Determination and Removal of Endocrine Disruptors in Wastewater by Activated Carbon

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Abstract: This study aimed to evaluate the EDC (endocrine disruptors compounds) in the city of São Paulo's water sources, from samples collected at predetermined sampling points and to evaluate the adsorptive capacity of these compounds in different types of activated carbon. The effects of these EDC on humans are not well established due to the necessary large exposure time for the effect's manifestation. After tests using powdered and granular activated carbon, all samples were filtered under vacuum using cellulose acetate membrane (0.45 μm) to remove eventual impurities, and posteriorly carried out the solid-phase extraction SPE (solid-phase extraction) and chromatographic analysis. The results lead to the conclusion that both powdered activated carbon have removal effectiveness of these compounds by adsorption. Furthermore, great amount of endocrine disruptors were found at several sampling points in river and city's water reservoirs, which shows different levels of pollution of water sources, some of them responsible for the watersupply of the city of São Paulo, Brazil.

Key words: Activated carbon, endocrine disruptors, adsorption, solid-phase extraction, chromatographic analysis, micropollutants.

1. Introduction

Increasing cities' urbanization due to increased population, has generated several environmental impacts worldwide, and these changes have happened quickly and uncontrollably [1], highlighting the disposal of municipal wastewater, which collects much of the by-products regarding this intense urbanization.

In this regard, measures to control the pollution have, as one of the main objectives, to protect water bodies from compounds and materials dumps that can cause eutrophication processes, oxygen depletion, toxicity, among other negative impacts, which consequently reduce biological diversity and also turn it dangerous for drinkable water and other human consumption purposes. Among these compounds, whose most part has final destination directly to the environment, are highlighted PPCPs (pharmaceutical and personal care products) [2, 3]. With the increasing in production and consumption of more and new chemicals compounds,

which in many cases have yet unknown properties, but harmful to the health and the environment, or even recognized with deleterious properties, there is a worsening of the problem because of the inefficiency in collection and treatment of municipal sewage and industrial effluents in developing countries, which are often thrown inadequately into the environment. These facts enable emerging concentration of contaminants in the environment [4].

The emerging contaminants class, such as PPCPs, covers various groups of anthropogenic and natural substances, among which are included drugs, toilets, industrial byproducts and hormones that, even if in the environment at low concentrations ($\mu\text{g/L}$ to pg/L), are capable of causing organisms' harmful effects. Some of these emerging contaminants, such as estrogens, phytoestrogens, alkylphenols, brominated flame retardants, are classified also as endocrine disruptors. These compounds are harmful to ecosystems and, despite its occurrence in different environmental compartments, do not have specific regulation in Brazil and many countries and, therefore, are not properly

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monitored [4, 5].

The endocrine disruptors are substances that have the ability to interact and cause changes in endocrine system's functions, simulating the activity of endogenous hormones that may cause damage to the body and their descendants. These substances are divided into three groups: the natural estrogens, synthetic estrogens and xenoestrogens [4].

Synthetic estrogens are steroids with a modified molecular structure, found in drugs, especially those assigned to hormone therapies replacement, treatment of neoplasms and contraceptives. They have high potential to interfere and cause damage to the human endocrine system, and the 17 α -ethinylestradiol (EE 2), derived from the 17 β -estradiol (E2), is one of its greatest representatives found in oral contraceptives. In the case of natural estrogens, these substances constitute a portion of the hormones produced by the organism, mainly the 17 β -estradiol (E2), estrone (E1) and estriol (E3). These hormones are related to feminine characteristics and other important processes, such as growth, development and behavior, immune and cardiovascular systems, with influence to brain development. Xenoestrogens, for example, BisphenolA monomer, they are generally less harmful and more widely found in the environment, but also possess the ability to mimic and block the endogenous estrogens activity [4]. Natural and synthetic estrogens act in ng/L order of magnitude and alkylphenol compounds (nonylphenol) demonstrate estrogenic activity in mgL⁻¹ concentrations [6, 7].

Although the effects of these substances in humans are not well known due to the long time required for the manifestation of the effects, the impacts of environmental estrogens in aquatic life are already known [8]. Studies regarding environmental estrogens involving animals confirm that these compounds are manifested differently, when compared in terms of toxicology to the term "dose-response" traditional, so, these studies produce significant responses at extremely low concentrations [9]. Several studies have

studied the major toxic effects of pharmaceutical compounds and natural hormones [10], although these emerging compounds presence is the subject of studies in STPs (sewage treatment plants) in Europe and United States, little has been studied under Brazilian conditions.

2. Treatment Technology: Activated Carbon Adsorption

Several solid materials have been applied to remove endocrine disruptors in water, and among those, the processes and techniques currently in using activated carbon, are promising due to their high efficiency in removing various contaminants [11, 12]. PAC (powdered activated carbon) is more widely used in relation to GAC (granular activated carbon); it is more common to apply PAC in conventional treatment stations, because it is easier to control the dosage and needs a lower investment cost. However, it cannot be regenerated after its use and is difficult to remove from water after treatment. Also, the GAC use is more restricted to situations in which the water is very polluted. Nevertheless, this type of treatment has the advantage of GAC's regeneration, in addition to providing the development of biological activity enabling the organic compounds removal which has greater biodegradability than adsorption [8].

Activated carbon is a microporous adsorbent with a high surface area and the presence of various functional groups on this surface, due to the thermal treatment made under high temperature. It can be derived from various carbonaceous materials (vegetable, mineral and animal) and is widely used in the treatment of industrial effluents for adsorption of organic compounds. The compounds adsorption by activated carbon occurs mainly by physical or chemical processes (chemisorption). In physical adsorption, there is a predominance of Van der Waals Forces between the adsorbent (activated carbon) and the adsorbate (organic micropollutant). In relation to chemisorption, the adsorbent and the adsorbate create a

chemical bond due to the electrostatic forces' predominance (polarization interactions), resulting in molecular structure change [8].

To characterize the affinity sorption between endocrine disruptors and organic material (activated carbon), it is used the octanol-water partition coefficient (K_{OW}). Two sorption types can occur: hydrophobic interactions related to absorption, which is given by $\log K_{ow}$ value; and adsorption, that is determined by acid dissociation constant (pKa). Substances with higher $\log K_{ow}$ and molecular weight tend to be adsorbed, while those with lower $\log K_{ow}$, due to their less potential for adsorption, tend to appear in higher concentrations in water sources [4].

3. Experimental Methods

This research aimed to evaluate: (1) the effectiveness of analytical standards removal of environmental estrogens estrone (E1), 17 β -estradiol (E2), estriol, 17 α -ethinylestradiol (EE2), and xenoestrogens BPA (bisphenol A) and NP (nonylphenol), dissolved in deionized water in fixed concentrations by adsorption on powdered activated carbon (106/90 and 108/90) and granular activated carbon (8x30 and 12x40); and (2) surface natural water samples were collected at sampling points of Billings and Guarapiranga Reservoirs and Tietê river in São Paulo, Brazil, conducted bimonthly by CETESB (Environmental Company of São Paulo State). After all analysis with activated carbons, the concentration of these compounds was also determined at selected sampling points.

Samples were stored in amber glass bottles (1 L) with polished cover, and transported from CETESB to the laboratory in a thermal container with ice. Posteriorly, the filtration of the sample was made by cellulose acetate membrane with 0.45 μ m porosity. Sulfuric acid was added to preserve them stored under

4 °C refrigeration until their use in the experiments.

4. Material and Methods

4.1 Compounds' Determination

The identification and quantification processes of estrogens estrone (E1), 17 β -estradiol (E2), estriol, 17 α -ethinylestradiol (EE2), and xenoestrogens BPA (bisphenol A) and nonylphenol (NP), were conducted in stages [13-15]. Firstly samples of 500 mL were filtered in vacuum through a cellulose acetate membrane (0.45 μ m porosity); the compounds of interest were extracted from the water samples through a solid-phase extraction using Strata X cartridges. Subsequently, the matrix interferences were removed (particularly those with high polarity), isolating the analytes which were retained in the cartridge with 5 mL of deionized water (flow rate of 3-5 mL/min), leaving it for 5 min in the vacuum.

The samples were eluted and concentrated using the organic solvent ACN (acetonitrile) (HPLC-UV (high performance liquid chromatograph with ultravioletgrade)). The elution was carried out using two volumes of 5 mL ACN in each cartridge connected to the vacuum manifold (flow rate of 3-5 mL/min), and posteriorly collected in vials of 10 mL capacity for each one. After all ACN flowed, the cartridges remained about 5 min in vacuum to ensure the passage of entire solvent. Thus, each eluted sample in the vial, with 10 mL ACN concentration, remained in contact with a nitrogen flow (gas) to evaporate until completely dry. Then the samples were reconstituted in the same vial with 0.5 mL MeOH (HPLC-UV grade) to desorb the analytes of interest and concentrate it in 0.5 mL (Fig. 1). Consecutively, 0.5 mL samples were analyzed using a HPLC-UV detection.

The whole process was adapted from Araújo [16], Lanças [14], López de Alda and Barceló [17], Raimundo [18], and Verbinnen et al. [19].

Fig. 1 Experimental equipment used in the SPE (solid-phase extraction) phase.

5. Adsorption by Activated Carbon

5.1 PAC (Powdered Activated Carbon)

Two types of PAC produced from vegetable source (Pinus), one of them 106/90 (600 mg/g iodine number) and other 108/90 (800 mg/g iodine number). Powdered activated carbon was used to treat 500 mL standard solutions of deionized water containing 1 mg/L compound of interest's solution (estrone, 17β -estradiol, estriol, 17α -ethinylestradiol, Bisphenol A and nonylphenol).

Experiments were performed in triplicate for both types of powdered activated carbon totaling 54 tests, varying the activated carbon concentration and its contact time with the standard solution. Solutions with powdered activated carbon treatment were submitted to constant agitation (120 rpm) in the jar test equipment (Fig. 2). After mixing, the samples were filtered under

vacuum using cellulose acetate membrane ($0.45\ \mu\text{m}$ porosity) to remove the PAC, and posteriorly carried out the solid phase extraction SPE and chromatographic analysis aforementioned.

5.2 GAC (Granular Activated Carbon)

Two types of GAC were used in experiments, one with smaller granulometry ($8 \times 30\ \mu\text{m}$) and other with larger granulometry ($12 \times 40\ \mu\text{m}$). Experiments were conducted in glass column $5 \times 30\ \text{cm}$ (internal diameter $\times$ height), filled with 33 g of each GAC to the percolation of 500 mL standard solution in descending continuous flow, varying the time of standard solution passage by flow control. Although the CAG can be regenerated, in this present study, it was discarded and completely replaced at the moment that its saturation started in the columns.

Fig. 2 Jar test equipment.

Table1 *p*-values for each factor.

Compounds	<i>p</i> -values time	<i>p</i> -values concentration	<i>p</i> -values time × concentration
Estriol	0	0	0
Estrone (E1)	0.434	0.458	0.008
17 α -ethinylestradiol (EE2)	0.442	0.476	0.001
17 β -estradiol (E2)	0.284	0.006	0
Bisphenol A (BPA)	0	0	0
Nonylphenol (NP)	0.313	0	0.761

After the conclusion of the assays, the samples were filtered under vacuum using cellulose acetate membrane (0.45 μ m porosity) to remove any eventual impurities from the GAC, and posteriorly also carried out the SPE and chromatographic analysis aforementioned.

6. Results and Discussion

6.1 Evaluation of Estrogens Removal Process by Powdered Activated Carbons (PACs)

ANOVA (analysis of variance with interaction) was used to evaluate the estrogens removal by powdered activated carbon (106/90 and 108/90), which enabled the comparison of quantitative factors (treatments): time (contact time between PAC and samples), concentration (PAC concentration in the sample) and time versus concentration (interaction between two factors). The factors showed statistical significance (probably true), so, they influenced the estrogens

removal by adsorption when *p*-value was lower than 0.05. Thus, the factor's significance will be greater for removing the compound of interest.

6.2 Powdered Activated Carbon 106/90 (PAC 106/90)

Tests performed by PAC 106/90, only estriol and Bisphenol A presented *p*-value less than 0.05 for time factor, i.e., the contact time between these compounds and activated carbon influenced its removal. In this case, one hour of contact showed more effective removal for both compounds. In relation to the concentration factor, the *p*-value less than 0.05 was obtained for removal of estriol, 17 β -estradiol, bisphenol A and nonylphenol, and 50 mg/L of activated carbon was the most effective concentration in the removal process (Table 1).

However, the interaction analysis between time and concentration was significant for all compounds

(except for nonylphenol) with 1 and 2 hours of variation in removal time, and 50 mg/L activated carbon as the most effective concentration. This result was expected because there is a contact surface loss with minor amounts of PAC. The mechanical agitation of samples by jar test may also have interfered in the kinetics of chemical reaction hindering the chemisorption process by collisions between the adsorbent (PAC) and adsorbate (estrogens/xenoestrogens), with a possible predominance of physical adsorption. In physical adsorption, weaker chemical bonds (Van der Waals Forces) between activated carbon and estrogen/xenoestrogens enable the occurrence of desorption, affording the return of the compounds to the sample again.

6.3 Powdered Activated Carbon 108/90 (PAC 108/90)

For removal using PAC 108/90, estriol was the only compound that presented a *p*-value less than 0.05 for the time factor, which indicated that contact time between compounds and activated carbon, in general,

was not decisive in the removal process. Nevertheless, concentration factor was significant for removal of estriol, 17 α -ethinylestradiol, 17 β -estradiol, bisphenol A and nonylphenol (*p*-value less than 0.05 or very close to 0.05), with 50 mg/L of activated carbon as the most effective concentration in the process. In relation to factor interaction between time and concentration, the greater significance was obtained from removal of estriol and bisphenol, with 4 hours and 50 mg/L of activated carbon as the most effective time and concentration, respectively (Table 2). As previously seen for PAC 106/90, concentration was the greater significant factor (50 mg/L activated carbon as more effective), due to the larger surface contact and therefore more binding sites for compounds.

6.4 Evaluation of Estrogens Removal Process by GACs (Granular Activated Carbons)

From the experiments using granular activated carbon (GAC), it was verified the efficiency of removal process (Table 3) by two different granulometry (8 $\times$ 30 and 12 $\times$ 40).

Table 2 *p*-values for each factor.

Compounds	<i>p</i> -values time	<i>p</i> -values concentration	<i>p</i> -values time $\times$ concentration
Estriol	0.013	0	0
Estrone (E1)	0.387	0.195	0.483
17 α -ethinylestradiol (EE2)	0.253	0.053	0.180
17 β -estradiol (E2)	0.318	0.053	0.439
Bisphenol A (BPA)	0.404	0.001	0.001
Nonylphenol (NP)	0.920	0.015	0.523

Table 3 Removal efficiency by GACs.

Compounds	CAG 8 $\times$ 30 (%)	CAG 12 $\times$ 40 (%)
E1	21.1	38.2
E2	17.4	41.4
E3	14.1	41.9
EE2	14.1	36.8
BPA	19.6	48.2
4n-NP	12.8	25.1

The removal efficiency percentages shown (Table 6) were lower than expected, according to studies using granular activated carbon [20], which obtained up to approximately 99% organic compound removal (2,4-D), using a concentration solution 100 times higher and lower granular activated carbon mass, compared to the concentrations used in this study. The lower removal efficiency of compounds in this research compared to Loureiro [20], may possibly be related to the filling of GAC in the columns, due to occurrence of air bubbles in some tests, or because the sample flowed quickly through the column. However, as it was expected, the GAC with large granulometry (12×40 mm) showed more effectivity in compounds removal than GAC with smaller granulometry (8×30 mm).

6.5 Determination of Estrogens/Xenoestrogens in São Paulo's Water Sources

The determination step for estrogens estrone,

17β -estradiol, estriol, 17α -ethinylestradiol and xenoestrogens bisphenol A and nonylphenol, from sampling points at Billings and Guarapiranga Reservoirs, and Tietêriver (near to Remédios and Bandeiras Bridges), in São Paulo, Brazil, showed a high level of water quality degradation as expected. The raw sewage, untreated wastewater and the variety of waste released directly in the water sources can be responsible for this degradation in São Paulo.

Figs. 3-6 show the distribution of estrogens/xenoestrogens at sampling points selected in the dams and Tietêriver.

Billings and Guarapiranga reservoirs presented higher levels for estrone and nonylphenol. In Tietêriver, at sampling point near to Bandeiras Bridge, bisphenol A and estriol showed the largest concentrations while at Remédios Bridge's sampling point higher amounts of estriol and estrone were detected.

Fig. 3 Distribution of estrogens and xenoestrogens at Billings reservoir (sampling point) during May, July, September and November, 2012.

Fig. 4 Distribution of estrogens and xenoestrogens at Guarapiranga reservoir (sampling point) during May, July, September and November, 2012.

Fig. 5 Distribution of estrogens and xenoestrogens at Bandeiras bridge (Tietêriver sampling point) during May, July, September and November, 2012.

Fig. 6 Distribution of estrogens and xenoestrogens at Remédios bridge (Tietê river sampling point) during May, July, September and November, 2012.

7. Conclusions

In the experiments with estrogens estrone, 17β -estradiol, estriol, 17α -ethinylestradiol, and xenoestrogens bisphenol A and nonylphenol, it was verified the removal effectiveness of these compounds by adsorption using powdered activated carbon 106/90 and 108/90. For both types of PAC, the concentration factor was decisive, wherein 50 mg/L carbon concentration was the most effective. However, for both types of granular activated carbon, only removal efficiencies were checked, which proved unsatisfactory, possibly due to operational problems.

As for the determination of interest compounds in water sources that supply São Paulo, they were found at selected sampling points. This situation concerns for water's degradation and pollution that currently do not receive specific treatment to remove these compounds, and it has been used for human consumption in the city.

Nevertheless, more research is necessary in order to

investigate estrogenic potential of more chemicals substances, as well as the search for viable technology for removing such compounds, with the goal to regulate and reduce these substances in the environment, besides avoiding the irregular disposal of several wastes.

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