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Endocrine disruptors in sludge wastewater treatment plants: environmental complications

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ABSTRACT. Concern over exposure to endocrine disruptors (EDs) and impacts on wildlife and human has gradually increased in importance in recent years. Wastewater systematically receives most if not all of these chemicals, so a deeper understanding of the fate of EDs in environment is sorely needed. We evaluated EDs concentration in the affluent and effluent from the Sludge Wastewater Treatment Plants (SWTPs) Penha and Ilha do Governador. It was used as evaluation parameter the determination of some target compounds, such as: alkylphenols, 17β-estradiol, bisphenol A; complementing with measures of physical and chemical parameters: temperature, pH, dissolved oxygen, total suspended solids, volatile suspended solids, and total chemical oxygen demand. Samples were collected along the line of treatment of each SWTP, and after laboratory procedures, analyzed by LC-MS/MS and ELISA techniques. In some samples, we verified concentrations of chemical compounds with potential to disrupt the endocrine system of living organisms. Therefore, the release of this effluent, even within the effluent discharge standards (BRASIL, 2005) or in condition to be purified by the water course, may lead to physiological changes in the organisms of the receptor water body.

Keywords: ELISA, LC-MS/MS, water ecophysiology, aquatic ecotoxicology, human sewage, water pollution control.

Desreguladores endócrinos em estações de tratamento de esgotos: complicações ao meio ambiente

RESUMO. A preocupação sobre a exposição aos desreguladores endócrinos (DEs) e impactos sobre a fauna e os seres humanos, gradativamente, teve aumento em importância nos últimos anos. Águas residuais sistematicamente recebem a maioria destes desreguladores, se não todos destes produtos químicos, assim maior compreensão sobre o destino de DEs no ambiente é extremamente necessária. Foi avaliada a concentração de DEs no afluente e no efluente das estações de tratamento de esgoto (ETEs) da Penha e Ilha do Governador, Estado do Rio de Janeiro. Foi utilizado como parâmetro de avaliação a concentração dos seguintes compostos: alquilfenóis, 17β-estradiol, bisfenol A; complementando com a determinação dos parâmetros físico-químicos: Temperatura, pH, Oxigênio Dissolvido, Sólidos Suspensos Totais, Sólidos Suspensos Voláteis e Demanda Química de Oxigênio total. Foram coletadas amostras simples ao longo da linha de tratamento de cada ETE estudada e após procedimentos laboratoriais, analisadas por meio das técnicas LC-MS/MS e Elisa. Em algumas amostras, observaram-se concentrações de compostos químicos aqui pesquisados com potencial para desregular o sistema endócrino de organismos vivos. Portanto, conclui-se que o lançamento deste efluente mesmo enquadrado nas normativas de despejo (BRASIL, 2005) ou apresentando condições de depuração pelo curso hídrico, pode provocar a mudança fisiológica dos organismos do ecossistema aquático receptor.

Palavras-chave: Elisa, LC-MS/MS, ecofisiologia aquática, ecotoxicologia aquática, esgoto humano, controle de poluição hídrica.

Introduction

There is a growing concern among scientific community and public about a set of chemicals in the environment, which can interact with the endocrine system and cause adverse effects. Some studies report the presence of these compounds in wastewater treatment plants and in surface and groundwaters, due to the partial removal during the treatment process, and therefore one part is discharged into receiving water bodies (BILA et al., 2007; MEYER et al., 1999). These compounds are usually called as endocrine disruptors (EDs) because they can affect the health of human as well as of other organisms (COLBORN et al., 1993). On the other hand, the exposure to these products may affect all biological systems, including the reproductive system and its development (FERREIRA et al., 2006; RODRIGUEZ-MOZAZ et al., 2004).

ED is able to associate with all substance or blend of exogenous substances and to assume the same function of a natural hormone in living organisms, or to inhibit the normal functioning, by changing the endocrine system and causing adverse effects in organisms or descendants (TAPIERO et al., 2002).

According to Alves et al. (2007), EDs can lead to the development of some diseases such as cancer of the breast, uterus and prostate, abnormal sexual development, reduced male fertility, increased incidence of polycystic ovaries, changes in the thyroid gland, disorders in the ovary, fertilization and pregnancy. In animals they can disrupt the production and development of organisms, and may irreversibly induce female sexual characteristics in male fish, causing sterility or reduced reproduction (FOX, 2001).

Several substances are classified as EDs, among them: natural substances (phytoestrogens), synthetic chemicals (alkylphenols, pesticides, phthalates, polychlorinated biphenyls and bisphenol A), natural estrogens (17β- estradiol, estrone and estriol) and synthetic estrogens (17α-ethinylestradiol) (FERREIRA et al., 2006).

Besides EDs already known, several other new chemicals are synthetized every year and discharged into the environment with unknown consequences, and many of them have potential estrogenic activity. Indeed, more than 70 chemicals are referred as potential EDs. However, it is estimated that over 80,000 chemicals produced by men are commonly used, thus justifying their presence in the effluents of WTPs, as well as their breakdown products (BORGES; BARROS JÚNIOR, 2012; KOLPIN et al., 2002; LEE et al., 2005).

Polyethoxylated alkylphenols (PEAs) are widely used in domestic and commercial applications, being used as emulsifiers in industrial and domestic cleaning products (KOLPIN et al., 2002). During the treatment of urban and industrial wastewaters, PEAs are successively degraded to less biodegradable substances, e.g., the nonylphenol (NP) and octylphenol (OP), in order to be discharged into the aquatic system (CHENG et al., 2006).

In general, WTPs receive fresh sewage and subject it to a series of physical, chemical and biological processes aiming to remove undesirable substances from contaminated water, allowing its return to the environment with more suitable sanitary characteristics (BENTO et al., 2005; FALCIONI et al., 2005; FERREIRA et al., 2008). There is lacking information available relative to the effect of high discharges and their potential toxicity, especially in aquatic systems. Moreover, little information on the removal of EDs in WTPs prevents the estimation of material balances. In this way, several bioassays and analytical techniques have been employed to identify and examine the activity of several potentially estrogenic compounds (MARA, 2003).

17β- estradiol is responsible for the formation of female characteristics and 17α-ethinylestradiol is the main synthetic estrogen found in contraceptive pills and applied in hormone replacement therapies, these substances have high estrogenic potential and are classified as the main responsible for endocrine changes in organisms present in surface waters (OLEA-SERRANO et al., 2002). These compounds are detected in effluents of WTPs since the treatment process does not completely remove them from received wastewater (MORAES et al., 2008).

In this study, we analyzed the effluent treated in the SWTPs Penha and Ilha do Governador, in order to evaluate the yield and quality of the processes, intending thus to subsidize future projects of the SWTPs, allowing the improvement of performance and monitoring, resulting in better control of the process and the reduction of potential impacts caused on the environment by EDs.

Endocrine disruptors: framework and definition

The first studies that related the effect of some chemicals in animals emerged during the 1950's and 1960's by Rachel Carson who associated the impacts of the Dichloro-Diphenyl-Trichloroethane (DDT) in the environment with those in animals (CARSON, 1962). However, public concern over pollution problems only emerged with the publication of "Our stolen future: Are we threatening our fertility, intelligence and survival?".

In recent decades several studies have been conducted to relate properties of endocrine disruption of some compounds (natural and produced by men) mainly at the level of hormonal activity (SUMPTER; JOHNSON, 2005). There is no agreement on EDs and methods to determine, measure, analyze and test them in various types of organisms.

The appropriate choice and development to identify and characterize chemicals that can disrupt the endocrine system are very complicated. For example, the receiver of such compounds does not follow the standard rules for toxicity by the endogenous levels of hormones present in the body. The EDs may operate through pathways different from the other compounds producting distinct responses even at very low concentrations. On the other hand, there is a great number of available tests validated and based on a wide array of mechanisms completely different (WAISSMANN, 2002).

By detecting the significant levels of compounds able of endocrine disruption in the environment emerged the need to define EDs. Nevertheless, all the attempts to define EDs include the concept of deregulation or disruption – any effect (adverse or not) on the endocrine system.

This is related to small biochemical changes or small changes at cell level that can lead to great damages in the long term. Actually changes in endocrine system occur systematically by natural environmental factors, and frequently small disturbances from anthropogenic compounds released in the environment do not even cause any pathology. The question is to what extent or to which concentration of EDs each individual exposed can adapt without any pathology. The consequences of the exposure can be displayed not only at individual level, but also at level of its offspring, i.e., there may be changes and effects at population level (WANG et al., 2005).

Identification, quantification and analysis: presence of ED in the environment

According to several authors there are between 50 and 70 different chemicals identified as being possible EDs (ISHIBASHI et al., 2001; MATTHIESEN; GIBBS, 1998). However, as more chemicals are tested, the number with estrogenic responses also increases, i.e., the list of compounds considered EDs is far from complete, and has not stopped increasing with the development of technology and new tests have arisen.

One of the most common ways of exposure to EDs is through the contact with contaminated water of rivers, bays, groundwaters etc. (FERREIRA et al., 2006). The EDs can reach the water by several ways: (a) punctual sources – effluents of wastewater treatment plants, effluents of industries, agricultural activities, leachates etc; and (b) diffuse sources – soil infiltration of compounds used in the agriculture and industry until reaching groundwater, recharge of aquifers with contaminated water, septic tanks, spread of sludge from wastewater treatment, etc (Figure 1).

The EDs similarly to other pollutants have a great variety of sources. These sources can have implications to the environment (due to their accumulation) and adverse effects to humans and other living organisms (BIRKETT; LESTER, 2003).

Surface waters are particularly susceptible to contamination by EDs, given the proximity to pollution sources and the low dilution factor, which originates a high degree of exposure of all organisms that come into contact. Furthermore, the treatment performed in the WTPs is not sufficient (FERREIRA et al., 2006). This because, in some cases, the degradation products of metabolism in the WTPs have a greater estrogenic potential. One example is the APEOs that degrade into NP. These compounds have been largely used in the last 50 years in a great array of domestic and commercial applications (TSUDA et al., 2000), as emulsifiers in domestic and industrial cleaning products (NICHOLS et al., 2001). During the treatment of urban and industrial wastewater, APEOs are successively degraded into less biodegradable forms, such as for example, NP and OP, which are thus released into aquatic ecosystems (MAGUIRE, 1999).

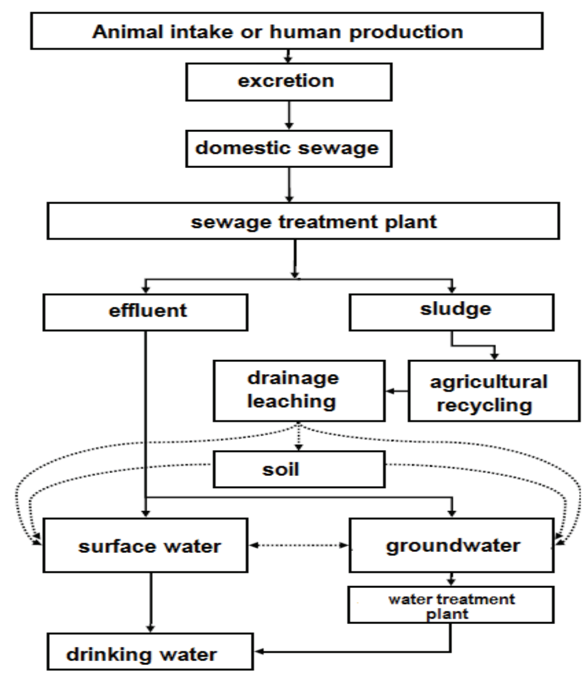

Figure 1. Potential routes of exposure to human hormones in the environment.

Moreover, alkylphenols (NP, OP) induce the production of vitellogenin in male individuals of several fish species (SCHWAIGER et al., 2000). Other estrogenic contaminants found in the water include ethinylestradiol, phytoestrogens, organochlorine compounds, among others vulnerable to disrupt the endocrine system of fish and other organisms.

The 17 β-estradiol is the main estrogen present in vertebrates, and in female fish it regulates the development and maintenance of gonads and somatic sexual characteristics and has a key role in the vitellogenesis (ASHFIELD et al., 1998). This justifies the great importance of studying of its concentration and the effects at the level of the endocrine system of fish. Another large group of EDs are the heavy metals, such as cadmium, lead and mercury, which despite not interfering with hormone activity, under high concentrations are toxic to gonadal cells of fish

(NELSON et al., 2007). Some of these substances were banned or no longer produced, but still found in the environment. In the Table 1 are listed the main substances known as EDs.

Among the EDs, natural and synthetic estrogens have called attention of some researchers, being found in the environment at concentrations in the range of μg L^{-1} and ng L^{-1}. Likewise, have been found in cosmetics and anabolics used in animal feed, and also considered EDs (ALVES et al., 2007). In this way, we can mention polychlorinated biphenyls (PCBs), bisphenol A (BPA), dioxins (PCDDs), furans (PCDFs), DDT and alkylphenol polyethoxylates compounds, such as OP and NP. BPA is an ingredient commonly found in several products for dental treatments, as also in internal coatings of food packages, which is a facilitator for human contamination (STUMPF et al., 1999). Natural estrogens, estrone and 17β-estradiol are naturally and daily excreted in the urine of women, female animals and men, and thus discharged in domestic sewage, as well as the 17α- ethinylestradiol is a synthetic estrogen used in contraceptive pills (BILA et al., 2007). Table 2 presents the daily excretion of estrogens by human.

Focusing on the study and evaluation of the estrogenic potential in the treatment steps of the SWTPs Penha and Ilha do Governador, it was determined some EDs, such as: alkylphenols (nonylphenol and octylphenol), 17 β-estradiol and bisphenol A. Samples were taken along the line of treatment of each SWTPs, which after processed were analyzed by LC-MS/MS and ELISA techniques.

Material and methods

Characterizing the STP

The SWTPs Penha and Ilha do Governador are submitted to the following operational parameters: pH, total suspended solids (TSS), fixed suspended solids (FSS), volatile suspended solids (VSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total Kjeldahl nitrogen, ammonia nitrogen, nitrate, nitrite and phosphorus.

SWTP Penha

The SWTP Penha operates with biofilters and activated sludge, treating a flow of around 1,600 L s^{-1}. The pre-treatment starts by medium and fine screening, with removal of solids, followed by removal of sands, oils, and greaset. Greases are incinerated. The primary treatment consists of an accelerated lamellar settling in four tanks, with optional physical and chemical treatment, and of three tanks of equalization/homogenization. The secondary biological treatment is performed by means of a continuous-flow activated sludge system with conventional aeration in six aerated tanks by surface aeration, followed by a secondary lamellar settling, in 12 settlers of rectangular plant, with biological sludge recirculation.

STP Ilha do Governador

The SWTP Ilha do Governador operates with activated sludge, treating a flow of around 525 L s^{-1}. The treatment process works with the steps: the elevated influent pass through a screening formed by grids – a coarse and a medium, responsible for removing coarse solids, followed by the removal of oils, greases and sands, in two grit chambers/degreasers. The resulting byproducts of this pre-treatment are later deposited in controlled landfill. The influent is then fed to a homogenization tank e with 4,000 m^3 working volume, whose function is to regularize peaks of pollutant load.

Table 1. Main substances of EDs.

Steroids	Alkylphenols	Polyaromatic compounds	Oxygenated organic compounds	Pesticides
17α- ethinylestradiol	Nonylphenol	Polychlorinated biphenyls (PCB)	Phthalates	Atrazine
17β-estradiol	Ethoxylated nonylphenol	Flame retardants	Bisphenol A	Linuron
Estrone	Octylphenol	Polyaromatic hydrocarbons (PAH		Hexachlorobenzene
Mestranol				Pentachlorophenol (PCP)
Diethylstilbestrol (DES)				

Source: Bila et al. (2007).

Table 2. Daily excretion (μg) per capita of estrogens by humans.

	17β-estradiol	Estrone	Estriol	17α-ethinylestradiol
Men	1.6	3.9	1.5	-
Women of childbearing age (10 - 49 years)	3.5	8	4.8	-
Menopausal women (above 49 years)	2.3	4	1	-
Pregnant women	259	600	6000	-
Women taking contraceptive	-	-	-	35

Source: Johnson et al. (2000).

The subsequent primary treatment consists of a preliminary step of physical and chemical treatment, with application of aluminum sulfate, hydrated lime and a polyelectrolyte on two lines of coagulation/flocculation tanks, with primary settling in two tanks of circular plant with bottom and surface scraper. The primary effluent is then fed to the secondary treatment, which is performed by means of activated sludge system, in six aeration tanks provided of two surface aerators each and sludge recirculation tank. The next step is the secondary settling in two settlers of circular plant, with bottom and surface scraper.

Sampling

Samplings were carried out along the line of treatment of the SWTPs Ilha do Governador and Penha. The sites sampled were: (1) influent, (2) post-preliminary treatment, (3) post-primary treatment and (4) final effluent.

Samples were taken in triplicate and stored in amber vials of 250 mL. During the 12h scheduled for sample collection, which occurred between 8 and 20h, it was gathered eight samples per site during the collection period. The collections in the SWTPs were performed in March 2010, and the results express the consolidated obtained from each analyzed site.

Laboratory analyses

After collection, samples were taken to the laboratory in chilled bags, filtered through a mesh of 230 μm to retain larger particles, and then through glass fiber filters Macherey-Nagel, MN GF -3, porosity of 0.60 μm. The methodology for the pre-treatment of samples and for extraction of different compounds was developed for analysis in Elisa (Indirect immunosorbent assay) by Takeda Chemical Industries Ltd. and for analysis in LC-MS/MS (Liquid chromatography-mass spectrometry) (PETROVIC et al., 2002).

In the analysis through Elisa, the extraction of BPA, 17*β*Estradiol and APE was undertaken using Oasis® HLB cartridge 3 cc 60 mg^{-1} 30 μm 100 box^{-1} (WAT094226, Waters), being eluted with 2 x 5.0 mL CH_3OH at 10% for the BPA and APE, and with 2 x 5.0 mL CH_2Cl_2 (100%) for the 17βEstradiol. Afterwards, samples were concentrated to 2 mL with liquid nitrogen. The processing scheme is shown in the Figure 2.

The Elisa assay is based on a reaction of competition between a specific monoclonal antibody and the compound under analysis. In this reaction, the surface of the wells is coated with a protein (monoclonal antibody) that binds exclusively to the ED (antigen) in analysis. The ED, under study, present in the samples analyzed and the conjugate (antigen – enzyme), (ED marked with an enzyme that changes color in reaction) were previously mixed and added to the microplate wells, taking place thus a competition for the limited number of bonds to the antibody.

Later, the excess of conjugate and the unreacted ED were removed from the wells using a wash solution (PBS/Tween). Then each well received a chromogenic substrate, in order to develop color under the presence of the enzyme conjugate. The amount of this bound to the antibody determined the color intensity, which in turn was measured by absorbance using an Elisa reader (BenchMark, Biorad).

The quantitative analysis was done by selected ion monitoring (SIM), using an external calibration. Calibration curves were built by a linear regression. The confirmation of the identity of the compounds in the effluent was performed by means of scan mode, by comparing the retention time and the mass spectrum of the compound analyzed with the respective standard.

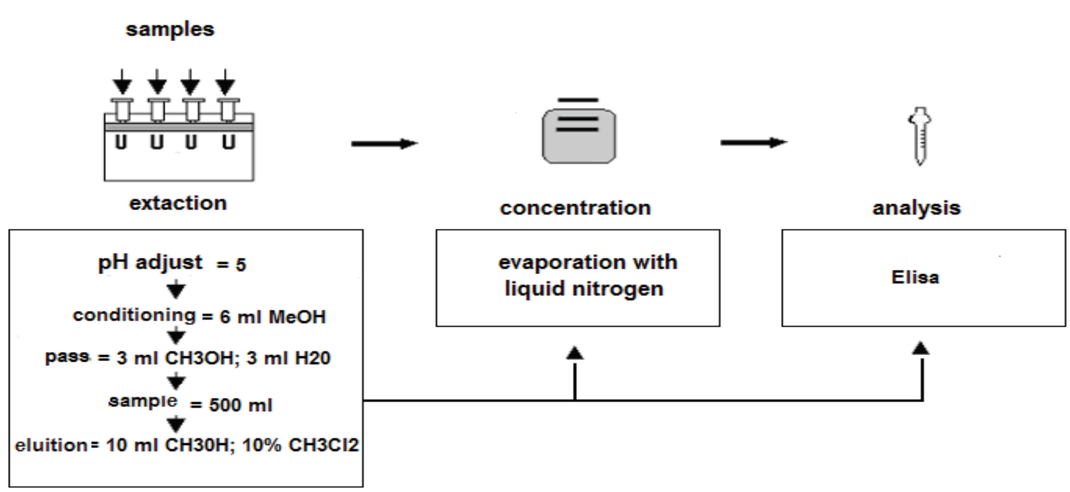

Figure 2. Scheme of processing and analysis of the samples.

Analyses by LC-MS/MS were accomplished in Agilent 1100 system, with automatic sampler coupled to a mass spectrophotometer MDS/SCIEX API2000 (Turbo Ion Spray®), with a reverse phase column - C18 (5 μm, 250 x 4 mm - LiChrospher 100 RP-18) and a pre-column (4 x 4 mm, 5 μm - Merck, Darmstadt, Germany). The injection volume was adjusted to 25 μL at a flow rate of 1 mL $min.^{-1}$.

Physical and chemical parameters were determined as follow: pH readings were made in situ using portable equipment (Digimed – DM/2P). The analyses of TSS, FSS, VSS, COD, BOD, total Kjeldahl N, ammonia N, nitrate, nitrite and phosphorus were performed according to 'Standard Methods for the Examination of Water and Wastewater' (AWWA, 1995).

Statistical analysis

Statistical analyses were run using the software Origin 7.5 (OriginLab Corporation).

Results and discussion

The results obtained for the EDs, from the effluents collected along the line of treatment of the SWTPs Penha and Ilha do Governador are presented in Tables 3 and 4, with analyses using the methodology LC-MS/MS; and in Tables 5 and 6, with analyses using the methodology Elisa.

The results obtained by several authors for 17β-estradiol in effluents of WTPs in Germany (5.2 ng L^{-1}), England (1.6 ng L^{-1}) Canada (2 ng L^{-1}), USA (6.5 ng L^{-1}) and Japan (0.5 ng L^{-1}), (DORABAWILA; GUPTA, 2005; KUCH; BALLSCHMITER, 2001; LEE et al., 2005) were within the range of 0.5 – 6.5 ng L^{-1}. Our results using the LC-MS/MS method for the same compound in the effluent of the SWTP Penha were in the average, with the range of 0.18 – 0.77 ng L^{-1} (X_{min}= 0.09 and X_{max}= 1.11) and of the SWTP Ilha do Governador with the range of 0.41 – 3.23 ng L^{-1} (X_{min}= 0.21 and X_{max}= 5.67). However, when the 17β-estradiol was examined by Elisa in the effluent of the SWTP Penha it was detected the range of 0.66 – 2.91 ng L^{-1} (X_{min}= 0.43 and X_{max}= 3.77), and in the SWTP Ilha do Governador, the range of 0.41 – 3.23 ng L^{-1} (X_{min}= 0.21 and X_{max}= 5.67). Considering that the abovementioned studies had not used the Elisa method, our results indicate a significant difference of method, showing better results that pointed values closer to the results observed in Germany and USA, and higher than in England, Canada and Japan.

Table 3. Analyses of concentrations of EDs by the LC-MS/MS method in the effluent of the SWTP Penha.

Sites	EDs	SWTP Penha LC-MS/MS method											
		X	Sd(yEr±)	Se(yEr±)	P_{25}	P_{75}	P_{95}	X_{min}	X_{max}	R	Median	Var	CoefVar
1	17 β estradiol (ng L^{-1})	0.77	0.20708	0.07322	0.61	0.88	1.11	0.56	1.11	0.55	0.7	0.04288	0.26764
2		0.65	0.19241	0.06803	0.48	0.68	1.04	0.46	1.04	0.58	0.63	0.03702	0.29264
3		0.92	0.19456	0.06879	0.72	1.07	1.19	0.69	1.19	0.5	0.925	0.03786	0.21006
4		0.18	0.04422	0.01563	0.16	0.21	0.23	0.09	0.23	0.14	0.185	0.00196	0.24397
1	BPA (μg L^{-1})	4.44	0.91526	0.32359	3.37	5.14	5.31	2.78	5.31	2.53	4.68	0.8377	0.20608
2		2.12	0.5646	0.19962	1.86	2.54	2.64	0.98	2.64	1.66	2.21	0.31877	0.26585
3		2.72	0.6904	0.24409	2.14	2.81	4.05	1.79	4.05	2.26	2.705	0.47666	0.25382
4		1.05	0.31802	0.11244	0.89	1.04	1.79	0.71	1.79	1.08	0.99	0.10114	0.30216
1	APEOs (μg L^{-1})	52.76	7.47733	2.64363	45.86	59.27	63.84	44.89	63.84	18.95	49.395	55.91039	0.14172
2		69.94	6.42292	2.27085	63.67	76.12	78.25	62.77	78.25	15.48	69.29	41.25396	0.09182
3		47.08	6.93132	2.45059	42.33	51.19	57.48	37.38	57.48	20.1	45.49	48.04316	0.1472
4		10.34	2.10333	0.74364	8.45	11.58	13.86	8.19	13.86	5.67	9.78	4.42401	0.20339

X-arithmetic mean; Sd- standard deviation; Se- standard error; P_{25} – Percentile 25; P_{75} – Percentile 75; P_{95} – Percentile 95; Xmin- minimum; Xmax-maximum; R – Range; Var – Variance; CoefVar- coefficient of variation (%).

Table 4. Analyses of concentrations of EDs by the LC-MS/MS method in the effluent of the SWTP Ilha do Governador.

Sites	EDs	SWTP Ilha do Governador LC-MS/MS method											
		X	Sd(yEr±)	Se(yEr±)	P_{25}	P_{75}	P_{95}	X_{min}	X_{max}	R	Median	Var	CoefVar
1	17 β estradiol (ng L^{-1})	0.96	0.22605	0.07992	0.71	1.19	1.24	0.68	1.24	0.56	0.92	0.0511	0.23516
2		1.02	0.26259	0.09284	0.67	1.17	1.33	0.64	1.33	0.69	1.12	0.06896	0.25713
3		1.11	0.25182	0.08903	0.91	1.2	1.58	0.82	1.58	0.76	1.06	0.06341	0.22661
4		0.26	0.06413	0.02267	0.22	0.31	0.34	0.15	0.34	0.19	0.265	0.00411	0.24314
1	BPA (μg L^{-1})	1.77	0.53973	0.19082	1.28	1.96	2.77	1.26	2.77	1.51	1.575	0.29131	0.30429
2		1.94	0.76859	0.27174	1.39	2.31	3.08	0.66	3.08	2.42	1.815	0.59074	0.39567
3		0.78	0.22564	0.07978	0.6	0.86	1.17	0.46	1.17	0.71	0.77	0.05091	0.28928
4		0.41	0.15682	0.05544	0.23	0.51	0.67	0.22	0.67	0.45	0.39	0.02459	0.38017
1	APEOs (μg L^{-1})	32.15	10.79091	3.81516	21.69	38.69	47.23	18.64	47.23	28.59	31.44	116.44367	0.33563
2		25.55	8.94666	3.16312	16.57	31.31	38.45	15.33	38.45	23.12	24.895	80.0428	0.35011
3		29.08	10.20934	3.60955	18.78	35.62	43.81	17.43	43.81	26.38	28.33	104.23056	0.35105
4		5.15	1.91314	0.6764	3.17	6.44	7.91	3.03	7.91	4.88	5.1	3.66009	0.37148

X-arithmetic mean; Sd- standard deviation; Se- standard error; P_{25} – Percentile 25; P_{75} – Percentile 75; P_{95} – Percentile 95; Xmin- minimum; Xmax-maximum; R – Range; Var – Variance; CoefVar- coefficient of variation (%).

Table 5. Analyses of concentrations of EDs by the Elisa method in the effluent of the SWTP Penha.

		SWTP Penha											
Sites	EDs	Elisa method											
		X	Sd(yEr±)	Se(yEr±)	P_{25}	P_{75}	P_{95}	X_{min}	X_{max}	R	Median	Var	CoefVar
1	17 β estradiol (ng L^{-1})	2.91	0.67324	0.23803	2.17	3.37	3.77	2.02	3.77	1.75	3.07	0.45326	0.23136
2		2.57	0.59464	0.21024	1.92	2.96	3.32	1.77	3.32	1.55	2.71	0.3536	0.23138
3		2.13	0.58674	0.20745	1.86	2.54	2.76	0.95	2.76	1.81	2.21	0.34427	0.27531
4		0.66	0.22335	0.07897	0.43	0.68	1.09	0.43	1.09	0.66	0.625	0.04989	0.33841
1	BPA (μg L^{-1})	5.13	1.96571	0.69498	2.88	6.38	8.35	2.68	8.35	5.67	4.85	3.86403	0.38318
2		2.74	1.03593	0.36626	1.58	3.39	4.43	1.41	4.43	3.02	2.585	1.07316	0.37825
3		2.36	0.91872	0.32482	1.32	2.92	3.89	1.21	3.89	2.68	2.235	0.84406	0.38867
4		1.34	0.51826	0.18323	0.75	1.66	2.21	0.71	2.21	1.5	1.255	0.26859	0.38604
1	APEOs (μg L^{-1})	65.32	25.63814	9.06445	36.42	80.86	106.76	31.62	106.76	75.14	62.78	657.31427	0.39249
2		61.91	24.75357	8.75171	34.22	76.54	102.49	30.09	102.49	72.4	58.43	612.73913	0.39984
3		51.12	19.55092	6.9123	28.5	63.21	84.03	27.26	84.03	56.77	48.33	382.23864	0.38246
4		13.11	6.34904	2.24473	7.31	15.21	24.29	3.99	24.29	20.3	12.395	40.31037	0.48429

X-arithmetic mean; Sd- standard deviation; Se- standard error; P_{25} – Percentile 25; P_{75} – Percentile 75; P_{95} – Percentile 95; Xmin- minimum; Xmax-maximum; R – Range; Var – Variance; CoefVar- coefficient of variation (%).

Table 6. Analyses of concentrations of EDs by the Elisa method in the effluent of the SWTP Ilha do Governador.

		SWTP Ilha do Governador											
Sites	EDs	Elisa method											
		X	Sd(yEr±)	Se(yEr±)	P_{25}	P_{75}	P_{95}	X_{min}	X_{max}	R	Median	Var	CoefVar
1	17 β estradiol (ng L^{-1})	3.23	1.41932	0.5018	1.83	3.97	5.67	1.22	5.67	4.45	3.11	2.01446	0.43874
2		2.76	1.05107	0.37161	1.54	3.41	4.49	1.47	4.49	3.02	2.61	1.10474	0.38082
3		1.45	0.41074	0.14522	1.23	1.73	1.9	0.64	1.9	1.26	1.48	0.16871	0.28376
4		0.41	0.10849	0.03836	0.35	0.49	0.54	0.21	0.54	0.33	0.42	0.01177	0.26221
1	BPA (μg L^{-1})	3.32	1.45969	0.51608	1.88	4.08	5.81	1.23	5.81	4.58	3.195	2.1307	0.43983
2		2.29	1.08775	0.38458	1.37	2.81	4.03	0.56	4.03	3.47	2.2	1.18319	0.47552
3		1.38	0.61557	0.21764	0.78	1.69	2.45	0.51	2.45	1.94	1.325	0.37893	0.44566
4		0.72	0.31842	0.11258	0.41	0.89	1.27	0.27	1.27	1.00	0.695	0.10139	0.44072
1	APEOs (μg L^{-1})	45.10	19.56435	6.91704	28.19	55.55	78.34	15.19	78.34	63.15	43.52	382.76391	0.43375
2		40.13	18.21837	6.44117	22.81	49.47	71.15	13.22	71.15	57.93	38.75	331.90908	0.45403
3		33.45	14.94038	5.28222	18.31	41.23	58.89	12.17	58.89	46.72	32.295	223.21505	0.44668
4		10.17	4.73662	1.67465	5.76	12.53	18.33	3.13	18.33	15.2	9.805	22.43554	0.46552

X-arithmetic mean; Sd- standard deviation; Se- standard error; P_{25} – Percentile 25; P_{75} – Percentile 75; P_{95} – Percentile 95; Xmin- minimum; Xmax-maximum; R – Range; Var – Variance; CoefVar- coefficient of variation (%).

In relation to BPA, the obtained results with the LC-MS/MS method in the effluent of the SWTP Penha were in the average with the range of 1.05 – 4.44 μg L^{-1} (X_{min}= 0.71 and X_{max}= 5.31) and in the SWTP Ilha do Governador within the range of 0.41 – 1.77 μg L^{-1} (X_{min}= 0.22 and X_{max}= 2.77). With the Elisa method in the effluent of the SWTP Penha, we detected a range of 1.34 – 5.13 μg L^{-1} (X_{min}= 0.75 and X_{max}= 8.35) and in the SWTP Ilha do Governador, the range of 0.72 – 3.32 μg L^{-1} (X_{min}= 0.27 and X_{max}= 5.81). Significantly different results were also verified between methods, indicating a better result using the Elisa method. The BPA is a substance used in industries as a component of epoxy resins. In the environment, this compound has a high persistence. Values determined in the effluents of both SWTPs are within the interval registered by Heisterkamp et al. (2004), which detected levels between 0.085 and 28 μg L^{-1}, but the final effluent had on average 6.09 μg L^{-1}, higher than found in the present study.

Regarding the APEOs (NP and OP), LC-MS/MS method results in the effluent of the SWTP Penha were on average within the range of 10.34 – 52.76 μg L^{-1} (X_{min}= 8.19 and X_{max}= 63.84) and in the SWTP Ilha do Governador, between 5.15 – 32.15 μg L^{-1} (X_{min}= 3.03 and X_{max}= 47.23). Using the Elisa method, the range of 13.11 – 65.32 μg L^{-1} (X_{min}= 3.99 and X_{max}= 106.76) was found in the effluent of the SWTP Penha, and the range of 10.17 – 45.10 μg L^{-1} (X_{min}= 3.13 and X_{max}= 78.34) in the SWTP Ilha do Governador. In the last 50 years, the APEOs were largely used in industrial, commercial and domestic applications, with an annual production of 350,000 ton., in the USA, Europe and Japan, explaining their presence in the SWTPs (NICHOLS et al., 2001). This justifies the current growing concern about the indiscriminate use of these chemicals, especially due to the relative stability of some metabolits and degradation of products containing APEOs (TSUDA et al., 2000). Results achieved with the Elisa method were better than the LC-MS/MS.

All analyses pointed out a higher sensitivity to the Elisa method in detecting EDs. These two techniques were chosen to be tested given their relative accessibility and high reproducibility, subsidizing thus the research on EDs and contributing to make more reliable the conclusions made with the study.

Nevertheless, in all cases regardless of the method (Elisa or LC-MS/MS) the selected EDs were present in the effluents of both SWTPs, with varied concentrations, which are released into the Guanabara Bay, with levels with potential physiological effects on animal life (COLBORN et al., 1993; FERREIRA et al., 2006). But taking into account the dilution level in this bay, it is expected that the effects on the organisms are not relevant and/or immediate.

The Table 7 and Table 8 present the values of physical and chemical parameters of treatment control in the SWTPs Penha and Ilha do Governador, respectively. The behavior of these parameters was consistent with that expected; indicating the high efficiency of the process in the SWTPs. Importantly, the results of key parameters meet the requirement of Conama 357 (BRASIL, 2005), such as pH within the range of 5 and 9, and up to 60 mg L^{-1} of BOD and ammonia N amoniacal up to 20 mg L^{-1}.

Moreover, despite the efficiency of the SWTPs, there is a need for further procedures at the end of the process, aiming to reduce EDs to safe levels. Literature data on the removal efficiency of estrogens in WTPs reinforce the need for subsequent treatments in the effluent (LOPES et al., 2008). According to Andersen et al. (2003) in Arkansas (USA) the removal of EDs was more effective in the processes for nutrient removal, i.e., the tertiary treatment responsible for removing nitrogen and phosphorus. In this case, the sludge presented a retention time of 11-13 days with the growth of microorganisms able to degrade estrogens, which is not observed in conventional methods with the sludge retention time shorter than four days.

This study showed that concentrations of the studied compounds in the effluents of the SWTPs have been at worrying levels relative to levels of EDs in effluents of these countries, reflecting the need for research and implementation of a greater control in the WTPs, strengthening basic sanitation of the country, minimizing thus significant impacts to the environment.

Analytical techniques used herein allowed comparing values of EDs in the different process steps of the SWTPs. Data obtained with Elisa had high detectability and selectivity in relation to the LC-MS/MS method, with a greater sensitivity to the EDs, and therefore presented better detection limits. Parameters for validation observed for the procedure increased the reliability of the results, once there is no official method to simultaneously determine these chemicals. This procedure could also base monitoring studies on these compounds in effluents.

A suitable policy to reduce the threat of chemicals with potential to affect the hormone system requires a control on the availability and guidelines for using pesticides like endosulfan and methoxychlor, fungicides such as vinclozolin, herbicide like atrazine, alkylphenols, phthalates and BPA. In order to prevent the generation of dioxin is essential the gradual elimination of PVC, tetrachlorethylene and chlorinated pesticides.

Table 7. Physical and chemical parameters presented in the sampling sites of the SWTP Penha.

	SWTP Penha				
Parameter	Sampling sites				
	1	2	3	4	Efficiency (%)
pH	6.62	6.78	6.45	6.87	-
TSS (mg L^{-1})	4265.2	1910.7	108.6	92.1	97.8
FSS (mg L^{-1})	964.5	267.7	132.8	35.9	96.27
VSS (mg L^{-1})	3198.3	1625.8	121.8	41.0	98.71
COD (mg O_2 L^{-1})	25460	18636	254	233	99.08
BOD (mg O_2 L^{-1})	5745	3567	566	58	98.99
Total Kjeldahl N (mg N L^{-1})	333.7	165.9	13.1	11.8	96.46
Ammonia N (mg N-NH_3 L^{-1})	84.8	61.6	21.4	4.5	94.6
Nitrate (mg NO_3 L^{-1})	188.6	22.1	3.5	1.7	90.98
Nitrite (mg NO_2 L^{-1})	123.2	16.7	1.7	0.5	95.94
Total phosphorus (mg P L^{-1})	4.41	4.78	2.67	2.34	46.93

Table 8. Physical and chemical parameters presented in the sampling sites of the SWTP Ilha do Governador.

	SWTP Ilha do Governador				
Parameter	Sampling sites				
	1	2	3	4	Efficiency (%)
pH	6.34	6.43	6.77	6.93	-
TSS (mg L^{-1})	5642.7	1455.3	67.9	48.2	99.1
FSS (mg L^{-1})	678.9	199.4	111.1	24.4	96.4
VSS (mg L^{-1})	2433.7	1322	87.3	22.2	99.08
COD (mg O_2 L^{-1})	18453	12642	278	151	99.18
BOD (mg O_2 L^{-1})	5997	6622	475	44	99.26
Total Kjeldahl N (mg N L^{-1})	452.3	177.6	16.7	10.2	97.74
Ammonia N (mg N-NH_3 L^{-1})	112.5	48.7	19.8	7.6	93.24
Nitrate (mg NO_3 L^{-1})	231.4	77.2	4.1	1.5	93.51
Nitrite (mg NO_2 L^{-1})	98.2	22.6	2.4	0.3	96.94
Total phosphorus (mg P L^{-1})	3.77	2.15	1.87	0.87	76.92

Conclusion

This study adds knowledge to a little studied topic in the country, which produced data that allowed understanding the treatment and presence of EDs in two important SWTPs. Given the potential to disrupt the endocrine system it is necessary to continue the researches on methodologies for detection and on the action of EDs in the environment, and on the reproductive system of the fauna in aquatic systems. Something positive, Brazil already has public policies that place the country on par with European countries and USA, referring the control of EDs in SWTPs.

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