Characterization of the Interactions between Endocrine Disruptors and Aquatic Humic Substances from Tropical Rivers

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Interações entre substâncias húmicas aquáticas (SHA), de rios tropicais, e dois interferentes endócrinos (IE) foram estudados utilizando sistema de ultrafiltração em fluxo tangencial equipado com membrana de celulose de 1 kDa com a finalidade de separar os IE livres da fração ligada as SHA. A quantificação de 17α-ethinylestradiol e bisfenol A foi realizada utilizando cromatografia a gás acoplada a espectrômetro de massas (GC-MS). O tempo de equilíbrio entre as SHA e IE foi de aproximadamente 30 min, e as capacidades complexantes do 17α-ethinylestradiol e bisfenol A foram em torno de 18,53 e 2,07 mg g⁻¹ de carbono orgânico total (COT), respectivamente. A maior interação das SHA ocorreu para 17α-ethinylestradiol, em relação ao bisfenol A, devido a presença de hidrogênio na estrutura do 17α-ethinylestradiol, que pode interagir com os grupos oxigenados presentes nas SHA. Os resultados indicam que as SHA podem influenciar fortemente no transporte e reatividade dos interferentes endócrinos presentes em sistemas aquáticos.

Interactions between two endocrine disruptors (ED) and aquatic humic substances (AHS) from tropical rivers were studied using an ultrafiltration system equipped with a 1 kDa cut-off cellulose membrane to separate free ED from the fraction bound in the AHS. Quantification of 17α-ethynylestradiol and bisphenol A was performed using gas chromatography-mass spectrometry (GC-MS). The times required for establishment of equilibrium between the AHS and the ED were ca. 30 min, and complexation capacities for 17α-ethynylestradiol and bisphenol A were 18.53 and 2.07 mg g⁻¹ TOC, respectively. The greater interaction of AHS with 17α-ethynylestradiol, compared to bisphenol A, was due to the presence of hydrogen in the structure of 17α-ethynylestradiol, which could interact with ionized oxygenated groups of the AHS. The results indicate that AHS can strongly influence the transport and reactivity of endocrine disruptors in aquatic systems.

Keywords: water, pollution, endocrine disruptors, aquatic humic substances, complexation capacity

Introduction

Population increase, the development of new products, and intensified use of terrestrial and aquatic resources in industry and agriculture have resulted in the introduction into the environment of new compounds that may cause toxicity in humans and animals. Endocrine disruptors (ED) are often found in surface waters and effluents, where they can pose health risks even at low concentrations. In 1996, the European Commission defined this class of compounds as “exogenous substances that cause adverse health effects in the intact organism, or in its descendents, resulting in alterations of endocrine functions”1,2. ED can block cellular receptor sites or increase the production and/or secretion of hormones, hence interfering in the reproductive systems of living organisms.3,5 Many
compounds are suspected of interfering with the endocrine system, including natural and synthetic estrogens (lipid-soluble steroid hormones) such as 17α-ethynylestradiol, polycyclic aromatic hydrocarbons (compounds containing two or more conjugated aromatic rings, such as benzo(a)anthracene), polychlorinated biphenyls (highly toxic chlorinated compounds), pesticides (substances such as dichlorodiphenyltrichloroethane (DDT), used to repel or eliminate pathogenic insects), and plasticisers such as bisphenol A that are used in polymeric plastics.7

ED are conveyed to aquatic systems by various natural or anthropogenic mechanisms. Their presence in surface waters is often due to discharge of domestic and/or industrial effluents. ED contamination of aquatic systems is especially problematic because most conventional water treatment processes are unable to completely eliminate the compounds.8 Studies have found residues of pharmaceuticals and their metabolites, from ng L-1 to μg L-1 levels, in aquatic environments in countries including Germany, Brazil, Canada, Holland, UK, Italy, Sweden and the USA.9-13 However, since these chemicals belong to an emerging class of pollutants, the literature is still sparse concerning their behavior and interactions in hydric systems.

Aquatic humic substances (AHS), the most important class of complexation agents present in the natural environment, play an important role in the transport and behavior of ED.14 Characterization of the interactions of ED with AHS, exploring the formation and stability of possible ED-AHS complexes, can therefore provide important information concerning the availability of ED to aquatic biota.

This work presents the use of a procedure based on ultrafiltration to study the interactions between endocrine disruptors (17α-ethynylestradiol and bisphenol A) and aquatic humic substances extracted from two different tropical rivers, using measurements of equilibrium time, complexation capacity and competition between the ED for AHS complexation sites.

**Experimental**

**Chemicals and reagents**

All the reagents used were high-purity grade. The acid and alkaline solutions required for AHS isolation were prepared by diluting 30% hydrochloric acid or sodium hydroxide monohydrate (both Suprapure, Merck AG, Germany) in deionized water (resistivity 18.2 MΩ cm at 25 °C, Milli-Q system, Millipore, USA). The XAD-8 adsorbent (Serva Feinbiochemica, Germany) required for the isolation of AHS was pre-purified by successive soakings with 0.5 mol L-1 HCl, 0.5 mol L-1 NaOH and methanol (24 h each). Stock solutions of bisphenol A and 17α-ethynylestradiol (Sigma-Aldrich) were prepared by dissolution in acetonitrile (Merck AG, Germany).

**Water sampling and preliminary analysis**

The Itapanhau River is located in the Serra do Mar State Park (latitude: 23°50′23″S; longitude: 46°08′21″W), an environmentally protected area of the municipality of Bertioga, São Paulo State, Brazil. The Ribeira do Iguape River is situated close to the Juréia Ecological Station (latitude: 24°41′59″S; longitude: 47°33′05″W), in the municipality of Iguape, São Paulo State. These regions have a subtropical climate, with pluviometric index of ca. 2700 mm per year, humidity of ca. 80%, and ca. 150 and ca. 97 h per month of sun in January and July, respectively.

The pH, temperature, conductivity and dissolved oxygen content of water samples were determined in situ after calibration of the equipment (using standard reference solutions where appropriate). In the laboratory the water samples were digested at 120 °C using concentrated HNO3, and the concentrations of the metals Cu, Cd, Fe, Mn and Ni determined by graphite furnace atomic absorption spectroscopy (GFAAS). The dissolved organic carbon (DOC) contents of the AHS concentrate and the humic fractions were determined by catalytic combustion in an oxygen stream, with IR detection, using a total organic carbon analyzer - Shimadzu TOC 5000.

Table 1 lists the results of the preliminary characterization of the water samples. Extracts from two different rivers were used in order to explore the influence of environmental factors on the formation, decomposition and structural characteristics of AHS and, consequently, their interactions with 17α-ethynylestradiol and bisphenol A.

**Isolation of aquatic humic substances using XAD-8 resin**

The AHS were concentrated by filtration of the water samples through 0.45 μm cellulose-based membranes, acidified to pH < 2 with 0.5 mol L-1 HCl, and isolated on the XAD-8 collector, following the recommendations of Aiken et al.16 After elution with 0.1 mol L-1 NaOH, the AHS concentrate was acidified to pH 5.0 with 0.1 mol L-1 HCl solution, evaporated under vacuum, and oven-dried in re-circulating air at 55 °C.15

**Characterization of the aquatic humic substances**

The elemental composition (considering C, H, O, N and S) of the AHS was determined using a Thermo Finnigan
Table 1. Preliminary characterization of water samples collected from tributaries of the Itapanhaú and Ribeira de Iguape Rivers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Water sample</th>
<th>Itapanhaú</th>
<th>Ribeira de Iguape</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>4.4 ± 0.2</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td>23.0 ± 0.1</td>
<td>21.0 ± 0.1</td>
</tr>
<tr>
<td>Conductivity (µS cm⁻¹)</td>
<td></td>
<td>39.0 ± 0.4</td>
<td>303.0 ± 0.5</td>
</tr>
<tr>
<td>Dissolved oxygen (mg L⁻¹)</td>
<td></td>
<td>2.2 ± 0.2</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td>Total organic carbon (mg L⁻¹)</td>
<td></td>
<td>15.3 ± 2.1</td>
<td>37.8 ± 2.4</td>
</tr>
<tr>
<td>Absorbance at 436 nm (A)</td>
<td></td>
<td>0.085</td>
<td>0.220</td>
</tr>
<tr>
<td>Molar absorptivity (L cm⁻¹ mol⁻¹)</td>
<td></td>
<td>66.7</td>
<td>71.4</td>
</tr>
<tr>
<td>Fe (µg L⁻¹)</td>
<td></td>
<td>909.0 ± 0.4</td>
<td>1455.0 ± 5.82</td>
</tr>
<tr>
<td>Mn (µg L⁻¹)</td>
<td></td>
<td>250.1 ± 0.6</td>
<td>50.6 ± 0.3</td>
</tr>
<tr>
<td>Cu (µg L⁻¹)</td>
<td></td>
<td>0.83 ± 0.1</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>Pb (µg L⁻¹)</td>
<td></td>
<td>7.3 ± 0.5</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Ni (µg L⁻¹)</td>
<td></td>
<td>30.8 ± 0.7</td>
<td>6.8 ± 0.6</td>
</tr>
</tbody>
</table>

LOD (µg L⁻¹): 2.17 (Cu); 4.97 (Pb); 2.67 (Ni); 2.12 (Fe); 3.89 (Mn). LOQ (µg L⁻¹): 7.24 (Cu); 16.67 (Pb); 9.65 (Ni); 8.78 (Fe); 11.39 (Mn).

Flash EA 1112 elemental analyzer. ¹³C nuclear magnetic resonance (NMR) experiments, with crossed polarization (CP) and variable amplitude (VA) magic angle spinning (MAS), were performed using a Varian Unity Inova 400 spectrometer. The AHS samples were conditioned in a 5 mm diameter cylindrical zirconium rotor (Doty Supersonic), rotating at 6 kHz. The ¹³C VACP/MAS NMR spectra were acquired under the following experimental conditions: ¹³C resonance frequency of 100.05 MHz; crossed polarization spectral band of 20 kHz; 3.8 µs proton preparation pulse; 1 ms contact time; 12.8 ms acquisition time; 500 ms relaxation time. The chemical shift (δ) values were referenced to hexamethyl benzene (HMB), which possesses a well defined line at 17.2 ppm.

Interaction of aquatic humic substances and endocrine disruptors

The analytical procedure proposed by Burba et al.¹⁷ based on ultrafiltration, was used for studies of the interactions between the AHS and the ED (Figure 1). This technique employed a tangential ultrafiltration system (Sartorius Ultrasart X), fitted with a 1 kDa membrane (Gelman Pall-Filtron OMEGA) to separate AHS-ED complexes larger than 1 kDa from the free ED.

Determination of the time to equilibrium of complexation between AHS and ED

Kinetic studies of HS complexation provide important information concerning the minimum time necessary for a given species to form a complex with AHS. Volume of 8.0 mL of a solution of 5.0 mg L⁻¹ 17α-ethynylestradiol and bisphenol A were added to 192.0 mL of a solution of 20 mg L⁻¹ AHS, and 10 aliquots of 1.0 mL were collected after between 5 and 210 min. The ED were determined by gas chromatography-mass spectrometry, and the equilibrium time was obtained by plotting the concentration of the free species as a function of time.

Determination of the complexation capacity of AHS for ED

Titrations were performed using 20 mL volumes of 20 mg L⁻¹ AHS solution, adjusted to pH 5.0 using 0.1 mol L⁻¹ NaOH solution. The system was allowed to pump for about 5 min prior to addition of the ED solution, to condition the membrane. The first aliquot (ca. 2 mL) was then filtered, corresponding to time zero (before addition of the ED solution). Aliquots of the ED solution were then added to the AHS solution to achieve final ED concentrations of 10, 20, 30, 50, 70, 90, 110, 130 and 140 µg L⁻¹. The mixture was kept under agitation for 30 min between each addition, to assist attainment of exchange equilibrium. Aliquots (ca. 2 mL) were collected, and the ED concentrations determined by GC-MS. The complexation capacity of the AHS was obtained by plotting the concentration of ED added against that of free ED (separated using the ultrafiltration system).

Competition between endocrine disruptors for the AHS

Using quantities based on the complexation capacities of the AHS, 2 mL of each 20 mg L⁻¹ ED standard solution were added to 196 mL of a 20 mg L⁻¹ AHS solution, and the pH adjusted to pH 5.0 using 0.1 mol L⁻¹ NaOH solution. The system was allowed to pump for around 5 min, prior to addition of the ED solutions, in order to condition the membrane. The sample was then ultrafiltered, and ca. of 1.0 mL aliquots of the free ED solution were removed after 0, 10, 30 min, 1, 2, 3, 4, 5, 6, 7, 8, 24, 48 and 72 h.
Chromatography analysis

Optimization of the solid phase extraction (SPE) of analytes using Nexus cartridges (Varian) was performed according to the manufacturer’s recommended procedure for endocrine disruptors. Firstly, a 10 mL portion of each sample was passed through a 3 mL Nexus cartridge filled with 60 mg of the sorbent. After drying under vacuum, the cartridges were eluted with 5 mL of a methanol/ethyl acetate (10:90, v/v) solution. The extract was then derivatized, after evaporation of the eluent solution.

Derivatization of standard solutions and samples was performed in test tubes, following the procedure proposed by Jeannot et al. A 500 μL portion of the standard solution or sample was evaporated to dryness at 30 °C, after which 50 μL of N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) was added. The test tube was closed, and placed in a water bath at 60 °C for 30 min. 500 μL of Isooctane was added, and the tube placed in an ultrasonic bath for 10 min. After derivatization, the sample and standard solutions were made up to 1.0 mL in volumetric flasks, and were then ready for injection into the GC-MS.

A Varian (Les Ulis, France) GC-MS system was employed, consisting of a Model 450-GC gas chromatograph equipped with a Model 1079 split-splitless temperature-programmed injector and a 200-MS ion-trap mass spectrometer. Varian Saturn 2000 software was used for data processing. The injector contained a treated Siltek 2.0 mm i.d. liner filled with glass wool. Large volume (40 μL) injections of derivatized extracts or derivatized standard solutions were used. The injector temperature was initially set at 90 °C, where it was maintained for 1.5 min, then increased at a rate of 200 °C min^{-1} to 300 °C, where it was maintained for 12.4 min. The split-splitless valve operated in split mode from 0 to 1.5 min, in splitless mode from 1.5 to 3.0 min, then back in split mode from 3.0 to 15.0 min. Helium was used as the carrier gas, at a constant flow rate of 1.2 mL min^{-1}. Data acquisition was performed in full scan mode, from 50 to 650 m/z at 1 s scan^{-1}. The transfer line temperature was set at 280 °C. For the chromatographic conditions used, retention times and quantification ions were 7.97 min and m/z 357 for bisphenol A, and 12.73 min and m/z 368 and 285 for 17α-ethynylestradiol.

Results and Discussion

The AHS were characterized using atomic ratios (C/H and C/O) calculated from the elemental measurements, and functional groups obtained from the 13C NMR analyses (Table 2). Comparing the C/H atomic ratios, the Ribeira de Iguape AHS samples appeared to be more aromatic in nature than the Itapanhaú samples. The C/O atomic ratio is related to the carbohydrate content, with elevated values indicating a greater degree of humification, due to the loss of carbohydrate –COOH and –OH groups (both aromatic and aliphatic). Here, the C/O atomic ratios reflected very similar distributions of oxygenated groups in the structures of the AHS, which were also similar to those previously reported in the literature.

Observations of the types of carbon present in the samples (using 13C NMR spectra) showed the existence of structural differences between the AHS extracted from the two watersheds, which were also different to those reported in the literature. Such differences are related to the process of decomposition of the original

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IT</th>
<th>RDI</th>
<th>Sample</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Itapanhaú18</td>
<td>Rio Negro18</td>
<td>Rio Negro18</td>
<td></td>
</tr>
<tr>
<td>C/H</td>
<td>0.84</td>
<td>0.95</td>
<td>3.7</td>
<td>3.6</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>C/O</td>
<td>1.1</td>
<td>1.1</td>
<td>1.2</td>
<td>1.2</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Aliphatic (%)</td>
<td>34.2</td>
<td>31.9</td>
<td>25.0</td>
<td>23.1</td>
<td>32.0</td>
<td></td>
</tr>
<tr>
<td>Ether (%)</td>
<td>20.0</td>
<td>16.6</td>
<td>15.2</td>
<td>12.3</td>
<td>22.0</td>
<td></td>
</tr>
<tr>
<td>Aromatic (%)</td>
<td>23.1</td>
<td>22.8</td>
<td>33.6</td>
<td>38.0</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>Phenol (%)</td>
<td>3.2</td>
<td>5.3</td>
<td>4.8</td>
<td>6.2</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>Carboxyls, ethers and amides (%)</td>
<td>13.7</td>
<td>13.4</td>
<td>18.5</td>
<td>15.4</td>
<td>14.0</td>
<td></td>
</tr>
<tr>
<td>Aldehydes and ketones (%)</td>
<td>5.8</td>
<td>9.9</td>
<td>2.9</td>
<td>5.0</td>
<td>11.0</td>
<td></td>
</tr>
</tbody>
</table>
organic matter, with transformations being dependent on the type of substrate, the presence and characteristics of microorganisms, and other environmental factors such as solar radiation intensity, temperature, oxygen availability and pH.\textsuperscript{23,24} Sun et al.\textsuperscript{25} suggested that the abundance of aromatic rings in the structure of organic matter is an important factor influencing the sorption of endocrine disruptors. This emphasizes the importance of the characterization of the organic matter present in humic substances.

Figure 2 (A and B) shows typical curves obtained during determination of the complexation equilibrium time, for 17α-ethynylestradiol with AHS from the Ribeira de Iguape River (A), and for bisphenol A with AHS from the Itapanhaú River (B). According to Oliveira et al.,\textsuperscript{23} complexes with organic matter tend to stabilize as a function of time, due to inter- and/or intramolecular rearrangements and transfer of complexing species to complexation sites deeper within the molecules. Complexation equilibrium between the ED and AHS extracted from the different rivers was achieved after about 20 min (for both ED). These results are comparable to those obtained previously in studies of the complexation of HS, extracted from different matrices, with potentially toxic inorganic species, where the kinetics of complexation was around 30 min.\textsuperscript{15,26} During titration with endocrine disruptors, saturation of the strongest AHS binding sites occurs first, followed by saturation of the weaker sites. Figure 3 shows a typical titration of AHS with a standard solution of bisphenol A, using the tangential flow ultrafiltration procedure. The complexation capacity (CC) was determined by plotting the free ED concentration as a function of the total ED concentration. The curve shows a slope change, and the complexation capacity was obtained from the intersection of the two linear sections.\textsuperscript{26,27} No comparative results have been reported in the literature concerning the complexation capacities of AHS for endocrine disruptors. Table 3 presents the complexation capacities of AHS for some organic and inorganic substances, including the results of the present study.

The AHS from both sources showed greater affinity for 17α-ethynylestradiol than for bisphenol A. This can be explained by the behavior of the ED molecules at the AHS complexation sites, which is largely governed by the quantity of –OH groups present, with formation of hydrogen bond type links. Pacheco et al.\textsuperscript{29} suggested that HS form micelles having specific sizes, from which it can be inferred that even though 17α-ethynylestradiol has a higher molecular mass than bisphenol A, greater Van der Waals type interaction occurs within the HS micelles, due to the larger number of hydrogen atoms present in the molecule. Another important characteristic of HS is
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related to the presence of homologous groups that can form a coating around the AHS complexation sites, which can directly influence the stability of the complexes formed.\textsuperscript{31} Yamamoto \textit{et al.}\textsuperscript{32} determined the coefficients of sorption of several endocrine disruptors by dissolved organic matter, with the results showing a high affinity of the latter for estrogens (such as 17\textsubscript{a}-ethynylestradiol), and a mechanism involving interaction between d-electrons and hydrogen bonds.

The ED showed a lower affinity for AHS than that exhibited by metal species (Table 3), which could be related to the size and steric hindrance of the compounds. However, they showed affinities comparable to those of organic compounds such as 4-nitrocathecol and 1-naphthol.\textsuperscript{29} Sun \textit{et al.}\textsuperscript{25} investigated the coefficients of sorption of three endocrine disruptors by humic substances, and found that sorption was highly dependent on the physico-chemical properties of the humic substances. These findings provide support for the different complexation capacities obtained for the materials used in the present work.

Competition studies are used to evaluate the affinity, for a substrate, of any given compound in relation to others. Figures 5A and 5B illustrate the results obtained for the competition between the ED and the AHS extracted from the Ribeira de Iguape and Itapanhã rivers, respectively. For both samples there was release of bisphenol A and complexation of 17\textalpha-ethynylestradiol, in agreement with the complexation capacity data. The results demonstrate that 17\textalpha-ethynylestradiol has a greater affinity than bisphenol A for the AHS. Physico-chemical data suggest that the humic molecule possesses a flexible structure as a result of intra/intermolecular interactions and hydrogen bonding. According to Swift,\textsuperscript{33} this flexible structure results in molecules that approximate spheres (micelles), with a Gaussian molar mass distribution and a high mass density in the center, which decreases towards the extremities of the sphere. Furthermore, Hayes \textit{et al.},\textsuperscript{34} and Ghosh and Shnitzer\textsuperscript{35} state that the dimensions of the sphere depend on factors such as charge density, solvation, ionization of acid groups, branching and cross-linking. Hence, for both of the ED studied, it can be inferred that their main interaction with the AHS was due to hydrogen bonding of the oxygenated groups and –OH groupings present in the molecules. 17\textalpha-ethynylestradiol occupied complexation sites in the humic substance micelles that were previously occupied by bisphenol A, due to the presence of a greater number of hydrogen atoms available for Van der Waals type interactions. The results also show that greater ED complexation occur using AHS extracted from the Ribeira de Iguape River, which is in agreement with the complexation capacity data.

**Conclusions**

Studies of complexation between AHS and the endocrine disruptors 17\textalpha-ethynylestradiol and bisphenol A showed that complexation stability was reached after around 20 min, and that the complexation capacity for 17\textalpha-ethynylestradiol (16.15-18.53 mg g\textsuperscript{-1} TOC) was greater than that for bisphenol A (1.83-2.07 mg g\textsuperscript{-1} TOC). The highest complexation capacities were obtained using AHS extracts derived from the Ribeira de Iguape River, which were more aromatic and humified than those from the Itapanhã River.

**Table 3.** Complexation capacities (CC) of aquatic humic substances for endocrine disruptors and other organic and inorganic substances

<table>
<thead>
<tr>
<th>Species</th>
<th>CC (mg g\textsuperscript{-1} TOC)</th>
<th>AHS sample</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>79.40</td>
<td>Water from the Itapitangui Stream, São Paulo State</td>
<td>18</td>
</tr>
<tr>
<td>Cu</td>
<td>74.90</td>
<td>Water from the Iguape River, São Paulo State</td>
<td>18</td>
</tr>
<tr>
<td>Cu</td>
<td>91.51</td>
<td>Water from the Rio Negro, Amazon Basin</td>
<td>18</td>
</tr>
<tr>
<td>Al</td>
<td>55.90 - 67.45</td>
<td>New Zealand rivers</td>
<td>28</td>
</tr>
<tr>
<td>4-Nitrocathecol</td>
<td>20.30</td>
<td>Peat</td>
<td>29</td>
</tr>
<tr>
<td>1-Naphthol</td>
<td>28.40</td>
<td>HA</td>
<td>30</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>2.07</td>
<td>Ribeira de Iguape River</td>
<td>This work</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>1.83</td>
<td>Itapanhã River</td>
<td>This work</td>
</tr>
<tr>
<td>17\textalpha-Ethynylestradiol</td>
<td>18.53</td>
<td>Ribeira de Iguape River</td>
<td>This work</td>
</tr>
<tr>
<td>17\textalpha-Ethynylestradiol</td>
<td>16.15</td>
<td>Itapanhã River</td>
<td>This work</td>
</tr>
</tbody>
</table>

LOD (µg L\textsuperscript{-1}): 8.37 (bisphenol A); 1.23 (17\textalpha-ethynylestradiol). LOQ (µg L\textsuperscript{-1}): 27.45 (bisphenol A); 4.58 (17\textalpha-ethynylestradiol).
in the 17α-ethynylestradiol molecule available for Van der Waals type interactions, enabling the molecule to occupy sites previously complexed by bisphenol A.

These results demonstrate that the availability of endocrine disruptors can be directly influenced by the presence of humic substances in aquatic systems. Consequently, studies of the interactions between AHS and endocrine disruptors are vital for a better understanding of the transport and reactivity of this type of contaminant in the environment.

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