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RESPONSE OF ESTROGENIC ENDOCRINE DISRUPTORS TO PRESENCE OF COLLOIDS IN SEDIMENTS AND WATER COLUMN

ODPOWIEDŹ STEROIDOWYCH HORMONÓW PŁCIOWYCH NA OBECNOŚĆ KOLOIDÓW W OSADACH DENNYCH I TONI WODNEJ

The presence of the endocrine disrupting compounds (EDCs) in the environment has become an emerging interest worldwide, because of their possible effects on ecosystems. A number of studies have correlated its occurrence to adverse effects in wildlife and fish, however, these compounds might also interfere with the reproduction of humans, live-stock and wild-living animals. The synthetic estrogenic compounds were found to be highly persistent in the environment. For example, 17 α -ethynylestradiol (EE2), which is a widely used in hormone replacement therapy and oral contraceptive formulations, has been detected in the nanograms per liter range in discharged domestic effluents, surface and ground waters, and subsequently in drinking water. As a result of its substantial potency and estrogenic activity, 17 α -ethynylestradiol (EE2) has been chosen as the probe compound in this study. The aim of this research is to achieve a deeper insight in the partitioning of synthetic steroidal estrogenic compounds into colloidal materials. The focus of this study was to identify and evaluate the extent of partitioning of EE2 to sorbents such as clay minerals (illite and montmorillonite), iron oxide (goethite) and humic acid. The sorption of EE2 was studied under different pH values, carefully chosen to reflect its effect on aqueous chemistry as well as sorbent and sorbate charge. This investigation showed that 17 α -ethynylestradiol sorption to various suspended particles had a great dependency on pH. Highest sorption values were achieved at the isoelectric points of individual suspended material, where uncharged surfaces were most abundant. The highest sorption of 99% was achieved by montmorillonite, following by illite (63%) and goethite (52%). It was found that the interaction between the synthetic steroid estrogen (EE2) and various suspended materials was governed by binding the non-polar molecule to particle surface hydroxyl groups through hydrogen bonding. Additionally, hydrophobic interaction with siloxane surface, ascribed to van der Waals forces, contributed to the overall sorption mechanism. The outcome from this study relates to the partitioning of EE2 to the suspended material in a large spectra of environmental conditions, e.g. to uppermost sediments, to suspended materials transported by water current, effect of erosion, effect of effluent quality discharged from municipal and industrial WWTP, resuspension due to flowrate change (flooding), waterbed, and to the river morphology. The results also show a possible transport of EE2 into groundwater across geological strata.

1. Introduction

Recently, the occurrence and fate of pharmaceutically active compounds in the aquatic environment has been recognized as one of the emerging issues in environmental chemistry and as a matter of public concern. Among emerging contaminants, Endocrine Disrupting Compounds (EDCs) have become of particular concern as they induce aquatic toxicity, dangerous for humans and wild animals; due to their biological effect, at very low concentrations [1,2,3,4,5]. Potential sources of EDCs found in the environment are numerous, primarily as a consequence of their secretion by wildlife and humans, in addition to the introduction of anthropogenic pharmaceuticals from the disposal of unused or expired drugs, and accidental spills during manufacturing or distribution.

Among the EDCs there are five groups of compounds that are of priority within European Union and USA research activities: (1) Alkylphenol Polyethoxylates (APEO) and their associated metabolites [5,6,7,8,9,10,11], (2) Polychlorinated Compounds: dioxins, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) [5,7,9,11,12,13,14,15], (3) Polybrominated Diphenyl Ethers, PBDEs, [9], (4) Phthalates [5,9,11,13,16], and (5) Steroid Sex Hormones (estrogens, androgens, progestogens) [5,6,7,8,9,11,13]. Of these groups, it appears that the Steroid Hormones represent the predominant form of estrogenic activity in wastewater effluents [6].

17 α -ethynylestradiol (EE2), in particular, is a widely used pharmaceutical that constitutes the major ingredient of hormone replacement therapy and oral contraceptive formulations. This synthetic estrogen was found to be more potent than natural estrogens [17,18] and it was also found to be highly persistent in the environment [19,20]. EE2 has been detected in the nanogram per liter range in discharged domestic effluents, rivers, drinking, surface and ground waters [1,21,22,23,20]. It was evident that despite these low concentrations, EE2 may exert adverse effects, especially on biota and wildlife [18,24]. As a result of its substantial potency and estrogenic activity, 17 α -ethynylestradiol (EE2) has been chosen as the probe compound in this study.

The aim of this research is to achieve a deeper insight in the partitioning of synthetic steroidal estrogenic compounds into colloidal materials. The focus of this study was to identify and evaluate the extent of partitioning of EE2 to sorbents such as clay minerals (illite and montmorillonite), iron oxide (goethite) and humic acid. The sorption of EE2 was studied under different pH values, carefully chosen to reflect its effect on aqueous chemistry as well as sorbent and sorbate charge.

The outcome from this study relates to the partitioning of EE2 to the suspended material in a large spectra of environmental conditions, e.g. to uppermost sediments, to suspended materials transported by water current, effect of erosion, effect of effluent quality discharged from municipal and industrial WWTP, resuspension due to flowrate change (flooding), waterbed, and to the river morphology.

2. Materials and Methods

2.1. Chemicals and materials

17 α -Ethinylestradiol (Tab 1) \geq 98% purity was obtained from Sigma-Aldrich, Oakville, ON. Internal standard (D4), 17 α -Ethinylestradiol-2,4,16,16-d4, was purchased from CDN Isotopes, Point-Claire, QC.

Tab. 1. Physico-chemical properties of EE2

Chemical Formula	C ₂₀ H ₂₄ O ₂
Molecular Weight	296.39 (g/mol)
Water Solubility	4.8 (mg/L)
Log K _{ow}	4.15
Melting Point	183 (°C)
Vapor Pressure	4.5 E-11 (mmHg)
pKa	~10.2

HPLC-grade water, methanol, and acetonitrile (Fisher Scientific, Ottawa, ON), and dichloromethane (Sigma-Aldrich, Oakville, ON) were all analytical reagents of greater than 99% purity. Stock solutions of both EE2 and the internal standard were prepared in methanol, and were kept under refrigerated conditions (-16 °C) when not in use. Materials known for a high sorption effect, such as clays and hydrous-oxide minerals are used in this study. Illite, having octahedral-tetrahedral of 2:1 mineral structure, was used as a representative of the family of mica-like clay minerals. Furthermore, montmorillonite (2:1) characterized by very high specific surface area, and exchange capacity was chosen as another clay mineral. Goethite (FeOOH) was selected as a representative of the hydrous-oxide minerals. Humic acid was considered to represent organic substances as one of the sorbents in this research. Target sorbents were obtained from Sigma-Aldrich, Oakville, ON. Some of the properties of these sorbents are listed in Table 2.

Tab. 2. Physico-chemical properties of suspended materials

Suspended Particles	Surface Area ^{a,b} m ² /g	Isoelectric Point
Illite	70 - 100	7.5 ^c
Montmorillonite	600 - 800	2.5 ^d
Goethite	50	7.3 ^d , 9.0 ^c

^aBrandy and Weil, 1996 [30]; ^bMitchell, 1993 [31]; ^cEssington, 2004 [32]; ^dAppelo and Postma, 1993 [33]

2.2. Analytical techniques

Given the low concentrations (sub- $\mu\text{g/L}$ to low- $\mu\text{g/L}$) an analytical procedure that attains maximum extraction and detection efficiency of the synthetic estrogenic compound, EE2, from aqueous media was developed.

Unless otherwise indicated, all experimental samples were centrifuged at 2700 rpm for 30 minutes, and the supernatant (30 mL) was collected and extracted by solvent extraction. Before commencing the extraction step, the internal standard, D4, was added to collected supernatants, with concentrations comparable to those of EE2 that are expected to prevail in the aqueous phase. All aqueous phases were liquid-liquid extracted, three times, with 3 mL dichloromethane (DCM), by shaking vigorously for 30 seconds at each extraction step, and taken for analysis [25].

All samples were analyzed with high performance liquid chromatography (HPLC, Hewlett Packard, Series 1100) system equipped with a reversed-phase column (Luna C8 (2), 3 micron, 50 x 1 mm) from Phenomenex, Torrance, CA. The synthetic estrogen, 17 α -ethynylestradiol, was detected using a Micromass-Quattro II mass spectrometer (MS), with a Z-Spray electrospray source [25].

2.3. Experimental Approach

Experimental protocol included a series of tests having in objective to assess the effect of pH on EE2 sorption to selected water column components and define changes within the bonding system. Subsequently, a series of batch-equilibration experiments were performed to assess the effect of variation of suspension pH on the partitioning of EE2 between aqueous and solid phases. Furthermore, FTIR analysis to investigate potential changes to mineral surfaces (due to changes in pH), and UV/Vis analysis to assess the behavior of dissolved EE2 in response to changes in pH.

Samples consisting of clay minerals (illite, montmorillonite), oxides (goethite) and humic acid were weighed (3.5 g) individually in a preweighed glass centrifuge tubes (50 mL) equipped with Teflon-Silicon discs and screw-caps. Milli-Q water was added to each tube in a volume of 35 mL. Solid mass (g) to solution volume (mL) ratio was fixed at (1:10) throughout the course of this study. The pH of the suspension was adjusted to the required value by the addition of acetic acid (AcOH) or sodium hydroxide (NaOH), and the suspension was pre-equilibrated for 1 h. After equilibration, a proper dilution of the hormone solution was added to receive the final concentration in each tube of 10 $\mu\text{g/L}$ (equivalent to 100 ng/g of dry material). Tubes were capped and mixed on an end-over-end shaker at controlled temperature, 30°C, for one hour. At the end of the experiment, sample tubes were centrifuged, and the supernatant was collected, extracted and analyzed for EE2 concentration. Additionally, control samples were analyzed under the same conditions of each investigated suspended material, with the exception that no particle mass was added to the controls.

For each suspended material, sorption of EE2 was studied under three suspension pH values, carefully chosen to reflect the effect of variation of aqueous chemistry and particle-surface charges on the rate and extent of sorption of the organic estrogen, EE2, to various suspended particles. For each material, three pH values were chosen to represent; neutral conditions (~ 7), acidic range (~ 3), and basic range (~ 11), respectively. The selection of the studied pH values for individual suspended material was made with respect to the pH at their isoelectric points (Table 2), whenever possible.

Fourier Transform Infrared Spectroscopy (FTIR) was used to evaluate potential changes that might take place within suspended particles – EE2 system under variable environmental conditions. At the end of the batch tests, solutions were centrifuged and the remaining solid phases were collected, air dried, and taken for FTIR analysis. All analyses were performed using Nicolet Nexus 470 FTIR spectrometer equipped with smart miracle AMTIR crystal. The analyses were done for the range of bands between 4000 cm^{-1} and 400 cm^{-1} .

UV/VIS analysis was intended to assess the behavior of EE2 in the liquid phase under variable environmental conditions. Due to the extremely low concentration of EE2, alternatively, elevated concentrations of dissolved EE2 were particularly prepared for this stage of research. Solutions of EE2 were prepared in distilled water to give a final concentration of 4 ppm. The pH of these solutions were adjusted to the required values (3, 7, and 11) by the addition of acetic acid (AcOH) or sodium hydroxide (NaOH) and taken for analysis.

3. Results and Discussion

The effect of pH on the behavior of the partitioning of EE2 onto clay minerals, iron oxide and humic acid was investigated while acidic, neutral, and alkaline solution environments were acclimated and tested in a batch equilibration experiment. Results showed that while illite (Figure 1) displayed the highest sorption capacity (ca. 63%) around neutral pH (pH \sim 7.0), and decreased sorption as pH diverged from the neutral condition in either direction (i.e. pH \sim 4 or pH \sim 11). Montmorillonite exhibited maximum sorption in the acid range (ca. 99%), and decreased steadily as solution pH departed away towards more basic state (Figure 2). It can be seen from Figure 3 that the uptake of EE2 onto goethite was the greatest around neutral solution pH (ca. 52%), but declined as solution pH was changed either above or below pH 7.0.

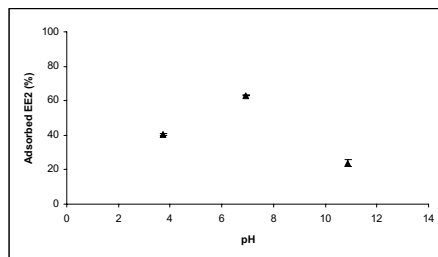


Fig. 1. pH effect on EE2 sorption to illite

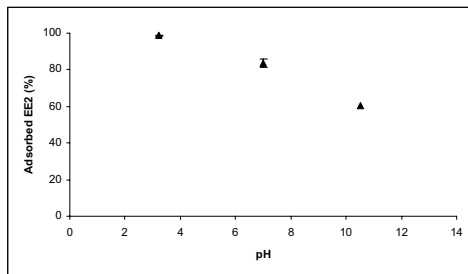


Fig. 2. pH effect on EE2 sorption to montmorillonite

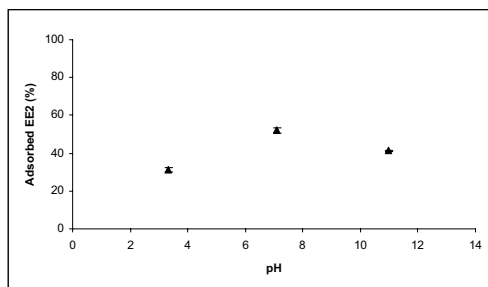


Fig. 3. pH effect on EE2 sorption to goethite

Uptake of EE2 by humic acid slightly changed between basic and neutral solution. Nevertheless, a large increase in sorption was observed as solution pH was dropped below 7.0 (Figure 4).

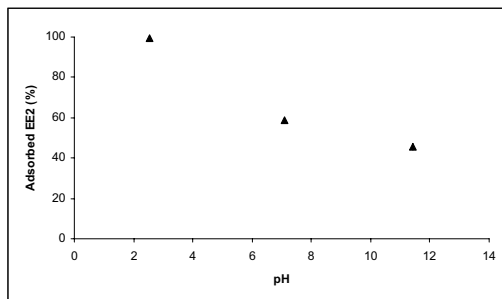


Fig. 4. pH effect on EE2 sorption to humic acid

FTIR analysis demonstrates that most of the detected OH groups were abundant within the mineral structure (dimers). It also shows that altering pH values had a pronounced effect on changes in the mineral arrangement as the ratio of absorbance of bands at 3600 cm⁻¹ (OH of dimers) and at 3400 cm⁻¹ (bulk OH) varied in response to changes in pH values (Table 3).

The FTIR spectrum (Fig. 5) of the clay mineral montmorillonite shows that montmorillonite has a molecular structure similar to that of illite. However, the major differ-

ence between the molecular structures of montmorillonite in comparison to that of illite is the ratio between OH groups of dimers and the bulk water OH's (Fig. 6).

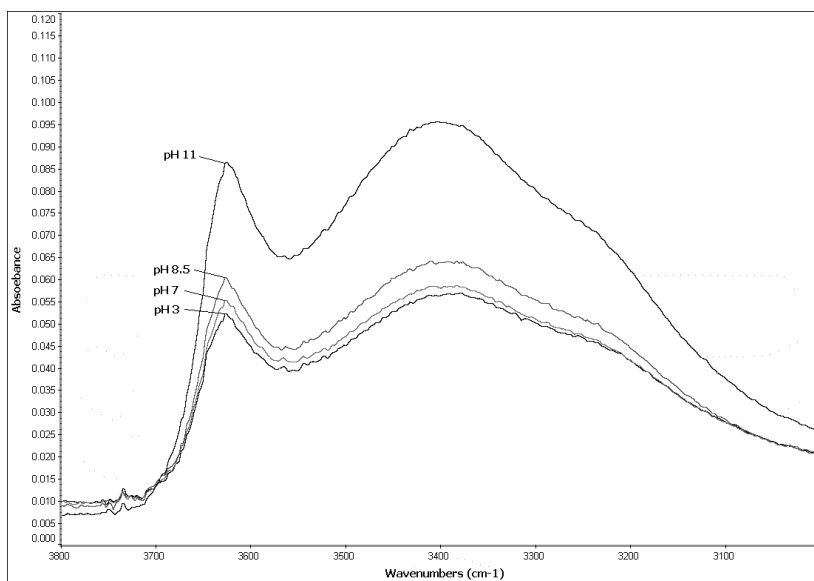


Fig. 5. FTIR spectra of montmorillonite under different pH values (3800 to 3000 cm^{-1})

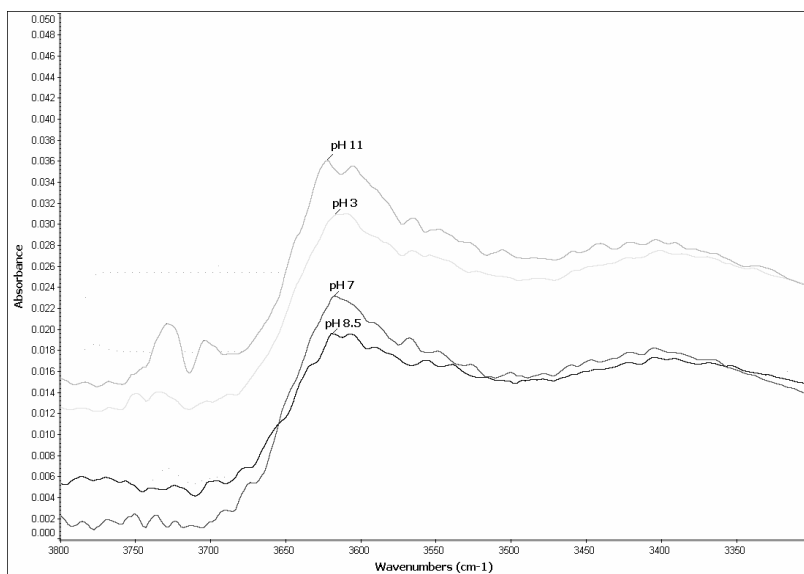


Fig. 6. FTIR spectra of illite under different pH values (3800 to 3000 cm^{-1})

Table 3 shows that the calculated ratios were lower in the case of montmorillonite relative to the ratios associated with illite.

Tab. 3. Ratio of absorbance of bands at 3600 cm⁻¹ (OH of dimers) and at 3400 cm⁻¹ (bulk OH) varied in response to changes in pH values (FTIR analysis)

Suspended Material	pH 3	pH 7	pH 11
Illite	4.3	4.6	5.2
Montmorillonite	2.3	2.2	1.95

This difference coincides with the fact the montmorillonites are typical swelling clay minerals that provide a greater access for bulk water to penetrate within their mineral sheets. Here again, the calculated ratios show that the molecular structure of montmorillonite and illite was influenced in response to changes in pH values.

Results showed that the iron oxide (goethite) prepared under different pH values (3, 7, 9, and 11) was found to be the mineral most influenced by changes in pH values. UV/Vis measurements of EE2 changes

UV/Vis analysis was performed to assess the behavior of dissolved EE2 in response to changes in pH. The UV spectra of EE2 at pH 7 showed three distinctive bands that were representative of the phenolic OH group in the benzene ring of the hormone's structure. Results also indicated that under aggressive acidic conditions (pH 3), the OH group had undergone some changes; as was observed by the hyperchromic effect. On the other hand, the spectra of EE2 at pH 11 exhibited a bathochromic effect (shift of a spectral band to longer wavelengths). This bathochromic shift is assumed to be the result of the formation of phenolate complex with sodium (introduced by sodium hydroxide, NaOH to adjust the pH).

In the current study it is evident that EE2 sorption to clay minerals has a great dependency on pH, as can be seen in Figure 2 and Figure 3. This observation could be associated with the resulting surface charge on clay surfaces. Suspended particles possess electrical charges originating from different sources. The sign and magnitude of these charges have a great influence on particles behavior. The flat basal surface of mineral particles carries a permanent negative charge due to isomorphic substitutions in the crystal lattice of clay minerals. However, due to imperfections and distortions of the clay minerals, their edge surfaces are characterized by the resultant variable charges [32,33]. At these edges, the broken bonds generate the amphoteric Al-OH and Si-OH surface function groups. The arising charge on these surface hydroxyls results from protonation and deprotonation processes depending on the pH of the solution.

This assumption coincided with the results of the FTIR analysis, which showed that the occurrence and distribution of OH groups of clay minerals was significantly subject to pH values. Consequently, the amount of variable charge is directly related to the pH of the particle solution. Maximum sorption onto illite occurred around mid pH (ca. 7) while sorption onto montmorillonite was maximal at acid range pH (ca. 3). These pH values corresponded with isoelectric points of illite and montmorillonite of ca 7.5 and 2.5, respectively. Accordingly, non-polar EE2 primarily interacted with uncharged surface hydroxyl groups. The increased charge on clay surfaces at pH different from their point of zero charge resulted in decreased rates of adsorption. The effect of pH on the structure of EE2 was further examined by the UV analysis. The results endorsed that at very alkaline conditions, the chemical structure of EE2 was altered to form phenolate complexes, which agrees to the dissociation of the phenolic group. The resulting electrostatic repulsion would account for the more pronounced drop in sorption at higher pH. Consequently, it is believed that sorption mechanism of EE2 onto clay minerals was

probably governed by a combination of hydrogen bonding with uncharged mineral surfaces and hydrophobic interaction with siloxane surface, attributed to nonspecific, short-range van der Waals interactions.

Iron oxides are amphoteric surfaces characterized by their variable charges. The hydroxyl groups at the goethite surface will protonate at low pH providing a positive charge on the mineral surface, and will release protons at high pH rendering the surface negatively charged. Consequently, the increasing polarity of the goethite surface is responsible for the lower binding affinities of the non-polar molecule at both acidic and basic environments, as seen in Figure 4. This behavior indicates that EE2 mainly interacted with uncharged hydroxyl surfaces, as they are most abundant at the isoelectric point (ca. pH 7.3 - 8), plausibly by hydrogen bonding. Additionally, the reduced sorption capacities of goethite at extreme pH conditions could be attributed to the transformation of goethite into different salts, as was shown in the results of the FTIR analysis. For example, under acidic condition (pH ~ 3) adjusted by the addition of acetic acid, the chemical structure of goethite was altered to form acetate of iron, demolishing the hydroxyl sites available for sorption.

To understand the mechanism of interaction between EE2 and humic acid, it is important to examine the structure of humic material and its behavior under different pH states. The major constituents of humic materials structure are weakly condensed core of aromatic rings surrounded by a disordered network of aliphatic or alicyclic components. Humic materials incorporate a pool of functional groups such as carboxylates and phenolic hydroxyls [28,29].

Humic materials are highly sensitive to pH. At high pH, progressive deprotonation will take place, increasing the polarity of humic material. This appreciable deprotonation will alter the structure and decrease the ability of humic material to bind organic compounds. Furthermore, raising pH increases the solubility of humic materials, i.e. it facilitates dissolving more humic material. The increased solubility of humic material will, in turn, increase the concentration of EE2 in the extracted liquid phase indicating lesser sorption capacities of solid humic acid. On the other hand, at low pH, humic material will sorb hydrogen ions, lowering the negative charge on humic molecules and making it less hydrophilic. As a result, humic molecules will coagulate, increasing the size of the humic polymer and causing it to precipitate out of the solution. This charge decrease will also lead to increase the binding abilities of humic material to hydrophobic organic estrogens (Fig. 4).

In summary, it was found that the interaction between the synthetic steroid estrogen (EE2) and various suspended particles was governed by the combination of binding the non-polar molecule to uncharged particle surface hydroxyl groups through hydrogen bonding, in conjunction with hydrophobic interaction with siloxane surface, ascribed to van der Waals interactions. As such, altering the population of terminal hydroxyl groups accompanied with varying the electrical charge on the surfaces of suspended particles resulted, generally, in a lesser amount of EE2 being adsorbed to these charged surfaces. Thus, changing pH values from the corresponding isoelectric point of individual suspended material would affect the chemical structure and the electrical charges of particle surfaces and hence influence the extent of their interaction with EE2.

4. Conclusions

- It was evident that the sorption of the synthetic steroid estrogen (EE2) to various suspended particles had a great dependency on pH. Highest sorption values were achieved at the isoelectric points of individual suspended material; where uncharged particles surfaces are most abundant

- It was found that the interaction between EE2 and various soil constituents was governed by: binding the non-polar molecule to uncharged soil surface hydroxyl groups through hydrogen bonding, additionally, hydrophobic interaction with siloxane surface, ascribed to van der Waals forces, contributed to the overall sorption mechanism.

- It was, consequently, conceivable that shifting environmental conditions towards more pronounced acidic or alkaline conditions will have a great influence on the release and mobility of the estrogenic hormone within the water column.

- It was speculated that the origin of suspended materials and sediments plays a crucial role in the fate of EE2 in water bodies.

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