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Adsorptive fractionation of dissolved organic matter (DOM) by clay soil

1. Introduction

Dissolved organic matter (DOM) represents only a small fraction of SOM, however it is considered the most mobile and highly reactive SOM component (Bolan et al. 2011). DOM plays an important role in cycling and distribution of carbon in the ecosystem (Kaiser and Kalbitz 2012), as well as in biological activity in soils and water bodies (Kalbitz et al. 2000; Bolan et al. 2011). DOM affects various physico-chemical processes in soils, including the binding of trace elements (Ravichandran 2004) and organic pollutants (Totsche et al. 1996; Totsche et al. 1997; Müller et al. 2007; Barriuso et al. 2010), and co-precipitation of metal oxides (Shimizu et al. 2013). In addition, DOM is known to affect the transformation of redox active elements (Borch et al. 2010) and organic and inorganic contaminants by mineral surfaces (Brose and James 2010; Polubesova and Chefetz 2014).

DOM is a complex heterogeneous mixture of water-soluble natural organic compounds. It is operationally described as the organic matter fraction that is smaller than a specified size, usually 0.45 µm (Zsolnay 2003). Due to its complex nature, only a part of the DOM components have been chemically defined. In general, DOM includes abiotic and biotic degradation products of distinct classes of biomolecules (Hertkorn et al. 2007). It is composed of macromolecules such as enzymes, amino–sugar complexes, polyphenols, and humic substances, as well as low-molecular-weight compounds such as free amino acids and sugars (Chefetz et al. 1998). DOM composition has been reported to depend on its source and origin, e.g. aquatic systems, plant litter, manure, soils, and sediments (Thomsen et al. 2002; Kaiser and Kalbitz 2012). In agriculture, DOM is introduced into the soils through natural sources such as plant litter, soil humus, microbial biomass and root excretion. In addition, it is contributed by exogenous sources, such as irrigation with treated wastewater and organic amendments to soils such as biosolids.
One of the major processes affecting the fate of DOM in soils is sorption to mineral and organic soil components. Adsorptive reactivity of DOM is related to the type and content of its functional groups. For example, carboxylic groups have been found to play a major role in DOM adsorption to soil and minerals via a ligand-exchange mechanism with hydroxyl groups on metal-oxide surfaces (Gu et al. 1994; Edwards et al. 1996; Wang et al. 1997). Inner-sphere complexation of metal oxides with carboxylic functionalities of DOM has been reported to contribute to the accumulation of DOM in soils and to hinder its desorption (Kaiser et al. 1997). Other DOM constituents, such as aliphatic moieties and carboxylic groups bound through outer-sphere interactions, are easily desorbed (Oren and Chefetz 2012a; Oren and Chefetz 2012b).

Sorption affinity of DOM has been reported to vary among different soils and to be affected by the type of DOM. For example, Kothawala et al. (2012) reported that soils rich in Fe and Al exhibit high DOM adsorption capacity (1041–2022 mg kg\(^{-1}\)), whereas sorption capacity is much lower in soils that are poor in metal content (259–463 mg kg\(^{-1}\)). In addition to soil composition and DOM characteristics, which both affect DOM sorption, adsorption of DOM by soils is significantly affected by soil-solution chemistry (Shen 1999; Setia et al. 2013). In general, DOM adsorption increases with a decrease in pH and increase in ionic strength due to changes in DOM degree of ionization and conformation (Shen 1999; Kalbitz et al. 2000).

Since DOM consists of a mixture of components with varying structural characteristics, it was found that high molecular weight constituents of DOM as well as aromatic-rich moieties were preferentially adsorbed to mineral surfaces. This implies the DOM undergoes fractionation upon sorption due to interactions with soil components; these processes depend on DOM physico-chemical properties and surface properties of soil and mineral particles, and result in compositional change of DOM (Guo and Chorover 2003; Polubesova et al. 2008;
Heckman et al. 2011; Kothawala et al. 2012). However, qualitative and quantitative changes in DOM structural fractions as a result of DOM interactions with soil minerals have not been investigated. Thus the main objective of this study was to elucidate and to quantify the changes in DOM fractional composition and physico-chemical properties due to its adsorptive fractionation by mineral soil.

2. Materials and methods

2.1. Soil and DOM characterization

Fluvent soil (90-120 cm), which is rich in clay (67.5 ± 1.4 %) and poor in SOM (0.15% ± 0.02 organic carbon) was used in this research. The levels of Fe, Al and Mn oxides were 6.5 ± 0.2, 1.4 ± 0.2 and 0.9 ± 0.1 %, respectively and specific surface area was 327 ± 2 m² g⁻¹. Experiments to determine soil properties were performed in triplicate, the data represent average values with standard deviations. Detailed characterization of the soil can be found in Oren and Chefetz (2012a, 2012b).

Two types of DOM were used: DOM extracted from composted biosolids (hereafter denoted as compost DOM) and Suwannee River natural organic matter (SRNOM). Compost DOM was extracted by overnight agitation (200 rpm) of mature composted biosolids at 25°C with deionized water at a 1:10 water-to-compost ratio. The suspension was then centrifuged (12,000g, 20 min) and filtered through a 0.45-μm filter using an Acrodisc Supor membrane (PALL Corporation, Ann Arbor, MI, USA). The extracted DOM solution had a pH of 7.9, EC of 3.7 dS m⁻¹ and dissolved organic carbon (DOC) content of 800 mg C L⁻¹. DOC was measured by a VCPH model TOC analyzer (Shimadzu Scientific Instruments, Kyoto, Japan). SRNOM was purchased from the International Humic Substances Society (IHSS) and was used without further treatment.
All DOM analyses were performed prior and after adsorption to the studied soil; the initial concentration of total carbon (C_i; mg L\(^{-1}\)) and total carbon concentration in supernatants after adsorption (C_e; mg L\(^{-1}\)) were determined. Both bulk DOM samples were analyzed for their elemental composition by Flash EA 1112 CHN analyzer (Thermo Fisher Scientific Inc., Breda, The Netherlands) and for total acidity by titration (Inbar et al. 1990). Ultraviolet–visible (UV–VIS) spectroscopy analysis was performed for diluted DOM samples (DOC level < 30 mg C L\(^{-1}\)) with a Thermo Scientific Evolution 300 spectrophotometer. Absorption was measured at 254, 250 and 365 nm to calculate SUVA\(_{254}\) (i.e., the ratio between absorption intensity at 254 nm and the DOC concentration) and E\(_{250}/E_{365}\) ratio (the ratio of absorption at 250 and 365 nm, Peuravuori and Pihlaja, 1997; Weishaar et al., 2003). Spectrophotometric analysis was conducted with NaN\(_3\) (1.5 mM) as background solution; in our experimental conditions the UV absorption of NaN\(_3\) was not dominant. The ionic composition of the compost DOM solution was measured by an Acros-EOP ICP/AES (Spectro, Kleve, Germany). The major ions were: Ca\(^{2+}\), 35.1 mg L\(^{-1}\); Mg\(^{2+}\), 29.4 mg L\(^{-1}\); Na\(^+\), 99.4 mg L\(^{-1}\) and K\(^+\), 233 mg L\(^{-1}\).

2.2. Sorption–desorption experiments

Sorption and desorption of DOM (compost DOM and SRNOM) to the soil were measured by a batch-equilibrium technique using a solid-to-water ratio of 1:10 (w/v) at 25°C. Prior to the adsorption trials, the soil samples (2-3 g) were agitated for 2 h with deionized water (1:10 ratio) to reduce the level of native DOM released from the soil. After decanting the solution containing native DOM, elevated concentrations of exogenous DOM (5 to 115 mg C L\(^{-1}\)) were added to the wetted soil samples. Both compost DOM and SRNOM solutions were diluted with background solution containing 1.5 mM NaN\(_3\) (to inhibit microbial degradation) and a solution containing the dominant ions previously detected in the compost DOM extracts (Ca\(^{2+}\), 0.15 mM; Mg\(^{2+}\), 0.21 mM; Na\(^+\), 0.75 mM and K\(^+\), 1.02 mM, with Cl\(^-\) as
counterion). This enabled a constant ionic strength (3.8 mM) in all batch-equilibrium experiments at all DOM concentrations. Tubes containing soil samples and background solutions without DOM were used as controls. All treatments were conducted in triplicate. The pH was 7–8 for all adsorption experiments. After 4 days of agitation (equilibrium time as determined by Oren and Chefetz, 2012a, 2012b), the tubes were centrifuged (10,000g, 10 min), and 50% of the supernatant was decanted and replaced with fresh DOM-free background solution for the desorption experiments (for an additional 4 days). Three sequential desorption cycles were performed. The supernatants obtained in all sorption and desorption experiments were filtered (0.45 µm) and analyzed for DOC concentration.

2.3. Separation of DOM by resin

Separation of DOM samples based on different hydrophobicity and hydrophilicity was conducted to characterize DOM. DOM was separated using the method developed by Leenheer (1981). Resin-based separation was applied for the bulk DOM (i.e., DOM prior to introducing to soil) and for DOM in supernatants, obtained at the end of the sorption experiments after interaction with soil (i.e., non-bound DOM). We used polymeric resins to evaluate changes in the DOM structural fractions based on hydrophobicity and charge, Resins applied for DOM separation were purchased from Sigma-Aldrich (St. Louis, MO, USA). Separation was performed for three DOM concentrations (23, 57 and 94 mg C L\(^{-1}\) for the compost DOM, and 25, 55 and 86 mg C L\(^{-1}\) for the SRNOM).

DOM was fractionated into five fractions: hydrophobic acid (HoA), hydrophobic neutral (HoN), hydrophilic acid (HiA), hydrophilic base (HiB) and hydrophilic neutral (HiN). In brief, DOM (~6 mg C) was acidified to pH ~ 3 and loaded onto a Supelite DAX-8 resin column. Hydrophobic fractions were retained on the DAX-8 resin whereas hydrophilic fractions were eluted. The HoA fraction adsorbed to the DAX-8 resin was eluted by washing the resin with 0.1 N NaOH solution (0.25 pore volumes) followed by 1 pore volume of
distilled water. At this stage, the HoN fraction was retained by the resin. The pH of the hydrophilic fraction solution, which had been eluted from the DAX-8 resin, was adjusted to ~3, and then this solution was loaded onto cation-exchange resin (Amberlyst 15). The HiB fraction was retained on the resin while the eluted fractions (i.e., HiA and HiN), were loaded onto anion-exchange resin (Amberlyst A-21). At this stage, the HiA fraction was adsorbed to the resin, while the HiN fraction did not interact with the resin and eluted from the column. All samples were loaded at a flow rate that did not exceed 15 pore volumes per hour. DOC concentrations were measured for all eluted fractions. The DOM fractional distribution was determined by the calculation of the percentage of carbon of each fraction from the total carbon amount of DOM loaded on the resin.

2.4. Data analysis and statistics

DOM sorption coefficients were calculated using the Initial Mass approach (Nodvin et al. 1986; Kaiser and Zech 1997). With the Initial Mass approach, the amounts of DOM released into or removed (RE) from the solution are plotted against the initial amount of the DOM applied to the soil ($X_i$; Eq. 1).

$$RE = mX_i - b$$  \hspace{1cm} (1)

where $m$ is a measure of the sorbate (DOM) affinity to the sorbent (soil), and $b$ is the intercept, indicating the amount of sorbate released from the soil when no DOM is added ($X_i = 0$). The regression coefficient ($m$) can be used to calculate the distribution coefficient ($K_d$) for DOM sorption using Eq. 2.

$$K_d = \frac{m}{1 - m} \cdot \frac{V}{M}$$  \hspace{1cm} (2)

where $V$ and $M$ are the volume of the DOM solution and weight of the soil, respectively.

$K_d$ was also calculated directly from the adsorption isotherms for each data point by dividing the amount of adsorbed DOM by the DOM equilibrium concentration in the
supernatant. Statistical analysis (Student’s t-test, $P < 0.05$) was performed using JMP software (v. 7.0.1. SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

In this study, sorption and desorption behaviors were elucidated in batch experiments, the nature of adsorptive fractionation of DOM to soil was demonstrated by evaluating the changes in DOM composition and properties before and after interactions with soil.

3.1. Sorption and desorption of DOM

Both types of DOM exhibited pseudo-linear adsorption isotherms (Fig. 1) that did not reach maximum adsorption capacity within the range of applied initial concentrations. The isotherms of DOM sorption to soil constructed according to Initial Mass approach (Eq. 1) are presented in Fig. 2A. The SRNOM sorption affinity to soil ($m = 0.579 \pm 0.001$) was significantly higher than that for the compost DOM ($m = 0.285 \pm 0.002$). The obtained values were within the range reported for adsorption of other DOM types by soils (Kaiser et al. 1996). The $K_d$ values calculated using the Initial Mass approach (Eq. 2) were $3.98 \pm 0.03$ and $13.74 \pm 0.08$ L kg$^{-1}$ for the compost DOM and SRNOM, respectively. These values were similar to the average $K_d$ values calculated directly from the adsorption isotherms ($3.50 \pm 0.39$ and $11.78 \pm 1.08$ L kg$^{-1}$; Fig. 2B) for the compost DOM and SRNOM, respectively.
The higher adsorption affinity of the SRNOM to the studied soil is probably related to the nature of this DOM, which is richer in carboxylic groups and aromatic components than the compost DOM. The content of carboxylic functionalities was $9.81 \pm 0.89$ versus $7.79 \pm 0.38$ meq g C$^{-1}$ for the bulk SRNOM and bulk compost DOM, respectively. In addition, the bulk SRNOM exhibited a C/H ratio of $0.84 \pm 0.01$, while the value for the bulk compost DOM was much lower ($0.47 \pm 0.01$). The more acidic character and higher aromaticity of the SRNOM facilitated its adsorption to the soil through ligand-exchange and hydrogen-bonding with metal oxides and clay minerals (Gu et al. 1994; Mitchell et al. 2013). These two soil

**Fig. 1** DOM sorption and desorption (mean ± SE; filled and open symbols, respectively) isotherms for (A) compost DOM and (B) SRNOM.
constituents have been suggested as critical factors for the adsorption of DOM to soils (Gu et al. 1994; Kaiser and Guggenberger 2000; Oren and Chefetz 2012b).

**Fig. 2** Isotherms of DOM adsorption by soil (mean ± SE), (A) constructed using Initial Mass approach and (B) $K_d$ (mean ± SE) calculated as a function of amount of adsorbed DOM.

Desorption of DOM from soil and mineral surfaces is commonly reported as irreversible, exhibiting major sorption–desorption hysteresis (Benke et al. 1999; Guggenberger and Kaiser 2003). High sorption–desorption hysteresis level and low desorption capacity are mainly attributed to DOM interactions with metal oxides (Kaiser and Zech, 1999), while DOM desorbs more readily from a siloxane surfaces (Benke et al. 1999; Kahle et al. 2004). Thus, irreversible adsorption can be associated with stronger chemical bonds between DOM and soil.
oxides (ligand exchange) as compared to phyllosilicates (cation bridging). Similar to these studies, in our system both types of DOM exhibited negligible desorption (<10%), even after three desorption cycles. This is not surprising considering the composition of the studied soil, which was rich in Fe and Al oxides (Oren and Chefetz, 2012b). However, it is rather surprising that similar desorption behavior was obtained for both DOM samples, while the adsorption affinity of the SRNOM ($K_d$ value) was about three times higher than that of the compost DOM. We hypothesized that this issue can be explained by adsorptive fractionation, i.e. change in DOM characteristics and structural composition due to DOM adsorption to soil, which might result in preferential adsorption of the same DOM fraction for both DOM types. Detailed analysis and quantification of variable adsorptive changes in DOM physico-chemical properties and fractional composition (both in supernatants and adsorbed phase), as well as similarity of DOM desorption behavior, is discussed in the next sections.

3.2. DOM adsorptive fractionation: changes in DOM characteristics

To elucidate differences in DOM properties due to interaction with the soil, SUVA$_{254}$, and E2/E3 and C/H ratios were calculated for both compost DOM and SRNOM samples before and after adsorption by the soil (i.e., $C_i$ and $C_e$, respectively; Table 1). In terms of differences between two bulk DOM samples, SUVA$_{254}$, C/H and E$_2$/E$_3$ values indicated that the compost DOM is less aromatic and of a higher molecular size than SRNOM.

For both DOM samples at each of the three studied concentrations, SUVA$_{254}$ values were significantly reduced due to the interactions with the studied soil, suggesting preferential adsorption of aromatic-rich DOM moieties (Weishaar et al., 2003). A similar trend has been reported for adsorption of aquatic NOM and soil DOM to minerals and soils (Meier et al. 1999; Kothawala et al. 2012). Although both DOM types exhibited a similar trend, the reduction in the aromatic character of the DOM (i.e., the decrease in SUVA$_{254}$ values) as a result of adsorption was much more significant for compost DOM (SUVA$_{254}$ values decreased
by 43–50%) than for SRNOM (SUVA\textsubscript{254} values reduced by 17–25%; Table 1). This suggests that the SRNOM composition was less influenced by interactions with the soil, probably due to its higher aromatic content than that of compost DOM. The E\textsubscript{2}/E\textsubscript{3} values for non-bound DOM were significantly higher than those measured for the DOM before adsorption (Table 1). The E\textsubscript{2}/E\textsubscript{3} ratio decreases with the increase in molecular size due to stronger absorption of high-molecular-weight DOM at longer wavelengths (Peuravuori and Pihlaja 1997). This implies that larger-size DOM components were adsorbed, thus increasing the fraction of smaller-molecular-size DOM components in the supernatant. Our data is in agreement with previous studies indicating adsorption of higher molecular weight constituents to mineral surfaces (Chorover and Amistadi 2001; Ohno et al. 2007).

Similar to the observed changes in UV–VIS parameters, the changes recorded for the C/H ratio suggested preferential adsorption of aromatic-rich DOM moieties. For both DOM types at each of three studied concentrations, the C/H ratio of the bulk DOM was larger than that for the DOM retained in the supernatant after interaction with the soil. It is important to note that more pronounced changes in C/H ratio were observed for compost DOM than for SRNOM, and that these changes were of higher magnitude for the lower DOM concentration. We assume that higher selectivity and higher affinity of DOM aromatic components for the soil at low concentrations can be explained by the direct interactions of DOM with mineral surfaces. The collected data strongly suggest that the DOM was fractionated due to its adsorption by mineral soil. Although this phenomenon has been previously reported, quantitative changes in DOM fractional composition due to adsorption were not investigated. Thus, we studied adsorptive fractionation of DOM and preferential adsorption of DOM fractions by the soil.
Table 1 UV parameters and molar ratios (mean ± SE) for DOM samples at different initial (C_i) and equilibrium (C_e) concentrations

<table>
<thead>
<tr>
<th>DOM source</th>
<th>Concentration (mg C L(^{-1}))</th>
<th>SUVA (L mg C(^{-1}) m(^{-1}))</th>
<th>(E_2/E_3)</th>
<th>C/H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compost</td>
<td>C_i 32</td>
<td>3.64 ± 0.03</td>
<td>3.16 ± 0.01</td>
<td>0.43 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>C_e 23</td>
<td>1.97 ± 0.03</td>
<td>4.59 ± 0.01</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>C_i 81</td>
<td>3.71 ± 0.03</td>
<td>3.10 ± 0.06</td>
<td>0.35 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>C_e 57</td>
<td>1.97 ± 0.01</td>
<td>4.83 ± 0.07</td>
<td>0.24 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>C_i 128</td>
<td>3.72 ± 0.02</td>
<td>3.16 ± 0.01</td>
<td>0.41 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>C_e 94</td>
<td>2.11 ± 0.02</td>
<td>4.52 ± 0.03</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>Suwannee River</td>
<td>C_i 45</td>
<td>3.91 ± 0.03</td>
<td>4.81 ± 0.01</td>
<td>0.72 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>C_e 25</td>
<td>3.23 ± 0.01</td>
<td>5.09 ± 0.04</td>
<td>0.69 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>C_i 95</td>
<td>3.95 ± 0.02</td>
<td>4.75 ± 0.01</td>
<td>0.71 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>C_e 55</td>
<td>2.97 ± 0.02</td>
<td>5.36 ± 0.06</td>
<td>0.59 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>C_i 155</td>
<td>3.95 ± 0.05</td>
<td>4.73 ± 0.01</td>
<td>0.77 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>C_e 86</td>
<td>3.13 ± 0.03</td>
<td>5.70 ± 0.00</td>
<td>0.74 ± 0.01</td>
</tr>
</tbody>
</table>

3.3 Adsorptive fractionation of DOM: changes in structural composition

Structural changes in DOM composition in the supernatants following DOM adsorption, and amounts of DOM fractions adsorbed by the soil are shown in Fig. 3 and 4, respectively.

3.3.1 Hydrophobic fractions (HoA and HoN)

SRNOM was more hydrophobic in nature than the compost DOM: the hydrophobic fractions (HoA and HoN) made up 71\textendash83\% and 37\textendash48\% of the SRNOM and compost DOM, respectively. Although SRNOM was more hydrophobic than the compost DOM, both DOM
samples exhibited a similar change in composition following their adsorption to the studied soil. For both samples, a lower content of HoA in the supernatant (i.e., non-bound DOM) was obtained following interactions with the soil as compared to the level of this fraction in the initial bulk DOM solution (Fig. 3). The observed trend suggests that HoA is preferentially adsorbed by Al and Fe oxides. As the DOM concentration increases, larger DOM clusters are formed (Zsolnay 2003), and HoA makes up a larger portion of the DOM. Thus, HoA adsorption increases with increased DOM concentration for compost DOM (Fig. 4).

HoA is considered to be the most labile fraction of fulvic acid and is known to contain a large proportion of aromatic units associated with aliphatic moieties (Chefetz et al. 1998). HoA is also characterized by a high level of polar functional groups, such as hydroxyl and carboxyl (Qualls and Haines 1991), enabling its adsorption to the mineral soil through specific interactions mainly via ligand exchange and hydrogen bonding (Kaiser et al. 1996; Shen 1999). The preferential adsorption of HoA was further emphasized by its major fraction in the adsorbed phase (Fig. 4). It is important to note that for the SRNOM, HoA made up ~85% of the total adsorbed DOC at each of the three studied concentrations; however, for compost DOM, an increase in equilibrium concentration from 23 to 57 mg C L\(^{-1}\) resulted in enhanced HoA adsorption, from 47 to 72% of total adsorbed carbon. This suggests that HoA can be responsible for SRNOM higher hydrophobicity, and it may explain why the increase in SRNOM concentration does not affect the adsorption of HoA. For the less hydrophobic compost DOM, the increase in concentration results in enhanced adsorption of its hydrophobic component, i.e. HoA. Preferential adsorption of the HoA fraction implies that HoA can compete with other DOM fractions for the adsorption sites on the soil mineral surfaces. Our data are in agreement with Kaiser and Zech (1997), who reported that binding of the hydrophobic DOM fraction to soils and oxides is stronger than that of the hydrophilic
fractions, and that at increasing concentrations, the hydrophobic DOM fractions are capable of replacing indigenous hydrophilic DOM components.

The HoN level was not significantly different between the non-bound and bulk solutions (Fig. 3), indicating that this fraction is not selectively adsorbed. The HoN fraction made up only a few percent of the total adsorbed DOC (Fig. 4) for both DOM types, although it was present at a higher percentage in the bulk DOM in SRNOM (11–16%) as compared to compost DOM (7–10%; Fig. 3). This emphasizes its lower affinity to the soil. The HoN fraction consists of aliphatic and aromatic organic components and is characterized by higher hydrophobicity (Polubesova et al. 2007). The HoN fraction has been found to be adsorbed to montmorillonite to a larger extent than HoA, mostly due to its hydrophobic character, through van der Waals interactions (Polubesova et al. 2008). It is important to note that in our study, both HoA and HoN fractions were simultaneously introduced into the soil as DOM components. Thus, although HoN exhibited higher affinity than HoA to the pure mineral, when both fractions were co-introduced into the soil, preferential adsorption of HoA was clearly observed. This is probably due to HoA higher capacity for specific interactions of its carboxylic groups with Fe and Al oxides, while HoN is mainly adsorbed via van der Waals interactions with mineral surfaces. Our results demonstrated that the content of the HoN fraction is not affected by adsorption (Fig. 3) and that HoN is not selectively adsorbed (Fig. 4).
Fig. 3 Fraction distributions (mean ± SE) for compost DOM and SRNOM at three initial (C<sub>i</sub>) and equilibrium (C<sub>e</sub>) concentrations (filled and open columns, respectively). Asterisks indicate significant differences in percentages of C<sub>i</sub> and C<sub>e</sub> (Student’s t-test, P < 0.05).

3.3.2 Hydrophilic fractions (HiA, HiB, HiN)

Compost DOM was more hydrophilic than SRNOM; the HiN content in the bulk DOM was 37–43% and 13–16% for compost DOM and the SRNOM, respectively. The HiN level was almost unchanged after SRNOM adsorption, and exhibited a somewhat higher level in supernatants after compost DOM adsorption for two out of three concentrations tested (Fig. 3). This suggests that HiN adsorption to studied soil is unfavorable. The HiN fraction made up 17–40% of the total adsorbed carbon for compost DOM, as compared to SRNOM in which...
this fraction made up only 11% of the total adsorbed DOC at all applied concentrations (Fig. 3). The HiN fraction, the least polar among the hydrophilic fractions is composed mostly of poly- and oligosaccharides (Guggenberger et al. 1994; Chefetz et al. 1998), and is adsorbed to the soil mainly via H-bonding and van der Waals interactions. This explains the unfavorability of HiN adsorption compared to the HoA fraction which can interact specifically with mineral surfaces. For compost DOM, HoA was adsorbed up to four times more than HiN, although the initial levels of the two fractions in the bulk DOM were similar. It should be emphasized that for the compost DOM at the lowest equilibrium concentration (23 mg C L$^{-1}$), the adsorbed amount consisted of approximately equal amounts of HoA and HiN out of total adsorbed carbon (47 and 40%, respectively), whereas for SRNOM the proportion of adsorbed HoA and HiN is constant for all studied concentrations (Fig. 4). At low applied concentration, the influence of the native soil DOM (consisted of 16% HoA, 7% HoN, 1% HiB, 70% HiN, and 6% HiA of total native DOC) on fractionation of introduced DOM might become more pronounced for compost DOM due to its the lower hydrophobicity. Hence, high amount of native HiN may affect HoA adsorption. This phenomenon was less distinct at the corresponding equilibrium concentration for the more hydrophobic SRNOM (Fig. 4).

In five of six studied DOM samples, there was a significantly higher proportion of HiA in the supernatant after DOM adsorption than in the initial bulk DOM samples (Fig. 3). However, the overall level of this fraction was less than 16%, resulting in a negligible level of this fraction in the adsorbed phase (Fig. 4). The HiA fraction consists of low-molecular-weight humic-like acids and oxidized organic compounds (Chefetz et al. 1998), and is therefore capable of being adsorbed to soil minerals through specific interactions. However, due to its lower content in the bulk solutions, adsorption of this fraction was negligible. Among studied fractions, HiB, which consists mainly of aliphatic amines and amino acids (Barber et al. 2001), was present at the lowest level in both DOM types. The low level of this
fraction, as well as the insignificant differences observed in DOM solutions before and after DOM adsorption, resulted in a negligible level of HiB in the adsorbed phase.

![Graph](image)

**Fig. 4** Adsorption of DOM fractions (mean ± SE) by soil for compost DOM (A) and SRNOM (B) at different equilibrium concentration ($C_e$).

### 4. Conclusions

The results of this study highlight changes in the DOM composition due to adsorption to mineral soil. DOM originating from two different sources exhibited different sorptive affinities to the studied soil according to the content of aromatic and carboxylic moieties. However similarities in desorption hysteresis were recorded. This appears to be related to the sorption of HoA (hydrophobic acid fraction) which was governed by specific binding interactions. This highlights the dominant role of HoA–soil interactions, resulting in enhanced
hydrophobicity of soil mineral surfaces and enrichment of the aqueous phase with hydrophilic components.

Despite general important role in HoA adsorption to soil for both DOM, the assessment of fractional distribution DOM samples after adsorption demonstrated variability in quantitative distribution of the adsorbed fractions. This emphasizes the impact of the DOM chemical and structural properties on its adsorptive fractionation. This paper suggest that physico-chemical fractionation of DOM by mineral interfaces in soil must be considered in order to better predict DOM mobility and reactivity.

References


Behaviour of dissolved organic matter and organic pollutants upon interaction with the solid surface of colloidal materials (i.e., carbon nanotubes)

1. Introduction

The nanotechnology industry has greatly advanced during the last decade. Nanoparticles are classified as particles smaller than 100 nm in more than one dimension. A wide range of nanoparticles are manufactured, among them metal NPs and carbon nanotubes (CNTs). CNTs are produced by rolling up sheets of graphene into hollow tubes. Two main types of CNTs are manufactured: single-walled (SWCNTs) and multi-walled (MWCNTs). Materials exhibit unique properties at the nano-scale which may be harnessed for a wide range of applications. For example CNTs exhibit high tensile strength and thermal conductivity. Therefore, increasing amounts of nanoparticles are manufactured and incorporated into various consumer and industrial products. They are used in many fields such as food technology and biomedicine. The growing use of CNTs is worrisome; they may invade environmental systems during their synthesis, application and disposal. Once the CNTs are released, they may potentially interact with various substances in the aquatic environment such as organic pollutants and dissolved organic matter.

Dissolved organic matter (DOM) is a complex mixture of soluble organic compounds, filtered through 0.45 µm. DOM is ubiquitous in the environment, playing a significant role in many biogeochemical processes. Concentrations of DOM vary greatly in the environment; ranging from 0.5 mg C L\(^{-1}\) in oceans, to 1.5 mg C L\(^{-1}\) in lakes and rivers and up to 40 mg C L\(^{-1}\) in wastewater treatment plants effluents. It contains both aromatic and aliphatic structures with various functional groups such as hydroxyl, amide and carboxylic acid. The properties and composition of DOM vary with its origin, concentration and surrounding conditions.
Characterization of DOM is commonly achieved by mass spectrometry, nuclear magnetic resonance spectroscopy, size exclusion chromatography and UV-vis spectroscopy. DOM is also characterized by its physical separation into structural fractions, a technique first described by Leenheer (1981). The procedure is based on sorption of acidified DOM onto a weakly hydrophobic resin (DAX-8), dividing DOM into hydrophobic and hydrophilic fractions. Further separation into acidic, neutral and basic sub-fractions may then be achieved using ion-exchange resins. This preparative fractionation procedure has proven useful in the study of the interactions between DOM and other substances in the environment such as CNTs and organic pollutants. For example, the adsorption of carbamazepine by SWCNTs was decreased in the presence of DOM and each of its' hydrophobic fractions, but was not affected by the hydrophilic neutral fraction. Therefore we utilized the fractionation procedure to elucidate the role of the structural fractions of DOM in adsorption processes by CNTs.

DOM has been found to stabilize CNTs, its adsorption dependent on solution parameters and adsorbate/adsorbent properties. The main mechanisms proposed for DOM adsorption are Van der Waals forces, H-bonding and \( \pi-\pi \) interactions. Thus functionalities of MWCNTs were found to affect the adsorption of humic acid. The adsorption of natural organic matter (NOM) by oxidized CNTs was reported to decrease as the oxygen content was increased because the MWCNTs were less prone to form \( \pi-\pi \) interactions. High values of pH are known to decrease DOM adsorption by CNTs owing to increased electrostatic repulsion of the DOM. Increased ionic strength ultimately decreases electrostatic repulsion between DOM and CNTs due to enhanced double layer compression. Studies have also reported preferential adsorption of higher molecular weight fractions of NOM and aromatic-rich polar fulvic acid fractions by CNTs. The abovementioned studies suggest that
DOM fractionation might occur upon adsorption to CNTs, consequently changing its conformation and composition. However, very little is known about configurational changes and fractionation of native DOM upon adsorption to CNTs \(^{23,28}\) which we elucidated in this work.

DOM adsorption by CNTs is prone to influence the adsorption capacity and affinity of organic pollutants. Both suppressed and enhanced adsorption of organic pollutants by CNTs in the presence of DOM has been reported \(^{29-31}\). The presence of DOM resulted in a significant reduction in the adsorption of phenanthrene by CNTs, likely due to reduction in adsorption sites on account of competition and/or blockage by DOM \(^{29}\). On the other hand, humic acid coated CNTs exhibited only slight reduction in adsorption of phenanthrene, naphthalene and 1-naphthol. This was attributed to newly exposed adsorption sites \(^{30}\). The adsorption coefficients of sulfamethoxazole on humic acid-suspended CNTs were up to two orders of magnitude higher than those on aggregated CNTs. The authors ruled out adsorption of sulfamethoxazole to adsorbed humic acid on account of electrostatic repulsion. They suggested that humic acid adsorption exposed new adsorption sites for sulfamethoxazole adsorption by CNTs \(^{31}\). DOM and its' hydrophobic fractions were reported to reduce carbamazepine adsorption by CNTs, while the hydrophilic neutral fraction did not impact adsorption \(^{10}\). The inconsistent results regarding DOM impact on organic pollutant adsorption by CNTs are further clarified in the following study.

We provide a systematic research on the influence of DOM on the adsorption of atrazine and lamotrigine by SWCNTs under different solution. Both atrazine and lamotrigine are triazine based-compounds with many similarities in their physicochemical parameters. However each exhibits different practice; atrazine is a model herbicide \(^{32}\) whereas lamotrigine is a model pharmaceutical \(^{33}\).
2. Materials and methods

2.1. Materials

Pristine SWCNTs (outer diameter 1–2 nm and length 5–30 µm) were purchased from Chengdu Organic Chemistry Co. Ltd. (Chengdu, China). Supelite DAX-8, Amberlyst 15 and Amberlyst A-21 resins were purchased from Sigma-Aldrich (St. Louis, MO, USA). Suwannee River natural organic matter (SRNOM) was purchased from the International Humic Substances Society (IHSS) (St. Paul, MN, USA). Atrazine (1-chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine, 90% purity) was obtained from Agan Chemicals (Ashdod, Israel). Lamotrigine (6-(2,3- dichlorophenyl)-1,2,4-triazine-3,5-diamine, 98% purity) was purchased from EnzoBiochem Inc. (New York, NY, USA). Deionized water and analytical grade solvents (Sigma-Aldrich) were used in all experiments.

2.2. DOM extraction and separation into structural fractions

DOM was extracted from composted biosolids. The compost was sieved (2 mm) and agitated (250 rpm) overnight at 25°C with deionized water at a 1:10 solid-to-water ratio. The suspension was then centrifuged (12,000 g, 20 min) and filtered through a 0.45-µm filter using an Acrodisc Supor membrane (PALL Corp., Ann Arbor, MI, USA)\(^{18,24,34}\). Fresh aqueous extract (i.e., DOM) was prepared for each experiment. Carbon concentration of DOM (i.e., dissolved organic carbon; DOC) was determined using a V\(_{\text{CSH}}\) total organic carbon analyzer (Shimadzu, Japan; detection limit was 0.5 mg C L\(^{-1}\)). UV–VIS absorbance was measured with an Evolution 300 spectrophotometer (Thermo Scientific, Waltham, MA, USA) in order to obtain SUVA, \(E_2/E_3\) and \(E_4/E_6\) parameters. The \(E_2/E_3\) and \(E_4/E_6\) ratios were used to evaluate aromaticity, averaged molecular weight and polarity of the DOM samples\(^{35,36}\). Functional group analysis was conducted by titration procedure\(^{37}\).
DOM separation to structural fractions was performed at the end of the adsorption trials in three equilibrium concentrations: 13, 22 and 66 mg C L\(^{-1}\). At the end of the trial, the non-adsorbed DOM (i.e., DOM in the supernatant) and the bulk DOM (i.e., DOM at the same concentration as the non-adsorbed DOM, agitated under the same conditions without SWCNTs) were separated into five structural fractions: hydrophobic acid (HoA), hydrophobic neutral (HoN), hydrophilic acid (HiA), hydrophilic base (HiB) and hydrophilic neutral (HiN). The procedure was performed in triplicates according to a resin separation procedure developed by Leenheer (1981) with several modifications \(^{17,38}\). Briefly, separation between hydrophilic and hydrophobic fractions was conducted by loading the DOM onto Supelite DAX-8 resin. Acidified DOM (pH \(\approx 3\)) was loaded onto DAX-8 resin; at this stage the hydrophilic fractions were eluted from the column and hydrophobic fractions were retained on the DAX-8 resin. The HoA fraction was then eluted from the DAX-8 resin using 0.1 M NaOH while the HoN fraction was retained. The pH of the hydrophilic fraction was adjusted (pH \(\approx 3\)) with NaOH and loaded onto cation-exchange resin (Amberlyst 15). The HiA and HiN fractions were eluted while the HiB fraction was retained on the resin. The pH of the eluted solution was readjusted to 3 with 3 M NaOH and loaded onto anion-exchange resin (Amberlyst A-21). At this stage, the HiA fraction was retained on the resin while the HiN fraction was eluted. SRNOM separation into fractions was performed using the same method as for DOM at 20 mg C L\(^{-1}\). Furthermore, bulk DOM was separated into structural fractions to obtain a sufficient amount of material to be used as sorbates in batch-adsorption trials.

2.3. Adsorption of DOM by SWCNTs

Adsorption trials were conducted using batch-equilibration technique at 25\(^{\circ}\)C. DOM was diluted to a series of initial concentrations (100-3 mg C L\(^{-1}\)) which were
added to Pyrex vials with Teflon screw caps containing 4 mg SWCNTs. The solid-to-water ratio was set to 1:4 (mg:mL) in order to achieve at least 20% adsorption. The background solution was prepared using chloride salts to simulate DOM extract composition (Ca$^{2+}$, 4.4 mg L$^{-1}$; Mg$^{2+}$, 3.8 mg L$^{-1}$; Na$^{+}$, 12.5 mg L$^{-1}$ and K$^{+}$, 28.8 mg L$^{-1}$). Sodium azide (100 mg L$^{-1}$) was added to inhibit microbial activity. This enabled us to maintain a constant ionic strength (3.6 mM) and solution composition at all DOM initial concentrations. The pH in all solutions was 7–8. For the adsorption trials, DOM samples were agitated (120 rpm) for 5 days with SWCNTs (according to adsorption kinetic experiments). At the end of the equilibrium time the supernatants were filtered (0.45 µm filter) and DOC concentrations were measured. The complete removal of the SWCNTs by filtration was validated via measuring the absorbance at 800 nm ($^{20,24}$). Each concentration, including blanks (i.e., bulk DOM without SWCNTs) was conducted in triplicate. The amount of DOM adsorbed to SWCNTs was calculated by mass differences; mass losses for blank samples were negligible.

Adsorption trials for DOM fractions (HoA, HoN, HiA, HiB and HiN) were performed by the same procedure as for the DOM–SWCNT system but within a narrower initial concentration range (80-5 mg C L$^{-1}$). Ionic strength and composition were adjusted as needed to obtain conditions similar to those in the DOM–SWCNT system.

2.4. Adsorption of pollutants by SWCNTs in the absence and presence of DOM

Stock solutions of atrazine and lamotrigine were dissolved in acetone and methanol, respectively. The stock solutions were diluted to a series of concentrations (50 to 5000 µg L$^{-1}$ for atrazine and 150 to 6000 µg L$^{-1}$ for lamotrigine) in Pyrex bottles with Teflon screw caps using the same background solution as previously described. The SWCNT-to-water ratio was 1:100,000 for atrazine, and 1:50,000 for
lamotrigine. The amount of organic solvent was less than 0.5% in the final solutions to minimize co-solvent effects.

Samples were rotated vertically (200 rpm, 25 °C) until equilibrium, 2 days for atrazine and 1 day for lamotrigine. Triplicate samples and controls without SWCNTs and without the pollutant were set up for each concentration. After equilibrium was achieved, an aliquot of the sample was removed for quantitative analysis. In these experiments the validity of the mass-balance calculation was confirmed by solvent-extraction recovery experiments (with methanol). The calculated recovery for atrazine and lamotrigine was 101 ± 1% and 95 ± 2%, respectively.

Pollutant adsorption experiments were conducted with and without DOM at pH 4, 7 and 10 at constant ionic strength of 2 mM and 25 °C, and at increased ionic strength of 154 mM (pH 7 and 25 °C). DOM was introduced at different concentrations (~6 or 30 mg C L\(^{-1}\)). The ionic strength was increased with a background solution of simulated seawater \(^{39}\) diluted to 25% (154 mM). To examine competition between the organic pollutant and DOM, organic pollutant adsorption was examined in samples that were either pre- or post-exposed to DOM. For pre-DOM exposure, SWCNTs were first agitated in a DOM solution for 2 days, and then the solutions were spiked with the organic pollutant and further agitated for the required equilibration time. Similarly, post-DOM exposure experiments were performed by agitating the SWCNTs in the presence of the organic pollutant, then DOM was introduced by spiking. Organic pollutant adsorption by DOM-coated SWCNTs was evaluated in a DOM-free solution. DOM-coated SWCNTs were prepared by agitating SWCNTs in a DOM solution (6–7 mg C L\(^{-1}\)) for 2 days at the same solid-to-water ratio used for each organic pollutant. The DOM-coated SWCNTs were filtered from the solution and dried prior to use.
Bi-solute experiments were performed in order to examine competitive adsorption. In these experiments both organic pollutants were added to solution; the competitor was introduced at a constant concentration to varying concentrations of the main adsorbate. The experimental conditions for the bi-solute experiments were identical to those of the single-solute experiments and performed both in the absence and presence of DOM.

2.5. Solubility assays

The impact of DOM and solution chemistry (i.e., pH and ionic strength) on pollutant solubility ($C_s$) was examined for all tested conditions and DOM concentrations described above. An excess of solid atrazine or lamotrigine was added to the different solutions, then the vials were agitated for 24 h, filtered and analysed.

2.6. Detection of pollutant concentrations

Concentrations of atrazine and lamotrigine were determined using an Alliance e2695 HPLC system (Waters, Milford, MA, USA) equipped with a photodiode array detector. For the single-solute experiments, we used a reverse-phase C-18 column (LiCrospher® 100 RP-18, 25 cm x 4.6 mm, 5 µm) at a constant flow rate of 1 mL min$^{-1}$. Atrazine was eluted at a constant temperature of 35 °C using an isocratic program of 70% acetonitrile and 30% double-distilled water. Lamotrigine was eluted at a constant temperature of 45 °C using an isocratic program of 51% phosphate buffer (6.25 mM), 35% acetonitrile and 14% methanol. Atrazine was detected by absorption at 222 nm and lamotrigine at 307 nm. Limits of detection and quantification were 5 and 10 µg L$^{-1}$ for atrazine, and 5 and 15 µg L$^{-1}$ for lamotrigine, respectively. In the bi-solute experiments, the simultaneous detection of atrazine and lamotrigine was performed with a reverse-phase C-8 column (Kinetex® 100 Å, 10 cm x 4.6 mm, 5 µm) at a constant flow rate of 1 mL min$^{-1}$ at 42 °C. Gradient elution of the two pollutants was
conducted with a binary mobile phase of acetonitrile and double-distilled water, both 
acidified with formic acid (0.1%). The program was as follows (acetonitrile:water): 
13:87 for 2 min, up to 50:50 over 7.5 min, back to 13:87 in 0.1 min and held for 5.4 
min. All calibration curves were prepared with external standards of atrazine and 
lamotrigine. Limits of detection and quantification were 10 and 20 µg L⁻¹ for atrazine, 
and 15 and 25 µg L⁻¹ for lamotrigine, respectively.

2.7. Data analysis

Langmuir (Eq. 1) and Freundlich (Eq. 2) models were employed to fit the adsorption 
data of DOM by SWCNTs and the distribution coefficient (Kₜ) was calculated using 
Eq. 3.

\[
q = \frac{q_{\text{max}} \cdot K_L \cdot C_e}{1 + K_L \cdot C_e}
\]  

(1)

where \(q\) (mg C g⁻¹) is the adsorbed DOM, \(q_{\text{max}}\) is the Langmuir maximum adsorption 
capacity, \(K_L\) (L mg C⁻¹) is the adsorption affinity parameter and \(C_e\) (mg C L⁻¹) is the 
aqueous equilibrium concentration of DOM.

\[
q = K_f \cdot C_e^N
\]  

(2)

where \(K_f\) [(mg C g⁻¹)/(mg C L⁻¹)\(^N\)] is the adsorption affinity coefficient and \(N\) is the 
Freundlich exponential coefficient.

\[
K_d = \frac{q}{C_e}
\]  

(3)

The Polanyi theory-based Dubinin-Astakhov model (Eq. 4) was used to fit the 
adsorption data of the pollutants by SWCNTs.

\[
\log Q = \log Q_0 - \left( \frac{RT \cdot \ln(C_e / C_e)}{E} \right)^b
\]  

(4)
where $Q$ (mg g$^{-1}$) is the equilibrium adsorbed concentration of solute, $C_e$ (mg L$^{-1}$) is the aqueous equilibrium concentration of solute, $C_s$ (mg L$^{-1}$) is the solute solubility under the experimental conditions, $Q_0$ (mg g$^{-1}$) is the maximum adsorption capacity, E (kJ mol$^{-1}$) is the "correlating divisor" (commonly used to evaluate an average adsorption affinity), $R$ [8.314·$10^{-3}$ kJ mol$^{-1}$ K$^{-1}$] is the universal gas constant, $T$ (K) is absolute temperature and $b$ is a fitting parameter$^{23,40,41}$.

The partition coefficient of solute from the aqueous phase to DOM-containing solution was calculated from the solubility enhancement in the presence of DOM (Eq. 5), assuming a partition-like interaction$^{42-44}$. While specific interactions may occur between lamotrigine or atrazine and DOM, the partition coefficient was only used operationally to evaluate the impact of DOM presence on each pollutant.

\[
\frac{C_{s(DOM)}}{C_{s(aq)}} = 1 + K_{DOM} \ [DOM] 
\]  
(5)

where $C_{s(DOM)}$ and $C_{s(aq)}$ are the solutes' solubility in the presence and absence of DOM, respectively; $K_{DOM}$ (L mg C$^{-1}$) is the partition coefficient and [DOM] is the concentration of DOM (mg C L$^{-1}$).

The Setschenow constant was determined from the difference in solubility of the solute at low and high ionic strengths (i.e., simulated seawater) using the Setschenow equation (Eq. 6)$^{45}$.

\[
\log \frac{C_{s(aq)}}{C_{s(salt)}} = K_i^s \ [salt] 
\]  
(6)

where $C_{s(salt)}$ is the solubility of solute at higher salinity, $K_i^s$ (L mol$^{-1}$) is the Setschenow or salting-out constant and [salt] is the salt concentration in solution (mol L$^{-1}$).

Data analysis was performed using Matlab (v. 8.1.0 MathWorks Inc., Natick, MA, USA) and SigmaPlot software (v. 12 Systat Software Inc. San Jose, CA, USA).
3. **Results and discussion**

3.1. **Adsorptive fractionation of DOM by SWCNTs**

3.1.1. Compositional change of DOM upon adsorption by SWCNTs

Adsorptive fractionation of DOM upon interaction with SWCNTs was observed and found to be concentration-dependent (Fig. 1). Interestingly, an increase in DOM concentration resulted in an increase in the relative percentage of the hydrophobic fractions (HoA and HoN) for both non-adsorbed and bulk DOM (i.e., control). At elevated concentrations, DOM molecules are forced to interact due to reduced free space for their movement, forming larger, more hydrophobic clusters. In our study, these clusters are not likely to form micelle-like domains since the working concentrations were at least one order of magnitude lower than the critical micelle concentration reported for humic acid. The altered composition of the DOM was supported by the significant decrease in DOM total acidity as the DOM concentration increased (Table 1). Based on the observed differences in DOM compositional properties with concentration increase, the effect of SWCNTs on the chemical composition of DOM was evaluated for bulk (control) and non-adsorbed DOM at equal concentrations.
Table 1. Parameters of bulk and non-adsorbed dissolved organic matter (DOM). Mean ± standard error (for three or nine replicates) are presented.

<table>
<thead>
<tr>
<th>DOM Type</th>
<th>Conc. [mg C L(^{-1})]</th>
<th>SUVA [L mg C(^{-1}) m(^{-1})]</th>
<th>(E_2/E_3)</th>
<th>(E_4/E_6)</th>
<th>Total acidity [mmol g(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk</td>
<td>13</td>
<td>3.6 ± 1.8 (\times) 10(^{-2})</td>
<td>3.4 ± 1.1 (\times) 10(^{-2})</td>
<td>5.2 ± 3.0 (\times) 10(^{-2})</td>
<td>9.4 ± 6.5 (\times) 10(^{-1})</td>
</tr>
<tr>
<td>Non-adsorbed</td>
<td>2.6 ± 1.3 (\times) 10(^{-1})</td>
<td>3.7 ± 1.5 (\times) 10(^{-2})</td>
<td>4.2 ± 4.8 (\times) 10(^{-2})</td>
<td>7.1 ± 9.3 (\times) 10(^{-2})</td>
<td></td>
</tr>
<tr>
<td>Bulk</td>
<td>22</td>
<td>3.4 ± 2.7 (\times) 10(^{-1})</td>
<td>3.2 ± 1.3 (\times) 10(^{-2})</td>
<td>4.8 ± 2.0 (\times) 10(^{-1})</td>
<td>6.5 ± 2.6 (\times) 10(^{-1})</td>
</tr>
<tr>
<td>Non-adsorbed</td>
<td>2.6 ± 2.3 (\times) 10(^{-1})</td>
<td>3.7 ± 2.3 (\times) 10(^{-2})</td>
<td>4.6 ± 2.2 (\times) 10(^{-2})</td>
<td>5.5 ± 2.4 (\times) 10(^{-1})</td>
<td></td>
</tr>
<tr>
<td>Bulk</td>
<td>66 (^a)</td>
<td>3.6 ± 8.3 (\times) 10(^{-3})</td>
<td>3.2 ± 3.8 (\times) 10(^{-3})</td>
<td>5.2 ± 1.2 (\times) 10(^{-2})</td>
<td>4.3 ± 1.4 (\times) 10(^{-1})</td>
</tr>
<tr>
<td>Non-adsorbed</td>
<td>2.8 ± 1.8 (\times) 10(^{-4})</td>
<td>3.6 ± 5.6 (\times) 10(^{-3})</td>
<td>4.8 ± 4.0 (\times) 10(^{-2})</td>
<td>3.9 ± 2.3 (\times) 10(^{-1})</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Spectroscopic parameters were obtained by sample dilution to 20 mg C L\(^{-1}\).

Our results for the two types of DOM show distinct differences between the bulk and non-adsorbed DOM samples, indicating a significant change in composition of DOM induced by adsorption (Fig. 1). The spectroscopic parameters (SUVA, \(E_2/E_3\) and \(E_4/E_6\)) also indicated differences in DOM properties due to interaction with the SWCNTs (Table 1). Non-adsorbed DOM exhibited significantly lower SUVA values than those of the corresponding bulk DOM, suggesting preferential adsorption of aromatic-rich DOM components by SWCNTs, as reported for the adsorption of NOM to MWCNTs \(^{26}\). The \(E_2/E_3\) and \(E_4/E_6\) values of non-adsorbed DOM were significantly higher and lower, respectively, than those of bulk DOM. Similar differences were observed between spectroscopic parameters of bulk and non-adsorbed SRNOM (SUVA value decreased from 4.0 to 3.0 L mg C\(^{-1}\) m\(^{-1}\)). These differences imply that DOM fractions of higher molecular weight containing aromatic and polar moieties were preferentially adsorbed \(^{36}\). Similarly, higher \(E_2/E_3\) ratios have been reported for non-adsorbed fulvic acid after interaction with SWCNTs \(^{27}\). Total acidity was significantly lower for non-adsorbed DOM at 13 and 22 mg C L\(^{-1}\) (Table 1), probably due to adsorption of acidic hydrophobic compounds. At 66 mg C L\(^{-1}\), the total acidity...
values did not significantly differ between the bulk and non-adsorbed DOM. The overall observations (Table 1 and Fig. 1) suggest alterations in DOM composition due to its interaction with SWCNTs.

The HoA fraction exhibited significantly lower percentage in the non-adsorbed DOM than in the bulk DOM at all tested concentrations (Fig. 1). This strongly suggests preferential adsorption of this fraction to the SWCNTs. Indeed, analysis of the composition of the adsorbed DOM (Fig. 2) revealed that HoA was the major fraction (>50%) of the adsorbed materials at all studied equilibrium concentrations. The preferential adsorption of the HoA fraction likely resulted from the type of HoA-SWCNTs interactions. HoA contains the highest aromatic content (SUVA value of 2.8 L mg C\(^{-1}\) m\(^{-1}\)) of all other fractions (max. SUVA value of 1.5 mg C\(^{-1}\) m\(^{-1}\) was obtained for the other fractions). HoA is known to contain high amounts of humic and fulvic acids\(^ {49,50}\) which are both composed mainly of aromatic moieties that are rich in polar functionalities, such as carboxyl and hydroxyl\(^ {51}\), creating both electron-deficient and electron-rich hosting benzene rings, respectively. These rings may act as efficient \(\pi\)-electron acceptors and donors, respectively (Wang et al., 2011), interacting with the SWCNTs which act as both \(\pi\)-electron donors and acceptors\(^ {53}\).

Electrostatic attraction was probably negligible in our system due to the high pH (7-8) in which the HoA carboxylic functional groups (6.2 mmol g\(^{-1}\)) were deprotonated, facilitating electrostatic repulsion with the pseudo-negative charge of the SWCNTs\(^ 5\). This electrostatic repulsion seemed to have a minor influence on the adsorption. Moreover, at elevated pH, the carboxylate anions lose their H-bond-donating abilities and may not interact with the H-bond-accepting graphene sheets of the SWCNTs. However, H-bonds may form between the phenolic groups of HoA (1.8 mmol g\(^{-1}\)) and the SWCNT sheets serving as H-bond acceptors\(^ {27,54}\). Therefore, the HoA fraction has
higher capability for interaction with SWCNTs, which leads to preferential adsorption.

Figure 1. Composition of non-adsorbed and bulk dissolved organic matter (DOM) and Suwannee River natural organic matter (SRNOM) at different concentrations. Mean values and standard errors for three or nine replicates are presented. DOM fractions include: hydrophobic acid (HoA), hydrophobic neutral (HoN), hydrophilic acid (HiA), hydrophilic base (HiB) and hydrophilic neutral (HiN).

As opposed to HoA, the HiN fraction was present at a significantly higher percentage in the non-adsorbed DOM compared to the bulk DOM at identical concentration. It is important to note that the percentage of HiN in the non-adsorbed
fraction increased following adsorption, despite it being substantially adsorbed itself (Figs. 1 and 2). This suggests that this fraction has relatively low affinity to the solid phases thus it is less likely to compete over adsorption sites with other fractions. HiN consists mainly of poly- and oligosaccharides. It contains few charged functional groups, and it therefore interacts with SWCNTs primarily through Van der Waals interactions. Based on the properties of HiN, one would assume low adsorption to SWCNTs. Nonetheless, its adsorbed amount was substantial because HiN is ubiquitous in the DOM–SWCNT system (>40%). At the initial DOM concentration of 22 mg C L\(^{-1}\), HiN and HoA accounted for 46 and 28% of the DOM, respectively. Despite the initially higher amount of HiN in the DOM solution, at equilibrium, the amount of adsorbed HoA was nearly double that of HiN, emphasizing HoA’s superior adsorption (Fig. 2). The adsorption of HiN and HoA fractions may also have occurred to different adsorption sites on the SWCNT aggregates. The HoA fraction contains larger, more aromatic compounds than the HiN fraction, as was implied by SUVA (2.8 versus 0.6 L mg C\(^{-1}\) m\(^{-1}\), respectively) and \(E_2/E_3\) values (3.5 versus 4.5, respectively). Therefore, certain adsorption sites may not be accessible to HoA but mainly to smaller hydrophilic compounds such as those which compose the HiN fraction.

For HoN and HiB fractions, the differences between the percentages obtained in the bulk vs. non-adsorbed compost DOM samples of corresponding concentrations were not significant (Fig. 1). Low percentages of these fractions in compost DOM and the insignificant differences in their distribution due to interactions with SWCNTs produced large errors in their adsorbed amounts (Fig. 2). Therefore, no clear conclusions could be drawn regarding the adsorptive trends of HoN and HiB fractions of compost DOM.
Figure 2. Mean distribution of adsorbed dissolved organic matter (DOM) fractions at different DOM equilibrium concentrations. Bars represent standard errors for three or nine replicates. DOM fractions include: hydrophobic acid (HoA), hydrophobic neutral (HoN), hydrophilic acid (HiA), hydrophilic base (HiB) and hydrophilic neutral (HiN).

To validate the fractionation trends obtained for the compost DOM, we evaluated the fractionation of a different type of DOM (i.e., SRNOM). SRNOM was more hydrophobic in character, as compared to the compost DOM. The HoA, HoN, HiN, HiA and HiB fractions composed 48%, 11%, 19%, 22% and 0% of SRNOM, versus 29%, 1%, 46%, 22% and 3% of compost DOM, respectively. Although the two types of DOM exhibited different composition, both showed similar adsorptive fractionation trends - significantly lower percentage of HoA and higher percentage of HiN in the non-adsorbed phase compared to the bulk materials (Fig. 1 middle). Moreover, significantly lower percentage of HoN and higher percentage of HiA was present in the non-adsorbed SRNOM compared to the bulk SRNOM, respectively. Due to the low percentage of HoN in the compost DOM, precise results for this fractionation could not be obtained. The HoN level in the SRNOM was much higher, enabling more accurate measurements. Based on the SRNOM data it is clear that
HoN, similar to HoA, is likely to be adsorbed by the SWCNTs, resulting in its significant percentage reduction in the non-adsorbed SRNOM. The SRNOM data for HiA further supports the low percentage of HiA at the adsorbed phase (Fig. 2) suggesting that this fraction is either not adsorbed at a concentration higher than 13 mg C L\(^{-1}\) or is characterized as HoA at the adsorbed phase.

3.1.2. Adsorption isotherms of DOM and the isolated fractions

Adsorption of DOM to SWCNTs exhibited a nonlinear isotherm (Fig. 3a), likely due to heterogeneous adsorption sites on the SWCNTs\(^{55}\) and the heterogeneous DOM mixture\(^{26,29}\). In aqueous media, SWCNTs tend to bundle due to strong Van der Waals interactions, forming heterogeneous adsorption sites which include external surfaces, grooves and interstitial regions\(^{56,57}\). The maximum adsorption capacity for the DOM in our system was 90 mg C g\(^{-1}\) (Table 2); when normalized to surface area (420 m\(^2\) g\(^{-1}\)), the obtained maximum adsorption capacity was within the range of values reported for adsorption of tannic acid to SWCNTs\(^{21}\) and peat humic acid to MWCNTs\(^{58}\). On the other hand, maximum adsorption capacity of Aldrich humic acid to MWCNTs\(^{22}\) was over four times higher than the value obtained in our work. This is probably since Aldrich humic acid is rich in aromatic moieties\(^{59}\). The adsorption isotherms of fulvic acid\(^{27}\) and SRNOM\(^{26}\) to MWCNTs were less curvature than the isotherm obtained for DOM used in the current study. Various interactions, such as Van der Waals forces, electrostatic interactions, H-bonding and \(\pi-\pi\) electron donor/acceptor interactions have been proposed as adsorption mechanisms of DOM and DOM analogs by SWCNTs\(^{23,53,55,60}\). The adsorptive fractionation of DOM (Figs. 1 and 2) suggests that some of the DOM structures are adsorbed while others are less likely to interact with the solid phases of the SWCNTs. This implies that the obtained adsorption parameters (capacity and affinity) for DOM cannot be used to study
mechanistic interactions. Therefore, in this study we have investigated the adsorption behavior of the individual fractions.

**Figure 3.** Adsorption isotherms (a) and distribution coefficients (b) of dissolved organic matter (DOM) and DOM fractions by single-walled carbon nanotubes (SWCNTs). DOM fractions include: hydrophobic acid (HoA), hydrophobic neutral (HoN), hydrophilic acid (HiA), hydrophilic base (HiB) and hydrophilic neutral (HiN).

Similar to the bulk DOM, HoA and HoN exhibited nonlinear isotherms (Fig. 3a) and were all best fitted by the Langmuir model (Table 2). The nature of the isotherm was found to be dependent on the hydrophobicity of the adsorbed fraction. For the hydrophobic fractions (HoA and HoN), which are likely to form specific interactions (π – electron donor/acceptor and H-bonds) with the SWCNTs, adsorption ultimately reached maximum amount. Maximum adsorption capacity followed the order HoN > bulk DOM > HoA (Table 2), which may be explained by differences in molecular size. HoN exhibited smaller size than the DOM and HoA (as implied by E2/E3 values of 7.5, 3.4 and 3.5, respectively) and therefore had the highest \( q_{\text{max}} \) value (Table 2).
Table 2. Adsorption parameters for dissolved organic matter (DOM) and DOM fractions by single-walled carbon nanotubes (SWCNT). DOM fractions include: hydrophobic acid (HoA), hydrophobic neutral (HoN), hydrophilic acid (HiA), hydrophilic base (HiB) and hydrophilic neutral (HiN). Mean ± standard error presented.

<table>
<thead>
<tr>
<th></th>
<th>Langmuir model</th>
<th>Freundlich model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_L$ [L mg C$^{-1}$]</td>
<td>$q_{max}$ [mg C g$^{-1}$]</td>
</tr>
<tr>
<td>DOM</td>
<td>0.095 ± 0.004</td>
<td>90 ± 1</td>
</tr>
<tr>
<td>HoA</td>
<td>0.11 ± 0.015</td>
<td>77 ± 4</td>
</tr>
<tr>
<td>HoN</td>
<td>0.065 ± 0.008</td>
<td>121 ± 6</td>
</tr>
<tr>
<td>HiA</td>
<td>0.032 ± 0.006</td>
<td>98 ± 12</td>
</tr>
<tr>
<td>HiN</td>
<td>0.017 ± 0.003</td>
<td>122 ± 16</td>
</tr>
<tr>
<td>HiB</td>
<td>0.009 ± 0.003</td>
<td>91 ± 20</td>
</tr>
</tbody>
</table>

The higher $q_{max}$ value of DOM in comparison to HoA can be explained by adsorption of additional fractions such as HiN (Fig. 2), which also make up a substantial amount of the adsorbed DOM. Langmuir adsorption-affinity parameters were not significantly different between bulk DOM and HoA, likely owing to similar adsorption interactions of $\pi-\pi$ origin. The Langmuir adsorption affinity parameter of HoN was slightly lower than those of HoA and DOM, possibly due to its' lower aromaticity (HoN SUVA value of 1.5 mg C$^{-1}$ m$^{-1}$). Adsorption of the hydrophilic fractions (HiA, HiB and HiN) was best fitted by the Freundlich model (Table 2; Fig. 3a). Hydrophilic fractions contain less aromatic moieties (SUVA values of 1.5, 0.6 and 0.4 L mg C$^{-1}$ m$^{-1}$ for HiA, HiN and HiB, respectively) and are therefore likely to interact mostly via Van der Waals interactions. The differences between the types of isotherms of the isolated DOM fractions suggest that they behave like a heterogeneous mixture in the bulk DOM. The Freundlich adsorption coefficient of HiB was lower than those of HiN and HiA, perhaps due to the high solubility of the HiB fraction, which consists
mainly of aliphatic amines and amino acids\textsuperscript{51,61}. No statistical differences were found between the Freundlich parameters of HiN and HiA.

The distribution coefficients ($K_d$, Eq. 3) were significantly higher for DOM and its hydrophobic fractions than for its hydrophilic fractions (Fig. 3b). Similarly, Chen et al. (2007) reported that the adsorption affinity was higher for nonpolar aromatic compared to nonpolar aliphatic fractions. The $K_d$ of the hydrophilic fractions showed little dependence on adsorbed amount (Fig. 3b). Their binding, governed by nonspecific interactions, occurred mainly to the external surfaces of the SWCNTs. On the other hand, $K_d$ for the DOM and hydrophobic fractions was dependent on adsorbed amounts, with a high affinity noted for low adsorbed amounts. $K_d$ decreased as the adsorbed amount increased due to reduction in vacant adsorption sites. The results of this study emphasize that DOM is a heterogeneous mixture of weakly clustered constituents which interact differently with SWCNTs. Therefore, both SWCNTs and DOM are likely to contribute to the nonlinear adsorption isotherm of DOM.

3.2. Adsorption of atrazine and lamotrigine by SWCNTs: Impact of DOM

3.2.1. Influence of DOM and solution chemistry on pollutant solubility

Solubility is a governing factor in pollutant removal by adsorption; it is thus important to determine the solubilities of the studied compounds under all tested solution conditions, and to present the data based on their activity. Although both compounds are capable of forming strong H-bonds with water, only the solubility of lamotrigine was influenced by changes in solution chemistry and/or by the presence of DOM as compared to atrazine (Table 3). The solubility of lamotrigine was significantly higher at pH 4 compared to pH 7 or 10, with and without DOM. At pH values of 4, 7 and 10, the percentage of protonated lamotrigine was 100, 5 and
0.005%, respectively. Therefore, the increase in lamotrigine solubility at pH 4 was attributed to its positive charge, which enabled formation of strong ion-dipole interactions with the surrounding water molecules. In contrast, only 0.5% of atrazine was protonated at pH 4, indicating that it was essentially neutral within the tested pH range, enabling only H-bond interactions.

Table 3. Solubility (Cₜ) of atrazine and lamotrigine under different solution conditions in the absence (top) and presence (bottom) of dissolved organic matter (DOM). Mean ± standard error (for three replicates) is presented.

<table>
<thead>
<tr>
<th>DOM concentration (mg C L⁻¹)</th>
<th>pH</th>
<th>Ionic strength (mM)</th>
<th>Cₜ (mg L⁻¹)</th>
<th>Atrazine</th>
<th>Lamotrigine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without DOM</td>
<td>6</td>
<td>0</td>
<td>27.0 ± 0.7</td>
<td>150 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2</td>
<td>24.7 ± 0.6</td>
<td>207 ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2</td>
<td>26.4 ± 0.2</td>
<td>153 ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2</td>
<td>26.3 ± 0.1</td>
<td>145 ± 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>154</td>
<td>25.1 ± 0.8</td>
<td>118 ± 7</td>
<td></td>
</tr>
<tr>
<td>With DOM</td>
<td>5^a/6^b</td>
<td>4</td>
<td>24.2 ± 0.3</td>
<td>233 ± 11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6^a/8^b</td>
<td>7</td>
<td>25.0 ± 0.1</td>
<td>191 ± 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33^a/27^b</td>
<td>7</td>
<td>25.4 ± 0.3</td>
<td>228 ± 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6^a/7^b</td>
<td>10</td>
<td>25.2 ± 0.1</td>
<td>171 ± 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6^a/7^b</td>
<td>7</td>
<td>23.9 ± 0.3</td>
<td>202 ± 6</td>
<td></td>
</tr>
</tbody>
</table>

^a DOM concentration in atrazine solubility assays.
^b DOM concentration in lamotrigine solubility assays.

An increase in ionic strength (154 mM versus 2 mM at pH 7) resulted in a small decrease (5%) in the solubility of atrazine. However, the solubility of lamotrigine was significantly reduced at high ionic strength (by 23%), indicating a pronounced salting-out effect. The differences in the salinity impact on the solubility of each compound are demonstrated in their derived Setschenow constant (\( K_i^{\text{s}} \)), which was 0.259 ± 0.002 L mol⁻¹ for atrazine (\( K_{i-\text{ATZ}}^{\text{s}} \)) and 0.837 ± 0.003 L mol⁻¹ (\( K_{i-LTG}^{\text{s}} \)) for
lamotrigine. $K^s_{i-ATZ}$ was similar to the Setschenow constants reported for non-ionic organic compounds, such as phenanthrene, benzo[a]pyrene and biphenyl, in seawater, whereas $K^s_{i-LTG}$ corresponded well to values observed for a more polar compound such as 17β-estradiol.

Similar to the effects of pH and ionic strength, the presence of DOM only slightly decreased the solubility of atrazine under all tested conditions (Table 3). However lamotrigine's solubility was significantly enhanced in the presence of DOM as compared to the corresponding conditions without DOM. For example, at an ionic strength of 154 mM, the solubility of lamotrigine increased by 70% when DOM was introduced at an environmental relevant concentration of 7 mg C L$^{-1}$. In other words, the solubility-enhancing effect of DOM overcame the previously observed suppressive effect of increased salt concentration. This was also evidenced through $K^s_{i-LTG}$ which, in the presence of DOM, was $-0.182 \pm 0.002$ L mol$^{-1}$, compared to $0.837 \pm 0.003$ L mol$^{-1}$ in the DOM-free system. $K^s_{i-ATZ}$ did not differ greatly with and without DOM in solution, suggesting only weak interactions exist between atrazine and DOM. Moreover, unlike atrazine, lamotrigine solubility was dependent on DOM concentration, increasing by 25% and 50% at DOM concentrations of 8 and 27 mg C L$^{-1}$, respectively. The presence of DOM introduces both hydrophilic and hydrophobic domains into the aquatic environment, facilitating interactions with organic molecules. There seemed to be better physicochemical interactions between lamotrigine, but not atrazine, and DOM. The two compounds have similar H-bond donor abilities but the H-bond acceptor ability of lamotrigine is higher than that of atrazine), which may enable it to form more H-bonds with the DOM. The solubility of terbuthylazine, an atrazine analog, was also barely influenced by the presence of
DOM, suggesting that the triazine ring does not necessarily play a governing role in binding with DOM.

The apparent partition coefficients for lamotrigine to DOM ($K_{\text{DOM-LTG}}$) under all tested conditions at an ionic strength of 2 mM ranged between 0.02 and 0.03 L mg C$^{-1}$ (with no significant differences between them). At the increased ionic strength of 154 mM, $K_{\text{DOM-LTG}}$ was significantly higher ($0.10 \pm 0.02$ L mg C$^{-1}$). These results are in agreement with those obtained for pyrene and organochlorine pesticides in the presence of fulvic and humic acids. Therefore, we concluded that DOM serves as a binding agent for lamotrigine in aqueous systems, ultimately increasing lamotrigine's solubility. No partition coefficient ($K_{\text{DOM-ATZ}}$) could be derived for atrazine owing to the absence of an effect of DOM on its' solubility. Ilani et al. (2005) also reported a negligible partition coefficient of atrazine to DOM. The significant impact of DOM on the solubility of lamotrigine implies that DOM may have major effects on the mobility and bioavailability of such pollutants in natural aquatic systems. Such changes in solubility must be taken into account when evaluating potential methods for pollutant removal.

3.2.2. Adsorption of atrazine/lamotrigine by SWCNTs with and without DOM

In solution, SWCNTs form various adsorption sites, and multiple mechanisms of interaction may occur. Due to the significant differences in solubility between the two studied compounds (atrazine and lamotrigine) among the various solution conditions (Table 1), concentration data of atrazine and lamotrigine were normalized to their respective solubilities (i.e., reduced concentration; $C_e C_s^{-1}$). This enabled us to compare the two analytes' behaviors for similar activity rather than similar molar or mass concentration under different solution conditions (Fig. 4).
The adsorption isotherms of both analytes' (Fig. 4) were all fitted by the Dubinin-Astakhov model (Tables 4 and 5), which is commonly used to describe adsorption on energetically heterogeneous surfaces such as SWCNTs. In the DOM-free solution, the maximum adsorption capacity and affinity for both atrazine and lamotrigine derived no obvious trends as a function of pH (Table 4). Nonetheless, in the exhibited concentration range there was a reduction in the adsorption of lamotrigine at pH 4, likely due to its enhanced solubility (Table 3). Atrazine's adsorption capacity decreased at increased ionic strength, whereas that for lamotrigine was not influenced. This finding implies that increased ionic strength had an impact on adsorption sites available for atrazine. It is important to note that while adsorption of lamotrigine was not affected by increase in ionic strength, its solubility was reduced by 23%.

Table 4. Dubinin-Astakhov model fitted parameters for atrazine and lamotrigine adsorption by single-walled carbon nanotubes (SWCNTs) under different solution conditions (value ± standard error is presented).

<table>
<thead>
<tr>
<th>Single-/bi-solute system</th>
<th>pH</th>
<th>Ionic strength (mM)</th>
<th>log(Q_0) ((Q_0), mg g(^{-1}))</th>
<th>E (kJ mol(^{-1}))</th>
<th>b</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrazine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single solute</td>
<td>7</td>
<td>2</td>
<td>2.25 ± 0.05</td>
<td>18.80 ± 1.04</td>
<td>1.0 ± 0.1</td>
<td>0.996</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2</td>
<td>2.09 ± 0.03</td>
<td>17.48 ± 0.28</td>
<td>1.8 ± 0.2</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2</td>
<td>2.12 ± 0.07</td>
<td>25.33 ± 1.76</td>
<td>1.0 ± 0.1</td>
<td>0.982</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>154</td>
<td>2.02 ± 0.09</td>
<td>17.74 ± 0.73</td>
<td>2.1 ± 0.6</td>
<td>0.903</td>
</tr>
<tr>
<td>Bi-solute</td>
<td>7</td>
<td>2</td>
<td>2.01 ± 0.06</td>
<td>16.58 ± 0.47</td>
<td>1.8 ± 0.2</td>
<td>0.996</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single solute</td>
<td>7</td>
<td>2</td>
<td>2.35 ± 0.18</td>
<td>17.75 ± 3.19</td>
<td>1.0 ± 0.2</td>
<td>0.990</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2</td>
<td>2.01 ± 0.09</td>
<td>20.18 ± 1.64</td>
<td>1.0 ± 0.1</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2</td>
<td>2.07 ± 0.07</td>
<td>21.93 ± 1.15</td>
<td>1.4 ± 0.2</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>154</td>
<td>2.22 ± 0.23</td>
<td>19.58 ± 4.56</td>
<td>1.0 ± 0.3</td>
<td>0.970</td>
</tr>
<tr>
<td>Bi-solute</td>
<td>7</td>
<td>2</td>
<td>2.11 ± 0.04</td>
<td>14.78 ± 0.46</td>
<td>1.5 ± 0.1</td>
<td>0.998</td>
</tr>
</tbody>
</table>
In the observed concentration range, the presence of DOM clearly reduced adsorbed amounts and affinities of both atrazine and lamotrigine (Fig. 4). The approach of adsorption affinity and fitting parameter b toward 5.7 and 1 kJ mol\(^{-1}\) (Table 5), respectively, suggests increased linearity of the adsorption isotherms. This is attributed to increased homogeneity of available adsorption sites due to DOM adsorption\(^{23,72}\). At a \(C_e C_s^{-1}\) of 1%, adsorption of atrazine decreased by 72 and 93%, and that of lamotrigine by 38 and 66% at low (~6 mg C L\(^{-1}\)) and high (~30 mg C L\(^{-1}\)) concentrations of DOM, respectively (at pH 7 and ionic strength of 2 mM). Similar to our results, reduction in adsorption of synthetic organic chemicals by CNTs in the presence of natural organic matter\(^{29}\) and of pyrene by CNTs in solution containing humic acid\(^{40}\) has also been reported. The authors suggested two contrasting effects: blockage of adsorption sites by DOM and newly exposed surface area due to the dispersion effect of DOM. However both studies\(^{29,40}\) failed to consider changes in the solutes' solubility in the presence of natural organic matter and humic acid. Our data show that solubility of lamotrigine was 25% higher than that in DOM-free solution (Table 3) while solubility of atrazine was unaffected by DOM. Based on this and on the collected adsorption data, we conclude that the reduced adsorption of atrazine stems from direct site competition or blockage of adsorption sites by DOM (atrazine-DOM interactions were negligible). On the other hand, the decreased adsorption of lamotrigine may also be attributed to lower affinity of the DOM–lamotrigine complex to the SWCNTs.
**Figure 4.** Adsorption isotherms of atrazine and lamotrigine by single-walled carbon nanotubes (SWCNTs) in the absence (left) and presence (right) of dissolved organic matter (DOM) at different pH values (4, 7 and 10) and ionic strengths (2 and 154 mM). Symbols and bars represent means and standard errors, respectively.
In accordance with the trends witnessed in the tested concentration range (Fig. 1), the maximum adsorption capacity for atrazine decreased significantly in the presence of DOM at all tested pH values (Tables 2 and 3). But no difference was noted at the higher ionic strength of 154 mM with the addition of DOM. Our previous study showed that DOM adsorption at increased ionic strength alters the assembly of SWCNTs in solution which may expose additional sites for atrazine adsorption. Conversely, the maximum adsorption capacity for lamotrigine was not affected by DOM under any of the tested solution conditions, despite the observed trends. This suggests that lamotrigine may adsorb via cumulative adsorption onto already adsorbed DOM, or that DOM adsorption exposes new adsorption sites that are accessible to lamotrigine.

In the presence of DOM, the maximum adsorption capacity for both pollutants was lower at pH 4, whereas no distinct differences were obtained between pH 7 and 10. At decreased pH, adsorption of DOM is enhanced owing to increased H-bond formation and reduced electrostatic repulsion between DOM and the SWCNTs, resulting in increased competition and site blockage. This might explain the reduction in atrazine's maximum adsorption capacity. No difference in adsorption affinity was noted as a function of pH in the presence of DOM for either pollutant. With DOM at an ionic strength of 154 mM, maximum adsorption capacity for atrazine by SWCNTs was higher than at an ionic strength of 2 mM, whereas adsorption of lamotrigine remained unchanged between these conditions. Adsorption affinity for lamotrigine was not affected, but that for atrazine was significantly lower at 154 mM vs. 2 mM. Increased maximum adsorption capacity and decreased affinity for atrazine at 154 mM vs. 2 mM may be attributed to an increase in the homogeneity of the adsorption sites originating from a combined effect of DOM adsorption and increased ionic
strength. This is further demonstrated by the reduction in both E and b fitting parameters, indicating an isotherm shift toward linearity \(^72\).

3.2.3. Effect of DOM introduction stage on adsorption of atrazine/lamotrigine by SWCNTs

The effect of DOM on pollutant adsorption was further investigated by exploring the impact of the stage at which DOM was introduced. Indeed, DOM-introduction stage had a significant impact on the adsorption of both atrazine and lamotrigine by SWCNTs (Fig. 2). When the SWCNTs were pre-introduced to DOM, the adsorption of atrazine and lamotrigine was significantly inhibited. Adsorption of atrazine and lamotrigine decreased by 95% and 60%, respectively, at a \(C_e C_s^{-1}\) value of 1%. Negligible adsorption of atrazine in the pre-introduced DOM system was probably due to the occupation of adsorption sites by DOM. Adsorption affinity (E, Table 5) for lamotrigine did not differ from that with simultaneous exposure of the SWCNTs to DOM, implying that the DOM coating did not impact the interactions between lamotrigine and the SWCNTs. However, the maximum adsorption capacity (\(q_0\), Table 5) for lamotrigine decreased when DOM was pre-introduced, suggesting that DOM indeed reduced the availability of adsorption sites for lamotrigine, compared to simultaneous introduction of the two. Similarly, reduction in adsorption of 2-phenyl phenol was significantly higher than that of phenanthrene by CNTs pre-loaded with natural organic matter compared to bare CNTs \(^29\). Those authors explained that the competition between natural organic matter and 2-phenyl phenol is more severe due to the latter's nonplanar and hydrophilic character.
Figure 5. Adsorption isotherms of atrazine and lamotrigine by single-walled carbon nanotubes (SWCNTs) in the presence of dissolved organic matter (DOM) introduced at different stages. DOM concentration in simultaneous-, pre- and post-DOM introduction was approximately 6 mg C L\(^{-1}\). DOM coating was 80 and 62 mg C g\(^{-1}\) for experiments performed with atrazine and lamotrigine, respectively. All experiments were performed at pH 7 and ionic strength of 2 mM. Symbols and bars represent means and standard errors, respectively.

In post-DOM exposure (i.e., DOM was introduced to the system after the CNT–pollutant reached apparent equilibrium), adsorption affinity for both organic pollutants was significantly higher than that with the simultaneous introduction of DOM and pollutant, but similar to that obtained in the DOM-free solution (Table 5). The maximum adsorption capacity for atrazine did not differ between simultaneous and post-DOM introduction, but for lamotrigine, the capacity was slightly higher when DOM was post-introduced. In addition, maximum adsorption capacities were higher in DOM-free solution than when DOM was post-introduced, for both atrazine
and lamotrigine. This implies that the initial adsorption advantage given to the pollutants did not affect their maximum adsorption capacities, suggesting that there are DOM molecules with higher affinity to the SWCNTs which can replace the adsorbed pollutant and force its release back into solution.

To verify that reduced adsorption of lamotrigine by SWCNTs in the presence of DOM was not only the result of the pollutant's increased solubility, adsorption of atrazine and lamotrigine by DOM-coated SWCNTs was tested in a DOM-free solution. The adsorption of both compounds by DOM-coated SWCNTs was greatly reduced relative to the bare SWCNTs. When the changes in adsorbed amount of pollutant between the bare and DOM-coated SWCNTs were normalized to the adsorbed amount of DOM, the two data sets overlapped. This suggests that the adsorption of both pollutants is indeed reduced by blockage and occupation of adsorption sites by DOM. However, since lamotrigine interacts favorably with DOM, additional effects impact its adsorption when DOM is present in solution. Atrazine's adsorption affinity and maximum adsorption capacity by DOM-coated SWCNTs were lower than those by bare SWCNTs (both in a DOM-free solution), but did not differ from those obtained in DOM solution (Table 5). This further confirms that the suppression of atrazine adsorption is due to direct site blockage or occupation by DOM. On the other hand, the maximum adsorption capacity for lamotrigine did not differ between the bare and DOM-coated SWCNTs, supporting the notion that lamotrigine adsorption may ultimately occur via cumulative adsorption or onto newly exposed sites. The above findings illustrate the importance of the stage at which each potential adsorbate (i.e., pollutant and DOM) is introduced to the adsorbent (SWCNTs).
Table 5. Dubinin-Astakhov model fitted parameters for atrazine and lamotrigine adsorption by single-walled carbon nanotubes (SWCNTs) under different solution conditions in the presence of dissolved organic matter (DOM). Value ± standard error is presented.

<table>
<thead>
<tr>
<th>Single-/bi-solute system</th>
<th>DOM concentration (mg C L⁻¹)</th>
<th>pH</th>
<th>Ionic strength (mM)</th>
<th>( \log Q_0 ) (( Q_0 ), mg g⁻¹)</th>
<th>E (kJ mol⁻¹)</th>
<th>b</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atrazine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single solute</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 a</td>
<td>1.81 ± 0.06</td>
<td>13.46 ± 0.44</td>
<td>2.1 ± 0.2</td>
<td>0.980</td>
<td></td>
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</tr>
<tr>
<td>30</td>
<td>1.95 ± 0.23</td>
<td>8.59 ± 1.70</td>
<td>1.3 ± 0.3</td>
<td>0.962</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>6 b</td>
<td>1.86 ± 0.03</td>
<td>15.44 ± 0.21</td>
<td>2.2 ± 0.1</td>
<td>0.995</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOM coating c</td>
<td>1.69 ± 0.47</td>
<td>9.48 ± 4.75</td>
<td>1.0 ± 0.5</td>
<td>0.885</td>
<td></td>
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<tr>
<td>7</td>
<td>1.54 ± 0.17</td>
<td>12.95 ± 1.70</td>
<td>1.4 ± 0.4</td>
<td>0.947</td>
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<tr>
<td>7</td>
<td>1.88 ± 0.05</td>
<td>14.05 ± 0.40</td>
<td>1.9 ± 0.2</td>
<td>0.987</td>
<td></td>
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<tr>
<td>7</td>
<td>2.18 ± 0.18</td>
<td>9.64 ± 1.81</td>
<td>1.0 ± 0.2</td>
<td>0.981</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bi-solute</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2.16 ± 0.73</td>
<td>7.07 ± 5.09</td>
<td>1.0 ± 0.5</td>
<td>0.956</td>
<td></td>
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<tr>
<td><strong>Lamotrigine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single solute</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 a</td>
<td>2.14 ± 0.11</td>
<td>15.60 ± 1.26</td>
<td>1.4 ± 0.2</td>
<td>0.995</td>
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</tr>
<tr>
<td>32</td>
<td>2.17 ± 0.14</td>
<td>11.96 ± 1.27</td>
<td>1.3 ± 0.2</td>
<td>0.996</td>
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<td></td>
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</tr>
<tr>
<td>7 b</td>
<td>1.85 ± 0.05</td>
<td>19.98 ± 0.51</td>
<td>1.8 ± 0.2</td>
<td>0.996</td>
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</tr>
<tr>
<td>DOM coating c</td>
<td>1.65 ± 0.11</td>
<td>17.69 ± 0.87</td>
<td>2.4 ± 0.5</td>
<td>0.967</td>
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<tr>
<td>7</td>
<td>2.08 ± 0.28</td>
<td>11.23 ± 3.24</td>
<td>1.0 ± 0.3</td>
<td>0.987</td>
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<tr>
<td>7</td>
<td>1.89 ± 0.10</td>
<td>16.50 ± 0.89</td>
<td>1.3 ± 0.2</td>
<td>0.991</td>
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</tr>
<tr>
<td>7</td>
<td>2.03 ± 0.13</td>
<td>15.28 ± 1.61</td>
<td>2.0 ± 0.3</td>
<td>0.987</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2.14 ± 0.31</td>
<td>12.53 ± 3.97</td>
<td>1.0 ± 0.3</td>
<td>0.986</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bi-solute</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.98 ± 0.09</td>
<td>15.45 ± 0.80</td>
<td>1.9 ± 0.2</td>
<td>0.987</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Post-DOM introduction into the pollutant-SWCNT system.
b Pre-DOM introduction into the pollutant-SWCNT system.
c DOM coating of 80 and 62 mg C g⁻¹ for experiments performed with atrazine and lamotrigine, respectively.
3.2.4. Competition between pollutants with and without DOM

Competitive adsorption assays are necessary to imitate real conditions in contaminated water where there are likely to be multiple pollutants. The presence of a competitor significantly reduced the maximum adsorption capacity and affinity for both atrazine and lamotrigine (Table 4 and Fig. 6). This suggests that the two compounds compete over similar adsorption sites, however the adsorption of atrazine was less influenced by the presence of lamotrigine. At the reduced concentration ($C_e$/$C_s$) of 1%, adsorption of atrazine and lamotrigine decreased by 30% and 47% in the presence of a competitor, respectively. It is also evident that the adsorption of the competitor decreased as the concentration of the main adsorbate increased, ruling out cooperative adsorption. It is important to note that at the highest reduced concentration of atrazine, adsorption of the competitor lamotrigine declined by ~60%, whereas adsorption of the competitor atrazine was reduced by only 30% at the highest reduced concentration of lamotrigine (Fig. 6). This suggests that atrazine is a more effective competitor than lamotrigine, implying that there are adsorption sites which are preferentially occupied by atrazine.
Figure 6. Adsorption isotherms of atrazine (main solute) with lamotrigine as competitor (top), and lamotrigine (main solute) with atrazine as competitor (bottom) by single-walled carbon nanotubes (SWCNTs) in the absence (left) and presence (right) of DOM (7 mg C L\(^{-1}\)) at pH 7 and ionic strength of 2 mM. Symbols and bars represent means and standard errors, respectively.
The maximum adsorption capacity for both pollutants in the presence of a competitor was similar (Table 4), but adsorption affinity in the competitive system was higher for atrazine than for lamotrigine. Lamotrigine has few conformational possibilities in light of the single rotational bond between the triazine and the dichlorobenzene ring. Nonetheless, lamotrigine can effectively overlap with the curved SWCNT surfaces owing to its "butterfly"-like structure (dihedral angle of approximately 60°), similar to reports on adsorption of bisphenol A by CNTs. In contrast, atrazine has four rotational bonds, which may enable conformational adjustments so that the compound can wedge itself into interstitial areas and grooves. We hypothesize that atrazine adsorbs preferentially to interstitial spaces and groove sites due to its "flexibility" while lamotrigine interacts more with the external surface area due to its "butterfly"-like structure. Comparable results were obtained for competitive adsorption between carbamazepine and bisphenol A (as competitor) by SWCNTs, and between naphthalene and 2,4-dichlorophenol or 4-chloro-aniline (as competitors) by multi-walled CNTs. In addition, we postulate that the superior adsorption of atrazine over lamotrigine may stem from the fact that lamotrigine is a stronger Lewis base than atrazine. Although SWCNTs may serve as both electron donors and acceptors, they have been reported to interact favorably with electron acceptor molecules. Therefore, increased affinity of atrazine may be attributed to its' higher electron accepting abilities.

Competition between atrazine and lamotrigine in the presence of DOM (Fig. 6, right side) resulted in opposing trends to those witnessed in the DOM-free system (Fig. 6, left side). In the presence of DOM, adsorption of lamotrigine was far less influenced by a competitor than atrazine. Adsorption of atrazine and lamotrigine decreased by 73% and 14%, in the presence of a competitor, respectively, at the
reduced concentration (\(C_e C_s^{-1}\)) of 1%. Furthermore, decrease in adsorbed amount of the competitor atrazine was more distinct than that of the competitor lamotrigine (50% versus 30%, respectively), both compounds at their highest reduced concentrations. These results demonstrate once more the significant impact originating from the increased solubility of lamotrigine in the presence of DOM. With DOM in solution, lamotrigine likely adsorbs cumulatively upon already adsorbed DOM whereas atrazine competes with DOM over adsorption sites. Consequently, DOM served as a mediator for the adsorption of lamotrigine in the presence of atrazine and as a competitor for atrazine in the presence of lamotrigine. No significant differences were obtained between maximum adsorption capacities for the single and bi-solute systems in the presence of DOM. However the adsorption affinity was significantly higher for lamotrigine in comparison to atrazine, which may be allocated to the favorable interactions formed between lamotrigine and the adsorbed-DOM.

4. Conclusions

This report provides comprehensive data on the adsorptive fractionation of DOM by SWCNTs, as well as the effects of DOM on organic pollutant adsorption. Adsorption of DOM was governed by the SWCNT–HoA interaction, proving the dominance of this fraction in the adsorbed phase while the non-adsorbed DOM becomes more hydrophilic. This, in turn, might change the reactivity of the DOM in systems exposed to CNTs in light of its’ altered composition. As the hydrophilicity increases, it is less likely that the DOM will assist in adsorption to solid phases (via co- and cumulative adsorption). We anticipate similar adsorptive fractionation trends of DOM by other pristine carbon-based CNTs such as MWCNTs as they all exhibit similar surface properties.
Many studies have examined adsorption of organic pollutants by SWCNTs in different solution backgrounds with the assumption that pollutant solubility remains unchanged. The increase in the solubility of lamotrigine, but not atrazine, in DOM solution illustrated the importance of testing pollutant–DOM interactions when assessing pollutant removal in natural water systems. In addition, co-removal of multiple pollutants is essential in methods for water purification: we found that adsorption of lamotrigine was markedly reduced in the presence of atrazine however this result was overturned in the presence of DOM. We expect similar behavior from other triazine-based herbicides such as simazine, propazine and terbuthylazine. These pollutants are structurally similar to atrazine: they possess equivalent H-bond donor and acceptor abilities and the same amount of rotation bonds. On the other hand, in lamotrigine, there are more significant amendments to the triazine structure, owing to the additional dichlorobenzene ring. Thus, despite the fact that atrazine and lamotrigine are both triazine-based compounds, their expected behavior should not be generalized and they must be associated with two separate triazine classes. Adsorption of both pollutants was highly sensitive to the presence of DOM, which proved to be a major competitor for adsorption sites. Consequently, the prospective use of CNTs in organic pollutant removal from aquatic systems may be significantly impaired in the presence of DOM.
References

Sorption equilibrium and kinetics of 17β-estradiol and estrone with soil and environmental colloids

Jacob R. Prater, Michael L. Thompson, and Robert Horton

ABSTRACT

Estrogenic compounds occur in land-applied animal manure and pose a biological risk if they enter aquatic ecosystems through agricultural drainage or shallow groundwater, but the mechanisms and factors controlling their dissipation in soils have not been fully investigated. Estrogens are nonpolar and strongly adsorbed by soil organic matter, limiting their miscible transport, but presenting another possible transport vector, co-transport with colloids which may facilitate the transport of estrogens through soil and ultimately to surface water. The sorption and degradation kinetics of 17β-estradiol (E2) and estrone (E1) represent important dissipation pathways of these two endocrine-disrupting chemicals. As a first step in considering colloid mediated transport mechanisms, the objective of this study was to determine the intensity and kinetics of adsorption and the kinetics of degradation of 17β-estradiol (E2) and estrone (E1) with three Iowa soils, colloidal components of those soils, and colloidal components of swine manure. By using batch incubations, adsorption isotherms were measured for E2 and E1 binding to these just mentioned materials. Additionally, time series incubations (0.3 to 66 h) were conducted to determine the kinetics of both adsorption and degradation of E2 and E1 with each material. Adsorption isotherms showed linear relationships and measured partition coefficients (Kₐ values) ranged from 15 L kg⁻¹ to 34 L kg⁻¹ for the soil materials. The log Kᵦₒ values (organic carbon normalized Kₐ) for the soils were similar to values reported by others and ranged from 2.94 to 3.32. Kₐ values for colloidal material ranged from 72 L kg⁻¹ to 213 L kg⁻¹. The log Kᵦₒ values
for colloidal materials ranged from 2.79 to 3.33. Results from the time series incubations for both E2 and E1 indicate two sorption mechanisms or phases that contrasted in rate. Both sorption phases can be approximated as pseudo first-order processes, although the initial rapid phase may be more accurately described as instantaneous. Additionally, degradation of E2 can be approximated as a first-order process with rate coefficients varying from 0.006 to 0.06 h\(^{-1}\) and with a mean of 0.026 h\(^{-1}\) (sd = 0.016) in the tested materials. The calculated half-life of E2 had a mean of 47.0 h (sd = 31.4 h) with soils and 39.8 h (sd = 37.2 h) with soil colloids.

**Keywords:** estradiol, estrone, colloids

**INTRODUCTION**

Livestock, such as swine, as well as undomesticated animals excrete estrogenic hormones in their urine and feces. In the environment, the two most important and biologically potent of these estrogens are E2 and E1 due to their potential to disrupt the endocrine systems of aquatic organisms such as fish and amphibians (Oberdorster and Cheek, 2000; Corcoran et al., 2010) and in soil fauna and microbial systems (Markman et al., 2007; Chun et al., 2006). At small (ng L\(^{-1}\)) concentrations this disruption could lead to skewed sex ratios of fish and amphibians by causing males to exhibit phenotypically female characteristics (Purdom et al., 1994; Hansen et al., 1998). Additionally, these chemicals have been shown to occur in waste water effluent and biosolids after escaping degradation or removal during treatment (Aga, 2008). EDCs are present in untreated, land-applied manure obtained from concentrated animal-feeding operations (CAFOs) (Aga, 2008; Lange et al., 2002). Thus, understanding their fate in soil and water is paramount for maintaining water quality and healthy aquatic ecosystems. The unknown impact of such changes on aquatic ecosystems highlights the importance of understanding the dissipation of
estrogens in the environment. In general, E2 and E1 dissipate in three ways: dilution and transport, sorption, and transformation and degradation.

Recent studies have shown that estrogen transport could be dominated by preferential flow and chemical and physical non-equilibrium conditions (Sangsupan et al., 2006; Steiner et al., 2010; Kjaer et al., 2007). Some studies have pointed to the possibility of enhanced estrogen transport by colloids (Steiner et al., 2010; Arnon et al., 2008; Casey et al., 2008) with one study having demonstrated the phenomenon in a lab setting with intact soil cores (Prater et al., 2016).

Previous research has identified mechanisms of E2 and E1 sorption to soil which may be reversible or irreversible (Casey et al., 2003). Several studies measuring the dissipation of E2 with different soil materials suggest that dissipation can be predicted using pseudo first-order kinetics with results varying between soils (Lee et al., 2003; Colluci et al., 2001; Ying et al., 2003; Casey et al., 2005). Hildebrand et al. (2006) noted that sorption of E2 and E1 to soil is initially rapid and is then characterized by a slower phase of sorption. In contrast Casey et al. (2005) reported one sorption rate coefficient and one first-order process. Sorption rate coefficients for soils reported by Casey et al. (2005) were 0.178 h\(^{-1}\) and 0.210 h\(^{-1}\) for E2 and E1, respectively. Others have reported different first-order transformation and degradation rate coefficients covering a broad range. For example, Das et al. (2004) reported values of 0.0003 to 0.075 h\(^{-1}\) from a column experiment and Collucci et al. (2001) reported values of 0.013 to 0.13 h\(^{-1}\) from a batch experiment. Such ranges likely result from several factors including: experimental design (column or batch), degradation reaction model used, and soil conditions, including sample preparation and experimental parameters. Das et al. (2004) noted that even among different column experiments there was variability in calculated degradation rates. There is evidence that these rates and the direction of the reaction may be affected by other conditions,
namely the presence of oxygen and anaerobic or reducing conditions (Prater et al., 2015; Czajka and Londry 2006; Ying et al., 2003; Cheng and Tsai 2010). Also temperature and acidity may be factors in E2 and E1 degradation, with increasing rates at higher temperatures and decreasing rates with increasing acidity (Raman et al., 2001).

The sorption of E2 and E1 to soil has been interpreted to be dominated by hydrophobic partitioning owing to the correlation of partitioning coefficients to soil organic carbon (SOC) content (Lee et al. 2003). Still, two-site models seem to be supported by empirical data, but do not necessarily indicate different mechanisms (Casey et al. 2003). Casey et al. (2005) suggested that sorption of E2 and E1 was diffusion limited because the rate of adsorption was proportional to the square root of time. The molecular-scale mechanisms of these two apparent modes of sorption remain under debate. Perhaps two-site or two-phase behavior could be indicative of external and internal surfaces in the sorbent. This two-phase behavior could lead one to the conclusion that there is a rapid external surface sorption and diffusion limited internal surface sorption, consistent with the presence of clay minerals such as smectite which have internal surfaces in their interlayer spaces. Van Emmerik et al. (2003) noted a rapid initial sorption phase and reported that E2 could intercalate into the interlayer spaces of smectite. Intercalation of E2 into expandable clay minerals suggests that even small particles like colloids can have internal spaces where diffusion could play a role in limiting the rate of adsorption or desorption of estrogens. Whereas there has been evaluation of estrogen-soil interactions, these interactions have not been investigated with natural soil and manure colloidal material.

The role of colloids in inhibiting estrogen degradation has been noted by wastewater treatment engineers. Holbrook et al. (2003) discovered that the amount of colloidal organic material in wastewater effluent affected its estrogenic activity, and thus, that the capture of
colloidal material is a requisite step for reducing the estrogenic activity of wastewater effluent. This is noteworthy because, despite treatment (primarily of the liquid phase), estrogenic activity persisted. In soils that receive applications of manure that is not treated, two modes of transport exist, miscible transport and colloidal transport. Because estrogens can be degraded and sorbed by soil materials, it remains that perhaps in ideal soil conditions for crop production (high organic matter and good drainage) the dominant transport mechanism may be colloid facilitated transport of these compounds. The reason for this is two-fold: sorption hysteresis can lead to slower desorption from the colloidal material, and the colloids may protect estrogens from some forms of degradation.

Reported log $K_{oc}$ values for soil materials range from 2.3 to 4.2 for E2 and from 2.5 to 4.1 for E1 (Lee et al., 2003; Ying et al., 2003; Sarma et al., 2008; Sangsupan et al., 2006; Casey et al., 2005; Xie et al., 2008) while the reported values for river colloids are in the higher portion of the range of soils, 3.95 for E2 and 4.18 for E1 (Liu et al., 2005). Wastewater colloids have been reported to have similar values to river colloids ranging from 4.08 to 4.68 for E2 (Holbrook et al., 2003). Parameters describing sorption of estrogen compounds to fractionated soil colloidal material have not been reported, and only one other paper has reported on estrogen interaction with swine manure particulates (Amin et al., 2011). These researchers reported log $K_{oc}$ values for the smallest size fraction of swine slurry separates (colloidal size) of 3.02 L kg$^{-1}$ (Amin et al., 2012). The objective of this research was to determine the intensity of E2 and E1 sorption to three typical Iowa soils, to their associated colloids, and to swine manure colloids, highlighting any properties that might show linkage to sorption intensity with implications for the ability of colloidal materials to transport E2 and E1.
The reactions of estrogens with soil (sorption, transformation, and degradation) have been observed by many researchers but these interactions have not been investigated with soil and manure colloidal material. Despite investigation the conclusions about estrogen sorption, transformation, and degradation with and in soil have not always been the same. Because soils vary from region to region, chemical and physical interactions with estrogen are nuanced by soil properties. Different soils have yielded different shapes for sorption isotherms (linear and Freundlich), sorption reactions, and degradation reactions. Perhaps the reason for these variations is in the type of organic matter present, although numerous studies have pointed to roughly similar log $K_{oc}$ values (an organic carbon normalized partitioning coefficient ($K_d$)) for E1 and E2 (Lee et al. 2003; Casey et al. 2003; Hildebrand et al. 2006). This research investigates the interactions of organic carbon content, texture, and mineralogy on estrogen sorption intensity and rate as well as degradation in three Iowa soils, their colloids, and swine manure colloids. An example schematic of the sorption and degradation process can be found in Fig. 1.

**MATERIALS AND METHODS**

Three annually cultivated Iowa soils, representing a spectrum of particle size distribution and organic matter content (soil series: Hanlon, Clarion, and Zook soils) were sampled in the top 10 cm at each of three sites near Ames, IA. Each soil sample was dried and ground to pass through a 2-mm sieve. Classification information for these soils is found in Table 1. The Clarion soil sample is from the Iowa State Curtiss Research Farm and is characterized as a well-drained upland soil formed in till under native prairie vegetation. The Hanlon soil sample is from the Iowa State Hinds Research Farm and is characterized as well drained, having formed in
loamy alluvium under native prairie vegetation on natural levees along streams. The Zook soil sample is from the Ames Municipal Water Pollution Control Facility and is characterized as a poorly drained soil formed in silty and clayey alluvium and usually occurring in bottom lands. These three samples provide a particle size distribution from fine sandy loam to clay loam along with a broad spectrum of soil structural expression.

The soil colloidal material was dispersed by gentle agitation in distilled water using a rotary shaker at 15 rpm for 12 hours with a 1:8 soil to solution ratio (Kjaergaard et al., 1995). The resulting suspension was then fractionated using Stokes’ Law (particle density, $\rho_s = 2.65 \text{ g cm}^{-3}$) sedimentation for particles less than 1-μm (equivalent spherical diameter). The colloidal suspensions were then concentrated using auto irrigation pots (an automatic irrigation system adapted to be a porous ceramic filter apparatus) and subsequently freeze dried for later use.

Swine manure was collected from the flushing of a farrowing house at an Iowa State University research farm. The manure colloids were also fractionated using Stokes’ Law sedimentation for particles less than 1-μm (assuming equivalent spherical diameter and $\rho_s = 1.4 \text{ g cm}^{-3}$). Manure colloids were then concentrated onto 0.45-μm nylon filters and freeze dried for later use. Manure colloids were concentrated on nylon filters instead of auto irrigation pots in order to conserve the smaller amount of material and prevent the fouling of equipment.

After collection and fractionation, the soil and colloidal materials were analyzed for physical and chemical properties including particle size distribution using the pipette method (Gee and Or, 2002), soil organic carbon content by total combustion (Allison et al., 1965), pH (Peech, 1965), and cation exchange capacity using sodium saturation and ammonium acetate displacement (Chapman, 1965). A second measure of CEC was done without the removal of organic matter using the same method as the CEC measurement. The mineralogy of the colloid
samples was determined using x-ray diffraction, with the samples being treated with H$_2$O$_2$ to remove organic matter, saturated with Mg, plated onto ceramic tiles, saturated with glycerol, analyzed using CuK$_\alpha$ x-ray diffraction, and interpreted using the guidelines outlined by Whittig and Allardice (1986). An example of the x-ray patterns can be found in Fig. 2. Soil material physical and chemical properties are located in Table 2. Colloid properties are located in Table 3.

E1 and E2 were obtained from Sigma Aldrich (98% pure). Table 4 shows chemical structure (exhibiting a steroidal ring structure including a phenolic ring and few ionizable functional groups indicating their hydrophobicity) and properties of E2 and E1. The aqueous solubility of E2 and E1 are affected by both pH and ionic strength. Aqueous solubility increases significantly at the pH where the compounds are ionized ($pK_a \sim pH$). Ionic strength has the effect of possibly coagulating these compounds in a ‘salting out’ effect thus, decreasing their solubilities at higher ionic strengths (Shareef et al., 2006). For these reasons a background electrolyte concentration of 5 mM CaCl$_2$ was maintained and solution pH was controlled by the soil samples (ranging from 5.5 to 6.5), but was well below the $pK_a$ values of E2 and E1.

Estrogen stock solutions were prepared using methanol as a co-solvent (less than 1% of total volume) followed by dilution in 5 mM CaCl$_2$. The effect of methanol as a co-solvent in limited quantities on sorption has been shown to be negligible (Bouchard, 2003).

**Equilibrium Batch Studies**

Equilibrium batch studies were conducted to measure the sorption intensity of each of the soil and colloidal materials for E2 and E1. These batch studies were carried out in 50-mL glass centrifuge tubes with Teflon-lined caps using dried and ground soil or colloidal material. Solid-to-solution ratios were chosen according to an estimated sorption intensity based on measured
soil organic carbon (SOC) content. This was done to ensure that between 20% and 80% of the spiked estrogen (spikes of 0.05 – 2 mg L\textsuperscript{-1}) would remain in solution for efficient and accurate measurement. Solid-to-solution ratios were 1:100 for colloidal materials, 1:20 for Hanlon soil material, 1:40 for Clarion soil material, and 1:60 for Zook soil material. Significant effects of varying the solid-to-solution ratios on measured $K_d$ values seemed unlikely given the reasonably consistent log $K_{oc}$ values previously reported from sorption experiments with soils at different solid-solution ratios (Lee et al., 2003; Ying et al., 2003; Sarma et al., 2008; Sangsupan et al., 2006; Casey et al., 2005; Xie et al., 2008). Immediately before the sorption experiments, the soil and colloid samples were autoclaved (134°C for 60 min) to inhibit microbial activity. After autoclaving, the samples were equilibrated for 24 h on a reciprocating shaker. Following equilibration, each sample was centrifuged at 800 g for 16 min to separate the liquid and solid. An aliquot of the supernatant was then taken and adjusted to pH ~ 4 for analysis. Batch studies with soil were replicated 3 times and colloids were done singularly due to a short supply of the material. Solution and sorbed concentrations (determined by difference) were then plotted and fitted to linear isotherms with slopes denoted as partitioning coefficients ($K_d$). $K_d$ values and log $K_{oc}$ values (log of organic carbon normalized $K_d$ values) were used to make comparisons between soils and colloids. Calculations of $K_d$ values are found in Eq. 1, $E_s$ (mg kg\textsuperscript{-1}) being the estrogen (E2 or E1) on the solid phase and $E_{aq}$ (mg L\textsuperscript{-1}) being the estrogen in solution.

$$K_d = \frac{E_s}{E_{aq}} \quad [1]$$

**Kinetic Batch Studies**

Non-equilibrium, time-dependent batch studies were conducted to measure the kinetics of sorption and degradation of E2 and E1 in the presence of each of the seven soil and colloidal materials (Hanlon, Clarion, Zook, Hanlon WDC, Clarion WDC, Zook WDC, and swine manure.
WDC). Batch study samples were incubated in 50-mL glass centrifuge tubes with Teflon-lined caps using dried and ground soil and colloidal materials. Based on SOC content and measured sorption intensities (Prater et al., 2012a), solid-to-solution ratios were chosen (1:20 for Hanlon, 1:40 for Clarion, 1:60 for Zook, and 1:100 for WDCs) to ensure that between 20% and 80% of the added estrogen (~2 mg L⁻¹) would remain in solution to ensure measurable quantities and accuracy of measurement. Subsamples of the materials were autoclaved (60 min at 134°C) to inhibit microbial activity and denature enzymes prior to incubation for sorption measurements. The samples were incubated at 20°C on a reciprocating shaker with either an E2 or E1 spike. Incubation times for sorption measurements were 0.33, 2, 4, 8, 12, 24, and 48 h. Samples that were incubated to measure degradation were not autoclaved and were incubated under the same conditions described above, but for longer duration (0.33, 2, 4, 8, 12, 24, 32, 40, 48, 56, and 66 h). Following incubation each sample was centrifuged at 800 g for 16 min to separate solids and solution. Then an aliquot of the supernatant was taken and adjusted to pH ~ 4 for analysis of the dissolved estrogens. All samples were replicated twice with the exception of the swine manure colloids due to their very short supply.

Calculations of Rate Coefficients and Half-Life

Sorption rate coefficients were determined empirically using linear regression of natural log transformed plots of the sorption (sterilized conditions) data. Degradation rates were calculated using two different methods. The degradation rate of E2 to E1 was first calculated using the autoclaved sample data as a control and plotting the difference between the control and the untreated sample. An integrated form of the first-order degradation equation (Equation 2) was then used to calculate a rate coefficient using linear regression. This method of calculation
assumes there is negligible desorption of E2 from the solid phase and that degradation occurs primarily in the dissolved phase.

\[
(E_{2i} - (E_{2sp} - E_{2d})) = E_{2i}e^{-k_{deg}t} \quad [2]
\]

In Eq. 2, \(E_{2i}\) (mg L\(^{-1}\)) was the initial solution phase E2 concentration, \(E_{2sp}\) (mg L\(^{-1}\)) refers to the data from sterile sorption incubations, \(E_{2d}\) (mg L\(^{-1}\)) refers to the concentration from untreated degradation incubations, \(k_{deg}\) (h\(^{-1}\)) is the degradation rate coefficient, and \(t\) is time. The second method used to calculate E2 degradation to E1 was from the E1 data obtained from the degradation experiments. In this calculation we assume rapid sorption of E1 and a nearly instant equilibrium of E1 in solution with E1 in the sorbed phase. The calculation uses Eqs. 3 and 4 and include the use of a partitioning coefficient (\(K_d\) value) determined from the equilibrium batch studies.

\[
E_{2i}e^{-k_{deg}t} = (E_{2i} - (E_{1s} \frac{M}{V} + E_{1d})) \quad [3]
\]

\[
K_d = \frac{E_{1s}}{E_{1d}} \quad [4]
\]

In Eq. 3, \(E_{2i}\) (mg L\(^{-1}\)) was the initial solution phase E2 concentration, \(E_{1s}\) (mg kg\(^{-1}\)) is E1 on the solid phase, M (kg) is the mass of soil, V (L) is the volume of solution, \(E_{1d}\) (mg L\(^{-1}\)) is E1 in the solution phase of the degradation experiment, and \(E_{2i}\) (mg L\(^{-1}\)) is the E2 added initially.

Equation 4 is a calculation of the \(E_{1s}\) for the measured partition coefficient (\(K_d\)) from the equilibrium batch studies. Half-lives were then calculated from each of the above rate coefficients using Equation 5. These calculated half-lives represent a range of the half-life of E2 having been calculated with slightly different assumptions that may overestimate or underestimate half-life.

\[
t_\frac{1}{2} = \frac{\ln 2}{k_{deg}} \quad [5]
\]
In Eq. 5 \( t_{1/2} \) is the half-life.

**Analytical Techniques**

Concentrations of E2 and E1 in water were determined using high-pressure liquid chromatography (HPLC) (Beckman-Coulter HPLC, auto-sampler, and diode array detector, model #118, 507, and 168 respectively). This was accomplished using a pre-concentration and clean-up solid-phase extraction with a C-18 substrate (LiChrolut EN) as per Xie et al. (2008). Samples were then evaporated under a gentle \( N_2 \) gas stream at 40\(^\circ\)C and then reconstituted in methanol. High-pressure liquid chromatography instrument parameters were: sample loop, 50 \( \mu \)L, flow rate, 1.5 mL min\(^{-1}\); mobile phase, 45:55 Acetonitrile:H\(_2\)O (pH ~4); column, C-18 reverse phase (Alltima); UV detector, \( \lambda = 202 \) nm. Analysis with HPLC was based on the procedures used by Lee et al. (2003): Sample loop, 50 \( \mu \)L, flow rate, 1.5 mL min\(^{-1}\); mobile phase, 45:55 acetonitrile: H\(_2\)O (pH ~4); column, C-18 reverse phase (Alltima); UV detector, \( \lambda = 202 \) nm. The method detection limit was 2.5 \( \mu \)g L\(^{-1}\) in a 30 mL sample (defined as 3 times the background signal) and the limit of quantitation was 6 \( \mu \)g L\(^{-1}\) in a 30 mL sample (defined as the lowest standard used). HPLC analysis was deemed more efficient for our goals of measuring sorption and degradation properties compared to slower costlier methods with lower detection limits that are used to detect concentrations in purely aqueous systems.

**RESULTS AND DISCUSSION**

Water-dispersible colloids (WDC) had greater soil organic carbon (SOC) contents than the corresponding soil materials. Hanlon WDCs had 5 times more SOC than Hanlon soil materials, Clarion WDCs had 3 times more SOC than Clarion soil materials, and Zook WDCs
had 1.3 times more SOC than Zook soil materials (Table 2 and 3). X-ray diffraction analysis showed a very large peak for smectite indicating that the clay fraction of all three of these soil colloidal materials were dominated by smectite (Fig. 2). CEC without OM removal for the soil materials were 20.1 cmol kg$^{-1}$ for Clarion, 12.3 cmol kg$^{-1}$ for Hanlon and 58.9 cmol kg$^{-1}$ for Zook (Table 2). Particle size analysis indicated clay contents of 176 g kg$^{-1}$ for Clarion, 72 g kg$^{-1}$ for Hanlon and 400 g kg$^{-1}$ for Zook (Table 2).

**Equilibrium Sorption**

In all cases, WDCs had greater sorption intensities as indicated by the greater isotherm slopes and $K_d$ values than corresponding soil materials (Fig. 3 and Table 5). Soil organic carbon content is a good predictor of sorption intensity for these three Iowa soils and their WDCs as evidenced by log $K_{oc}$ values being very close to 3 for all materials (Table 5). Not all organic matter is equal with respect to sorption of these estrogens. With two alluvial soils (Hanlon and Zook) with similar log $K_{oc}$ values and one till-derived soil (Clarion), there is something different (perhaps different quantities of fats, waxes, and aromatic carbon forms) about organic carbon forms in these soils as evidenced by the range of log $K_{oc}$ values for both E2 and E1 (2.94 to 3.32 and 2.99 to 3.32 respectively). This range of log $K_{oc}$ values corresponds to a 1200 and 1100 L kg$^{-1}$ range in $K_{oc}$ values for E2 and E1, respectively. It is possible that Clarion, the upland soil, contains more fresh or undecomposed organic material being drier (upland landscape position) and that Zook also contains more fresh or non-pedogenic organic material having received biosolids applications in the past both leading to slightly lower log $K_{oc}$ values than Hanlon. This difference could mean that Hanlon contains more non-polar forms of SOC (fats, waxes, and aromatics) that might be associated with sorption of non-polar compounds like E2 and E1. However, these determinations are difficult to make without a more extensive data set of
sorption intensity and organic matter characterization. An extensive characterization of organic matter in these soils could be used to test whether or not certain forms of organic carbon have greater affinity for, or sorption potential of E2 and E1.

Water-dispersible colloids from individual soils had higher $K_d$ values than the corresponding soil materials (Table 5). This was particularly true in sandy, low-organic carbon Hanlon materials. The concentration of organic carbon (OC) in Hanlon colloids was 5 times more than in the bulk soil (Table 3). Average log $K_{oc}$ values for soil and WDCs (3.17 for E2 and 3.23 for E1) displayed standard deviations of 0.16 and 0.18 for E2 and E1, respectively. This puts the log $K_{oc}$ of manure colloids (2.79 for E2 and 2.81 for E1) 2.3 standard deviations from the mean of the soil and WDCs, an indication that the materials are distinctly different in terms of their interaction with E2 and E1. It is possible that the difference between the organic matter in soil and soil colloids and in swine manure colloids is the presence of less decomposed organic material. With the exception of Hanlon and E2, all log $K_{oc}$ values were higher for WDCs than the corresponding soil materials. The greater log $K_{oc}$ values for WDCs is consistent with the idea that the colloidal fraction of organic carbon is more degraded or more transformed pedogenically, thus, containing more recalcitrant organic matter with more hydrophobic components like aliphatic chains and aromatic rings. If it is the case that there is a larger proportion of recalcitrant organic carbon forms in these materials then they would likely have higher log $K_{oc}$ values due to having larger pools of non-polar organic matter. However, the greater log $K_{oc}$ values for WDCs could also be an effect of higher surface area per unit mass on smaller particles. Differences in soil $K_{oc}$ values for E2 and E1 by ecoregion (brown chernozems differing from other soils tested) have been noted in Alberta, Canada. Caron et al. (2010) also hypothesized that soils with more recalcitrant or more highly degraded organic matter may
exhibit higher $K_{oc}$ values. This is consistent with the mode of sorption being hydrophobic partitioning (as these more recalcitrant forms of SOC are more non-polar) which has been suggested by Lee et al. (2003) as the dominant sorption mechanism for these two estrogens to soils.

Conclusions about correlations between $K_d$ and clay content, fine clay content, and CEC, with and without OM removal (not shown) are difficult to draw from this data set, but they may indicate that there is some sorption activity associated with clay minerals due to a weak correlation between $K_d$ and clay and fine clay content, and CEC. Casey et al. (2003) also noted the relationship of $K_d$ to clay content, pointing to smectite and CEC as additional potential sorption predictors alongside organic carbon content. Van Emmerik et al. (2003) noted that there was some affinity of smectite for E2 and suggested that E2 could be intercalated into the interlayer spaces of Ca- and K-saturated montmorillonite. Bonin and Simpson (2007) also noted that expanding clay minerals had a role in sorption of E2 and E1 to soils alongside organic carbon, but with a smaller effect. Bonin and Simpson (2007) suggested that the effect of mineral sorption sites with a different mechanism of sorption than partitioning were negligible when increasing soil organic matter was present. This could explain some of the discrepancy between the log $K_{oc}$ values of Hanlon, Clarion, and Zook with the low SOC soil Hanlon displaying a larger influence from mineral based sorption due to its lower organic carbon content and the other two soils exhibiting more of the effect of their larger organic carbon content and thus a muted mineral sorption effect.

The interaction of swine manure WDCs with E2 and E1 were different from those of the soil materials and soil WDCs as indicated previously by their large deviation (2.3 standard deviations) in log $K_{oc}$ from the mean log $K_{oc}$ of the other materials. Sorption isotherms are
presented in Fig. 3. Swine manure colloids contained little if any mineral material and almost entirely consisted of microorganisms and partially digested organic matter. The chemical composition of the manure organic material was entirely different from the soil and soil colloids, with a much smaller C:N ratio than the soil derived materials (suggesting that the swine manure colloids were relatively protein rich) (Table 2 and 3). The difference in the interaction with estrogens is reflected by the lower log K_{oc} values for swine manure WDCs (Table 5). Consistent with the discussion above, lower log K_{oc} values for the swine manure colloids most likely reflect the presence of hydrophilic compounds such as cellulose and protein residues.

**Sorption and Degradation Kinetics**

Autoclaved samples exhibited no signs of degradation metabolites, so it was assumed that these treated samples had no degradation occurring. Both E2 and E1 were rapidly sorbed by soil and colloidal materials in autoclaved samples, approaching equilibrium after 10 to 12 h (Fig. 4). Equilibrium in the sorption of both E2 and E1 was reached by 24 hours, as reported by Xie et al. (2008) and Lee et al. (2003). Amin et al. (2012) noted that apparent sorption equilibrium was reached more rapidly with swine manure materials than with soil materials. This more rapid sorption to swine manure materials appears to be the case in the present experiments as well (with the exception of E2 with soil materials) with E2 and E1 reaching equilibrium with swine manure colloids within 8 h (Fig. 4). The longer equilibration time of E2 and E1 with soil and soil colloidal materials (12 h) could be due to the presence of minerals, namely smectite found to be the dominant clay mineral in these materials (Fig. 2), creating lower accessibility sorption sites. These lower accessibility sites might include hydrophobic nanopores in clay quasicrystals, sub-stack surfaces, similar to what was described by Hundal et al. (2001) when investigating the sorption of phenanthrene to reference smectites. Van Emmerik et al. (2003) has suggested that
E2 sorption to montmorillonite occurs in interlayer spaces and required a period of about three days of contact to reach equilibrium indicating that some sorption sites may require considerable time for reactions to occur.

Soil materials as well as soil and swine manure colloids exhibit two-phase sorption for both E2 and E1 (Fig. 4). Due to the lack of data points prior to 0.33 h, it is difficult to characterize the initial rapid sorption phase because its endpoint is not clearly defined from this data. Because of this uncertainty, only the rate coefficients of the second phase are reported. In order to make comparisons among the sorbents, boundaries were set to determine a reaction rate for the slower sorption phase. The boundaries used were the measurement at 0.33 h, where there is a clear change in rate and at 95% of the total sorption during the incubation, a proxy for equilibrium. Pseudo first-order rate constants ($k_2$ h$^{-1}$) for the second phase of sorption differed among the sorbents, with a mean of 0.066 h$^{-1}$ (sd = 0.04) for E2 with soil materials, 0.060 h$^{-1}$ (sd = 0.02) for E2 with soil colloidal materials, 0.035 h$^{-1}$ (sd = 0.015) for E1 with soil materials, and 0.047 h$^{-1}$ (sd = 0.006) for E1 with soil colloidal materials (Table 6). If it were calculated from the two measurements available (0 and 0.33 h), the initial, rapid phase had a rate coefficient as much as one order of magnitude larger than the second phase. It is possible that this initially rapid phase may be hard to distinguish under different experimental conditions (timing of sampling, initial concentration, and solid-to-solution ratio) which could lead to lumping both sorption phases.

The two sorption phases may differ mechanistically/chemically, with one being more rapid than the other. But such an interpretation may be overstating what occurs. Amin et al. (2012) suggested the possibility that the two phases of sorption represent similar chemical reactions that occur at exterior surfaces and interior surfaces. In other words, there may be one
mechanism, hydrophobic partitioning, but access varies to different sorbing surfaces, exterior and interior, with diffusion limiting the rate of the interior surface sorption. Van Emmerik et al. (2003) noted that montmorillonite could sorb E2 and that it could be intercalated into interlayer spaces of clay. The potential for intercalation of E2 into expanding clay minerals may provide some insight concerning the two apparent phases or mechanisms of sorption. Organomineral colloids (both fractionated and present in the unfractionated soil materials) consist of organic matter with clay and could provide interior spaces for a chemical to diffuse into and sorb. Additionally, these interior spaces may consist of hydrophobic nanopores in clay quasicrystals, sub-stack surfaces, similar to what was described by Hundal et al. (2001). A third possibility is that there are microaggregates present that contain interior surfaces with organic coatings. The presence of interior sorption sites might also explain some of the apparent irreversible sorption or incomplete recovery reported by Lee et al. (2003).

The proportions of sorbed E2 and E1 for each of the two phases were calculated as the mass sorbed during each phase relative to the total amount sorbed. The proportions of sorption in these phases show some variability across materials. The first phase constituted 32 to 83% of the total amount sorbed and the second phase constitutes 17 to 68% of the total amount sorbed (Table 7). The initial rapid phase represented a large proportion of the total sorption of both E2 and E1 to the soil materials and swine manure colloids while the soil colloids appeared to have more sorption in the slower phase than the other materials (Table 7). The larger proportion of sorption in the slower phase for soil colloids could be due to the greater clay content of these materials, creating more interior sorption surfaces where diffusion limited the rate of sorption. A larger array of soils with varying clay content could be used to test this hypothesis.
The presence of two distinct sorption phases differs from the work of Casey et al. (2003) who reported just one pseudo first-order process. In the present study, initial concentrations were at least one order of magnitude higher and solid-to-solution ratios were smaller than in the work of Casey et al. (2003). Additionally, the sorption rate coefficients reported by Casey et al. (2005) (0.178 h\(^{-1}\) and 0.210 h\(^{-1}\) for E2 and E1, respectively), using a soil similar to Hanlon in organic matter content, were also greater than the slow phase measured in this work.

The E2 degradation data reveal similar sorption behaviors to those measured separately in autoclaved samples, initially rapid then slowing before degradation dominates instead of equilibrium being reached (Fig. 4, 5, and 6). Unsterilized samples show a first-order degradation of E2 to E1 after sorption approached the equilibration time of the sterile samples (Fig. 5, and 6). Concomitant sorption and degradation resulted in a rapid initial decrease in solution E2 in conjunction with an increase in solution E1 followed by a slower decrease in E2 concentration approaching zero alongside a plateauing E1 solution concentration. This is similar to what Raman et al. (2001) reported from incubations of E2 with dairy manure. These results, showing degradation of E2 to E1 in a 3-day period, differ from those reported by Casey et al. (2005), which showed no degradation of E2 (i.e., no metabolites present in solution) in a similar period. Instead of E2 liquid concentrations approaching zero as in this study, Casey et al. (2005) measured E2 concentrations that approached an equilibrium.

Similar to the results of Raman et al. (2001) there was a small re-emergence of E2 in some samples toward the end of the incubation (data not shown), but the concentrations remained below the quantification limits used in this study (6 µg L\(^{-1}\) in a 30-mL sample). The re-emergence of E2 could be due to sorption hysteresis (slow release of E2 from the solid phase after solution-phase degradation) or to a reaction similar to that described by Prater et al. (2015),
i.e., reduction of E1 to E2 under reducing conditions. If the re-emergence of E2 is due to a desorption process, then we may infer that degradation took place preferentially in the solution phase rather than the sorbed phase, which is contrary to the assumption of Casey et al., 2005. This conclusion follows from the rapid rate of sorption and the lack of competition between E2 and E1 for sorption sites on soils containing organic matter noted by Bonin and Simpson (2007). If degradation occurred on the solid phase, desorption of E2 would be unlikely, because removal of E2 from the solid phase would presumably lead to disequilibrium resulting in more sorption of E2 rather than desorption. This interpretation does not mean that degradation was not associated with the solid phase, but rather that degradation did not occur primarily in the sorbed phase. In order to better understand the complex kinetics of E2 and E1 removal from the system, either the sorbed phase must also be measured to account for this desorption or a reliable model must be constructed to describe the behavior.

When the degradation rate of E2 was calculated using the two methods described previously (Equations 2-4) a range of degradation rate coefficients was produced varying from 0.006 to 0.06 h\(^{-1}\) with a mean of 0.026 h\(^{-1}\) (sd = 0.016) (Table 6). These rate coefficients, k\(_{\text{deg}}\), were similar to those reported by Das et al. (2004), 0.0003 to 0.075 h\(^{-1}\) from a column experiment and Collucci et al. (2001), 0.013 to 0.13 h\(^{-1}\) from a batch experiment. Half-lives of E2 calculated with Equation 4 range from 11.6 to 115.5 h with a mean of 43.4 h (sd = 34.6) (Table 5). Reported degradation rates are variable, revealing that soil conditions are an important factor.

**CONCLUSIONS**

Soils and colloids with large organic carbon concentrations have a strong affinity for sorbing estrogens. The \(K_d\) and log \(K_{oc}\) values for soils from this study are similar to other
published data, but the information on colloidal material fills a gap in current knowledge. First, the soil organic matter is strongly associated with the colloidal size fraction, so colloid-estrogen interactions are reflective of the whole soil of origin, as evidenced by the similar log $K_{oc}$ values. Second, the colloids have much greater sorption intensity (indicated by the $K_d$ values) than their soils of origin. Both of these findings, similar log $K_{oc}$ values and higher $K_d$ values for colloids than whole soils occur because most of the organic carbon in the soil is associated with the colloidal size fraction. Variations in the log $K_{oc}$ values among the soils may be related to differential decomposition due to soil formation processes resulting in a varying degree of hydrophobicity in the organic carbon present. Additional investigation into the forms of organic carbon as a result of soil formation as well as measurement of organic matter properties (nuclear magnetic resonance spectroscopy, fourier transform infrared spectroscopy, and thermal analysis) may reveal some of the reasons for differences in the sorption behavior of E2 and E1 with different soils.

Different types of organic material and clays may also present complexity in the creation of internal surface area in soil colloids. Sorption kinetics appear to show two processes with different rates. Both E2 and E1 were rapidly removed from the solution phase by sorption processes in these soil and manure derived materials. Degradation further removed E2 from solution in the soil and colloidal materials. These experiments did not indicate any degradation of E1, as E1 concentrations in degradation experiments plateaued or appeared to be approaching an equilibrium. The measured kinetics of E2 and E1 sorption showed a rapid phase and a second slower phase in all materials measured. We hypothesize that these phases arise from exterior and interior surfaces and that the two rates derive from surface sorption and diffusion-limited internal sorption. This hypothesis arises from the presence of smectite in the mineral fractions of all the
studied materials, with exception of the swine manure, and the work of Van Emmerik et al. (2003) showing intercalation of E2 into smectite. Testing of this hypothesis might require characterization of interior and exterior surface areas as well as the effective diffusion coefficients of these materials (a difficult task) and/or the use of manufactured materials with known interior and exterior surface areas.

The types of organic matter and surfaces may also influence degradation rates by protecting E2 and/or E1 from abiotic or biotic factors leading to oxidation. Degradation of estrogens may be rapid in soil under aerobic conditions, but it ceases and can even reverse under anaerobic conditions (Prater et al., 2015). 17 β-estradiol has a mean half-life of 47.0 h (sd = 31.4 h) with soils and 39.8 h (sd = 37.2 h) with soil colloids, as calculated from measurements in this study. Due to rapid sorption and a short half-life, E2 is not likely to be transported in soils unless conditions allow for reduced soil contact times or decreased degradation. If degradation primarily occurs in the solution phase, then sorption may protect E2 from some forms of degradation. This possible protection coupled with the high capacity of colloids for sorption of estrogens suggests that colloids may be important in the potential transport of E2.

If colloids were mobilized and transport pathways present, colloids could potentially carry estrogens through a soil profile and impact shallow groundwater or enter surface waters via tile drainage systems as suggested by Prater et al. (2016). Further, colloid facilitated transport could be a greater contributor to estrogen transport than simple miscible transport due to the smaller, but still substantive, $K_d$ values of the soil materials, which coupled with the greater mass of soil versus colloidal material, indicate that there would be a high degree of estrogen retention, via sorption, in soil and thus retardation of miscible estrogen transport in soil. Additionally, the initial rapid sorption phase indicates an area of further study due to its greater importance under
physical and chemical non-equilibrium transport conditions. Under these conditions, soil contact
times may be short and sorption rates developed from longer incubation times may
underestimate sorption and thus over predict miscible transport.

Conditions favorable to E2 and E1 transport include: anaerobic conditions resulting in
decreased degradation rates, preferential flow leading to decreased soil contact times, and the
presence of suspended organic colloids protecting E2 and E1 from degradation during transport.
Therefore, applying E2-containing manure to soils when wet conditions might ensue could risk
transport of estrogens. Thus in order to decrease the risk of estrogen transport, soil contact time
and oxygen availability should be maximized when applying manure which may contain high
levels of estrogens. In doing so sorption and degradation will be promoted and transport and
leaching reduced. Thus, applications of manure known to have high estrogen content should be
applied in drier conditions when soil gas exchange is high and soil water movement is slow,
especially avoiding areas where there is shallow groundwater, tile drainage systems, or karsts.
REFERENCES

Aga, D.S. 2008. Fate of pharmaceuticals in the environment and in water treatment systems. CRC Press Boca Raton, FL.


Table 1. Soil taxonomic description and characteristics.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Classification</th>
<th>Parent material</th>
<th>Surface horizon texture</th>
<th>Landscape position</th>
<th>Slope</th>
<th>Drainage class</th>
<th>Organic matter in surface horizon</th>
<th>pH in surface horizon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coarse-loamy, mixed, superactive, mesic Cumulic Hapludoll</td>
<td>Alluvium</td>
<td>Fine sandy loam</td>
<td>Floodplains</td>
<td>0 – 2%</td>
<td>Moderately well drained</td>
<td>1 – 3%</td>
<td>6.6 – 7.3</td>
</tr>
<tr>
<td>Hanlon</td>
<td>Fine-loamy, mixed, superactive, mesic, Typic Hapludoll</td>
<td>Till</td>
<td>Loam</td>
<td>Convex uplands</td>
<td>2 – 5%</td>
<td>Well drained</td>
<td>3 – 4%</td>
<td>5.0 – 6.5</td>
</tr>
<tr>
<td>Clarion</td>
<td>Fine, smectitic, mesic Cumulic Vertic Endoaquoll</td>
<td>Colluvium/Alluvium</td>
<td>Silty clay loam</td>
<td>Floodplains</td>
<td>0 – 2%</td>
<td>Poorly drained</td>
<td>5 – 7%</td>
<td>5.6 – 6.0</td>
</tr>
<tr>
<td>Zook</td>
<td>Fine, smectitic, mesic Cumulic Vertic Endoaquoll</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Soil physical and chemical characteristics including cation exchange capacity (CEC) with and without organic matter (OM).

<table>
<thead>
<tr>
<th></th>
<th>Hanlon</th>
<th>Clarion</th>
<th>Zook</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sandy loam</td>
<td>loam</td>
<td>clay/clay loam</td>
</tr>
<tr>
<td>pH</td>
<td>6.92</td>
<td>4.65</td>
<td>5.97</td>
</tr>
<tr>
<td>Sand (g kg⁻¹)</td>
<td>724</td>
<td>445</td>
<td>219</td>
</tr>
<tr>
<td>Silt (g kg⁻¹)</td>
<td>204</td>
<td>379</td>
<td>382</td>
</tr>
<tr>
<td>Clay (g kg⁻¹)</td>
<td>72</td>
<td>176</td>
<td>400</td>
</tr>
<tr>
<td>Coarse silt (g kg⁻¹)</td>
<td>137</td>
<td>212</td>
<td>132</td>
</tr>
<tr>
<td>Fine silt (g kg⁻¹)</td>
<td>67</td>
<td>167</td>
<td>250</td>
</tr>
<tr>
<td>Coarse clay (g kg⁻¹ of clay)</td>
<td>795</td>
<td>755</td>
<td>591</td>
</tr>
<tr>
<td>Fine clay (g kg⁻¹ of clay)</td>
<td>205</td>
<td>245</td>
<td>409</td>
</tr>
<tr>
<td>CEC (no carbonates or OM) (cmol kg⁻¹)</td>
<td>7.6</td>
<td>13.9</td>
<td>35.5</td>
</tr>
<tr>
<td>CEC (with OM) (cmol kg⁻¹)</td>
<td>12.3</td>
<td>20.1</td>
<td>58.9</td>
</tr>
<tr>
<td>Total carbon (g kg⁻¹)</td>
<td>7.2</td>
<td>18.4</td>
<td>30.1</td>
</tr>
<tr>
<td>Total nitrogen (g kg⁻¹)</td>
<td>1.0</td>
<td>1.8</td>
<td>2.7</td>
</tr>
<tr>
<td>C:N (ratio)</td>
<td>7.3</td>
<td>10.0</td>
<td>11.0</td>
</tr>
</tbody>
</table>
Table 3. Water-dispersible colloid (WDC) organic carbon and nitrogen contents.

<table>
<thead>
<tr>
<th></th>
<th>Hanlon WDC</th>
<th>Clarion WDC</th>
<th>Zook WDC</th>
<th>Manure WDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total carbon (g kg(^{-1}))</td>
<td>38.9</td>
<td>55.9</td>
<td>38.1</td>
<td>346.0</td>
</tr>
<tr>
<td>Total nitrogen (g kg(^{-1}))</td>
<td>5.3</td>
<td>6.4</td>
<td>4.3</td>
<td>63.2</td>
</tr>
<tr>
<td>C:N</td>
<td>7.4</td>
<td>8.7</td>
<td>9.0</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Table 4. Chemical structure and properties of 17 β-estradiol and estrone. pK\(_a\) is the pH at which 50% ionization occurs. Log K\(_{ow}\) is the log of the octanol-water partitioning coefficient.

<table>
<thead>
<tr>
<th></th>
<th>17β-Estradiol</th>
<th>Estrone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acronym</td>
<td>E2</td>
<td>E1</td>
</tr>
<tr>
<td>Structure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular mass (g mol(^{-1}))</td>
<td>272.4</td>
<td>270.4</td>
</tr>
<tr>
<td>Water solubility (mg L(^{-1}))</td>
<td>13-21.6</td>
<td>2.1-13</td>
</tr>
<tr>
<td>pK(_a)</td>
<td>10.2</td>
<td>10.4</td>
</tr>
<tr>
<td>Log K(_{ow})</td>
<td>3.1-4.0</td>
<td>3.1-3.4</td>
</tr>
</tbody>
</table>

Table 5. Sorption parameters, K\(_d\) (partition coefficient) and log K\(_{oc}\) (organic carbon normalized partition coefficient) and correlation coefficients. Solid-to-solution ratios for each sorbent were 1:20 for Hanlon, 1:40 for Clarion, 1:60 for Zook, and 1:100 for all water-dispersible colloids (WDC).

<table>
<thead>
<tr>
<th></th>
<th>17 β-Estradiol</th>
<th>Estrone</th>
</tr>
</thead>
<tbody>
<tr>
<td>K(_d) (L kg(^{-1}))</td>
<td>R(^2)</td>
<td>log K(_{oc})</td>
</tr>
<tr>
<td>Hanlon</td>
<td>15</td>
<td>0.98</td>
</tr>
<tr>
<td>Hanlon WDC</td>
<td>72</td>
<td>0.97</td>
</tr>
<tr>
<td>Clarion</td>
<td>16</td>
<td>0.99</td>
</tr>
<tr>
<td>Clarion WDC</td>
<td>74</td>
<td>0.99</td>
</tr>
<tr>
<td>Zook</td>
<td>34</td>
<td>0.98</td>
</tr>
<tr>
<td>Zook WDC</td>
<td>82</td>
<td>0.98</td>
</tr>
<tr>
<td>Manure WDC</td>
<td>213</td>
<td>0.99</td>
</tr>
</tbody>
</table>
Table 6. Rate coefficients ($k_2$) for the second phase of sorption, degradation rate coefficients ($k_{deg}$) calculated two ways (from E2 solution measurements and from E1 solution measurements), and half-lives ($t_{1/2}$) for soils, soil water-dispersible colloids (WDC) and swine manure (SM) WDCs. Correlation coefficients ($R^2$) are in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>17 β-Estradiol</th>
<th>Estrone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_2$ (h$^{-1}$)</td>
<td>$k_{deg}$ (h$^{-1}$)</td>
</tr>
<tr>
<td></td>
<td>Eq 1 ($R^2$)</td>
<td>Eq 2 ($R^2$)</td>
</tr>
<tr>
<td>Clarion</td>
<td>0.050</td>
<td>0.018 (0.91)</td>
</tr>
<tr>
<td>Hanlon</td>
<td>0.029</td>
<td>0.023 (0.96)</td>
</tr>
<tr>
<td>Zook</td>
<td>0.120</td>
<td>0.006 (0.96)</td>
</tr>
<tr>
<td>Clarion WDC</td>
<td>0.026</td>
<td>0.012 (0.91)</td>
</tr>
<tr>
<td>Hanlon WDC</td>
<td>0.070</td>
<td>0.032 (0.63)</td>
</tr>
<tr>
<td>Zook WDC</td>
<td>0.083</td>
<td>0.006 (0.87)</td>
</tr>
<tr>
<td>SM WDC</td>
<td>0.067</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 7. Proportions of E2 and E1 in each phase (1, initial rapid phase and 2, second slower phase) of sorption as a proportion of the total amount sorbed.

<table>
<thead>
<tr>
<th>Sorption Phase</th>
<th>E2</th>
<th>E1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Clarion</td>
<td>0.74</td>
<td>0.26</td>
</tr>
<tr>
<td>Hanlon</td>
<td>0.83</td>
<td>0.17</td>
</tr>
<tr>
<td>Zook</td>
<td>0.66</td>
<td>0.34</td>
</tr>
<tr>
<td>Clarion WDC</td>
<td>0.43</td>
<td>0.57</td>
</tr>
<tr>
<td>Hanlon WDC</td>
<td>0.34</td>
<td>0.66</td>
</tr>
<tr>
<td>Zook WDC</td>
<td>0.32</td>
<td>0.68</td>
</tr>
<tr>
<td>Manure WDC</td>
<td>0.65</td>
<td>0.35</td>
</tr>
</tbody>
</table>
Figure 1. Conceptual model of E2 and E1 reaction processes with soil.

Figure 2. X-ray diffraction pattern of Zook water dispersible colloids delineating the major mineral components.
Figure 3. Soil and water-dispersible colloid (WDC) sorption isotherms with E2 or E1.
Figure 4. Manure colloid sorption isotherms with E2 or E1.
Figure 5. Sorption kinetics of 17 β-estradiol (E2) and estrone (E1) with soil, soil water-dispersible colloids (WDCs), and swine manure WDCs.
Figure 6. Degradation of 17 β-estradiol (E2) to estrone (E1) with soil water-dispersible colloids (WDC). CC, HC, and ZC are Clarion WDCs, Hanlon WDCs, and Zook WDCs respectively.
Figure 7. Degradation of 17 β-estradiol (E2) to estrone (E1) incubated with soil.