Natural organic matter affects electrospray ionization during analysis of organic micropollutants in water

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Our research group is probing how water-borne organic micropollutants (OMPs) deriving from human activities interact with natural dissolved organic matter (DOM) in aquatic systems. We hypothesize that OMP-DOM interactions control contaminant bioavailability and environmental fate. Our results indicate that DOM molecular composition and concentration affect micro-pollutant speciation in water and affect our ability to accurately measure these trace constituents using standard electrospray ionization tandem mass spectrometry methods.

Processes ranging from vegetation decomposition to anthropogenic wastewater discharge result in infusion of reduced carbon compounds into soil, sediment and water. These dissolved organic matter (DOM) transfers to aquatic systems may be accompanied by releases of organic micropollutants (OMPs) from human activity. Several research groups have been documenting a widespread distribution of OMPs in watersheds worldwide. These include endocrine disrupting compounds that exhibit biological effect even at trace concentration. We hypothesize that the fate of these OMPs is coupled to their interfacial reactivity with naturally-occurring and anthropogenic DOM.

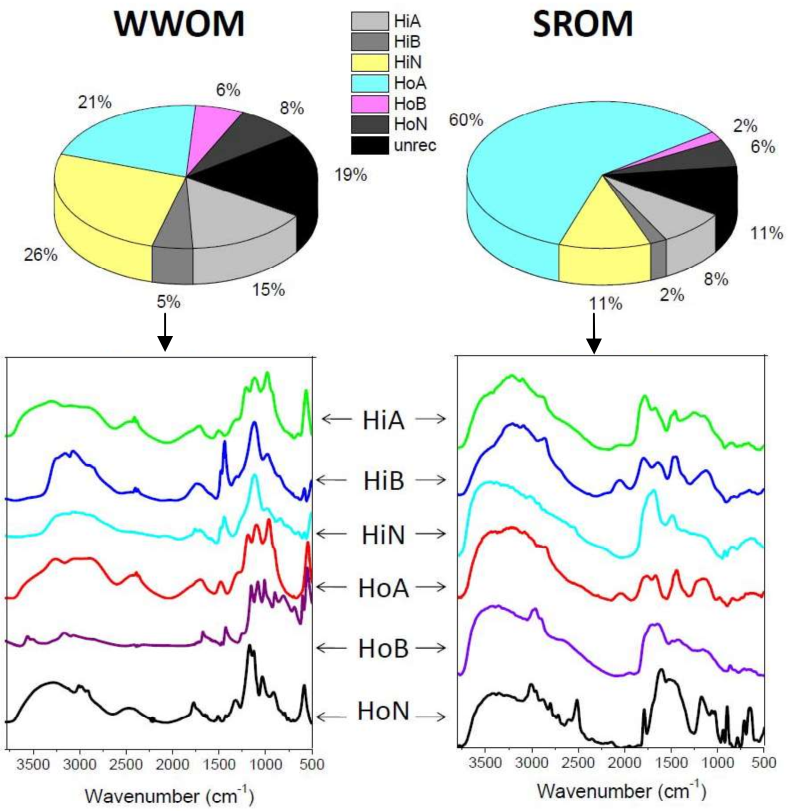

Figure 1. The molecular composition of DOM is strongly dependent on its source, and here we compare a representative municipal wastewater-derived organic matter (WWOM, left) with a standard wetland NOM from Suwannee River, GA (SROM, right) in terms of hydrophilic and hydrophobic fractions of total carbon (top) and infrared spectral signatures of those fractions (bottom). [1-3]

The composition of aquatic DOM is strongly dependent on its provenance; distinct differences in molecular hydrophilicity and functional group composition are observed among terrestrial, surface water, or anthropogenic sources. A variety of techniques are available to characterize its multi-component composition, including sorption chromatographic fractionation (Figure 1, top pie charts), where dissolved organic matter is fractionated into hydrophilic (Hi) and hydrophobic (Ho) acids (A), bases (B) and neutrals (N) on the basis or their resin sorbent affinities.[1]

For example, our anthropogenic wastewater organic matter sample (WWOM, Figure 1, left side) (Tucson, AZ) is enriched in microbial products that accumulate during biological wastewater treatment (bacterial proteins and polysaccharides). These biomolecular components are reflected in the large mass balance fraction of hydrophilic (Hi) constituents measured by resin adsorption (Figure 1, top left) and also in the large saccharide ring (C-O-C stretching) and carboxyl group bond vibrations at ca. 1000 and 1480 cm^{-1}, respectively, in the FTIR spectra of various Hi fractions (Figure 1, bottom left).

In contrast to the strong microbial signature of WWOM, terrestrial DOM is heavily imprinted with plant-derived biomolecules. In particular, terrestrial DOM is enriched in polyphenolic compounds that derive from partial oxidative biodegradation of lignin, a plant biomacromolecule comprised of phenol-propanoid subunits. Polyphenol-rich freshwater DOM is represented in the current experiments by the Suwannee River Organic Matter (SROM) standard (Figure 1, right side). Lignin phenolic compounds are selectively enriched in the large HoA fraction of SROM (Figure 1, top right), and also in the large broad phenolic-OH stretching vibration in FTIR spectra at 3000-3500 cm^{-1}.

When released to receiving waters, treated WWOM solutions also contain relatively high concentrations of OMPs that can interact with DOM constituents via hydrogen bonding, cation bridging, and hydrophobic interaction. These multi-faceted DOM interactions are likely to be most important for the polar and ionic OMPs that are only partially removed with solid residuals during secondary wastewater treatment processes. Once WWOM solutions are released into surface freshwaters, OMPs can potentially undergo transfer into polyphenol enriched SROM-like waters. Therefore, two questions that our group is addressing are: (1) *What are the fates of OMPs that are delivered into aquatic systems with wastewater organic matter (e.g., WWOM) containing fluids?* (2) *How is the speciation of these OMPs impacted by subsequent interaction with freshwater (terrestrial) organic matter sources (e.g., SROM) that they come into contact with upon discharge?*

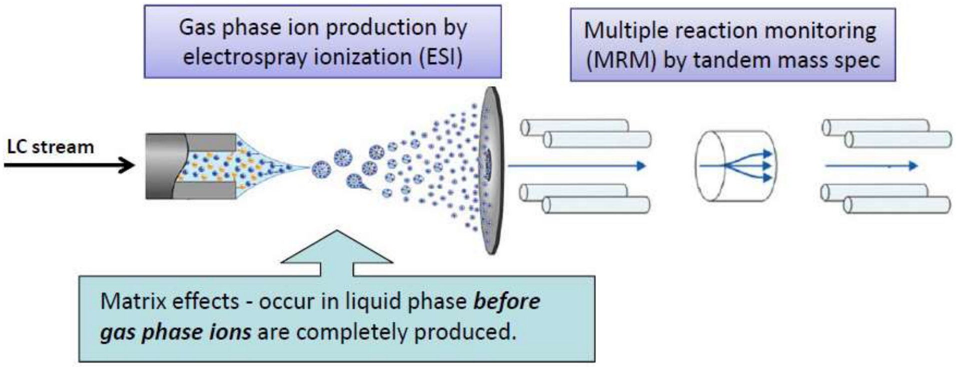

Figure 2. Upon introduction of the separated UHPLC elution stream , matrix interferences can occur as a result of charge transfer and/or complexation reactions in the liquid phase during production of gas phase ions.

A first step in answering this question is to develop appropriate methods for quantifying mixtures of these trace organic constituents in the presence of background organic matter. The preferred method of OMP analysis of natural waters involves direct ultra-high-pressure liquid chromatographic (UHPLC) injection, followed by tandem mass spectrometry (MS/MS) detection. If OMP concentrations are too low for direct aqueous injection, solid phase extraction (SPE) using hydrophilic-lipophilic balance resin is often used to enable preconcentration of analytes across a wide polarity range, but the quantitative efficiency of such sorbent-based preconcentration methods is not well known for DOM-containing solutions. In either case, once injected into the UHPLC, gas phase ion production by electrospray ionization (ESI) is followed by multiple reaction monitoring (MRM) in order to enable the confirmed quantification of target analytes on the basis of fragmentation reactions, OMP-DOM interactions in the liquid phase can potentially diminish via “matrix effects” the effective production of gas phase ions that would otherwise be quantified via MRM (Figure 2).

The “matrix effect” of varying concentration of bulk SROM and its four most prevalent fractions (HiA, HoA, HoB, HiN) on quantification of 14 different OMPs is shown in Figure 3 (from Wickramasekara et al.[2]). Data were generated by conducting a UHPLC-MS/MS infusion experiment that permits direct infusion of known concentrations of SROM fractions by a Y-connect inserted before electrospray ionization and introduction to the MS/MS, but post-UHPLC-separation of target OMP analytes. According to the increasing (negative) matrix effect with increasing SROM concentration, accurate MRM quantification of these contaminants is diminished in the presence of SROM components, particularly the hydrophilic acid (HiA) fraction of SROM. [3]

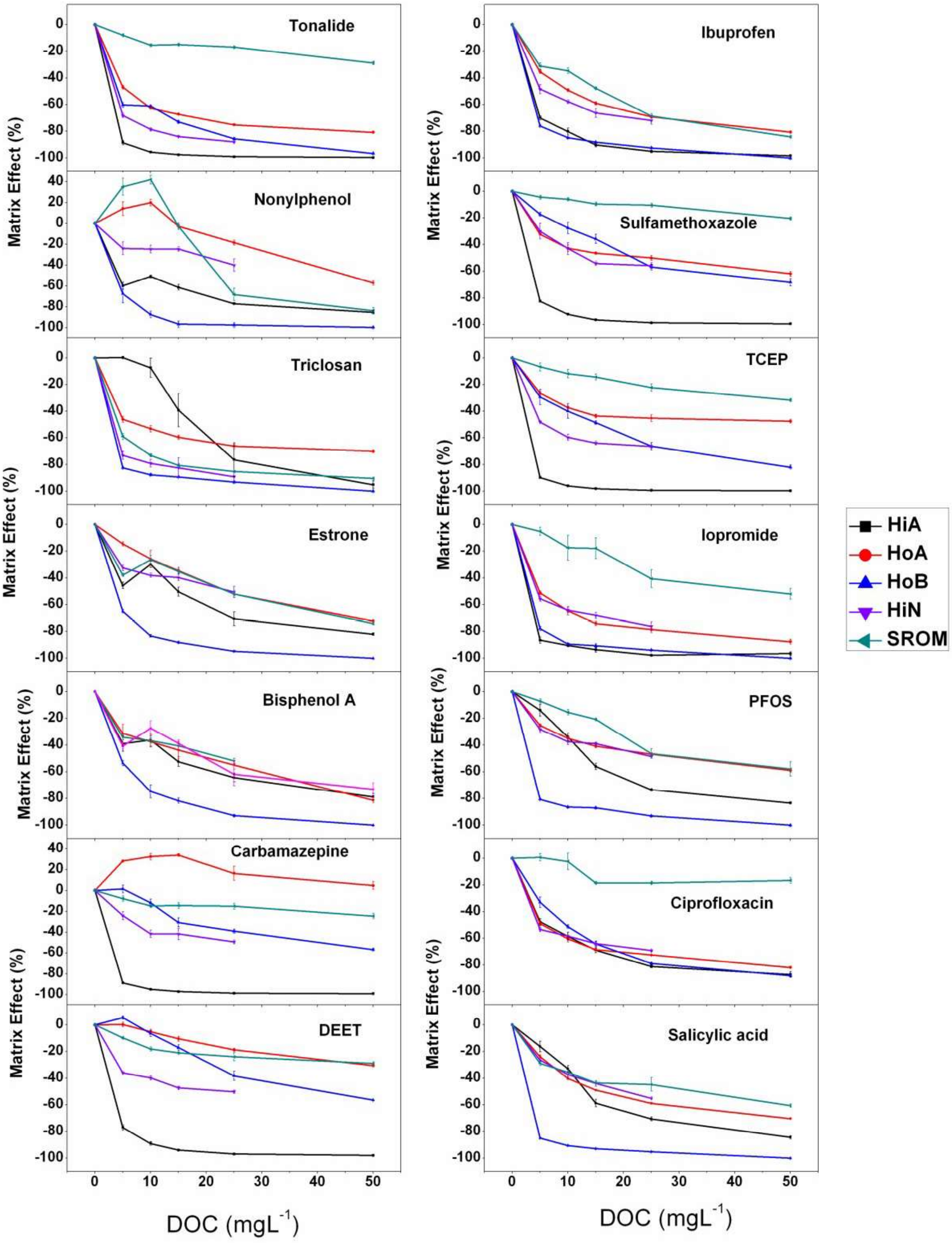

Figure 3. Calculated matrix effects for target analytes arising from post column infusion of bulk SROM and constituent fractions. Compounds are arranged in order of decreasing log Kow values (upper left to bottom right). Log Kow is the octanol-water partition coefficient, and is a measure of compound hydrophobicity. From Wickramasekara et al.[3]

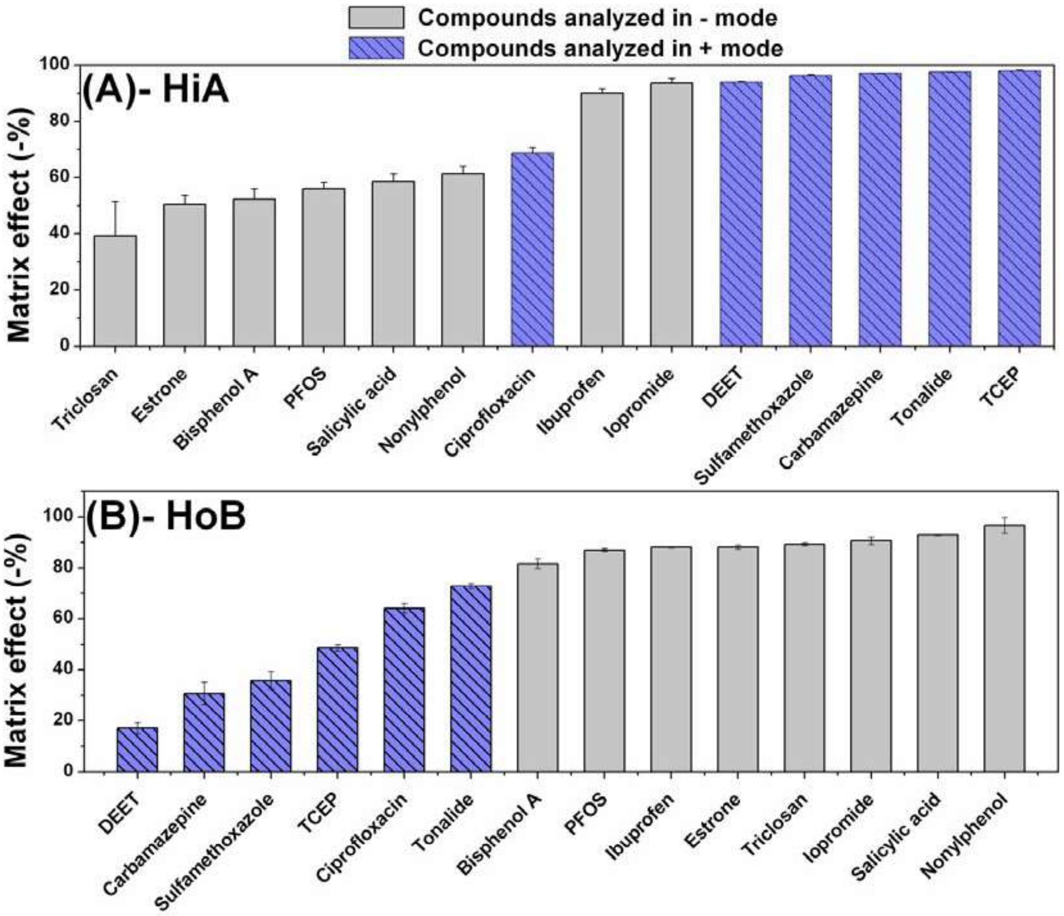

Figure 4. Ion suppression of target OMP analytes arising from (A) 15 mg L^{-1} HiA (carbon basis) fraction; (B) 15 mg L^{-1} HoB (carbon basis) fraction (arranged in order of increasing matrix effect). From Wickramasekara et al.[3]

During MS/MS analysis, electrospray ionization (ESI) is conducted in positive (+) ion or negative (-) ion mode depending on the target compound. Our results indicate that the impacts of a given SROM fraction on quantification by MRM depends on whether the compound is ionized in ESI negative or ESI positive mode. As shown in Figure 4, the hydrophilic acid (HiA) fraction, which is comprised dominantly of low molar mass carboxylated aliphatic constituents, introduces a stronger matrix effect for OMPs subjected to ESI + mode (Figure 4a). Conversely, the hydrophobic base (HoB) fraction, which contains dominantly hydrophobic proteins has a stronger impact on target compounds analyzed in ESI – mode (Figure 4b). Therefore, this study indicates that various fractions of freshwater dissolved organic matter differentially affect the ionization efficiency of important organic micropollutants in natural water samples. Hence, interactions with DOM may affect, not only the fate and transport of OMPs in aquatic systems, but also our ability to accurately quantify their presence in DOM-containing waters.

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