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Screening Concentration of E1, E2 and EE2 in Sewage Effluents and Surface Waters of the "Pampas" Region and the "Río de la Plata" Estuary (Argentina)

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Abstract Concentrations of estrone (E1), 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) were investigated for the first time in sewage effluents and receiving waters of the "Río de la Plata" estuary and neighboring areas by means of LC–MS/MS. E2 and EE2 were ubiquitous in the evaluated sewage effluent samples showing concentrations ranging between 122–631 and 65–187 ng/L, respectively. In surface waters, these estrogens were only detected in the "Girado" stream (Chascomús) at 369 and 43 ng/L, respectively. No significant relationship was found among the size of the served population and the concentration of the estrogens in the sewage effluent. The detection of these estrogens in receiving waters was dependent on the dilution capacity of the system. The studied estrogens were undetectable at the La Plata City water supply station. Conversely, concentrations found at the "Girado" stream indicate a potential ecotoxicological risk of these estrogens to the local aquatic biota.

Keywords Sewage effluents · Environmental estrogens · Endocrine disruptors · The Pampas · Río de la Plata

Endocrine disrupting chemicals (EDCs) are environmental pollutants that can interfere with the normal functioning of the endocrine system in wildlife and in humans. One of the main sources of EDCs to the aquatic environment is effluent discharges from sewage treatment plants (STP). In particular, natural and synthetic estrogens have been identified as the main contributors to the estrogenic activity of STP effluents, and their environmental relevance as endocrine disruptors has been demonstrated in several studies, e.g. induction of intersex and population collapse in fish (Sumpter and Jobling 2013).

A vast amount of information was generated during the last 20 years in Europe, North America, and Asia regarding the occurrence and concentration of environmental estrogens in sewage effluents with different degree of treatment and surface waters (Kolpin et al. 2002; De Mes et al. 2005; Liu et al. 2009). However information available for South America is still very limited.

The aim of this research is to report data from a first screening study on the concentration of E1, E2 and EE2 in

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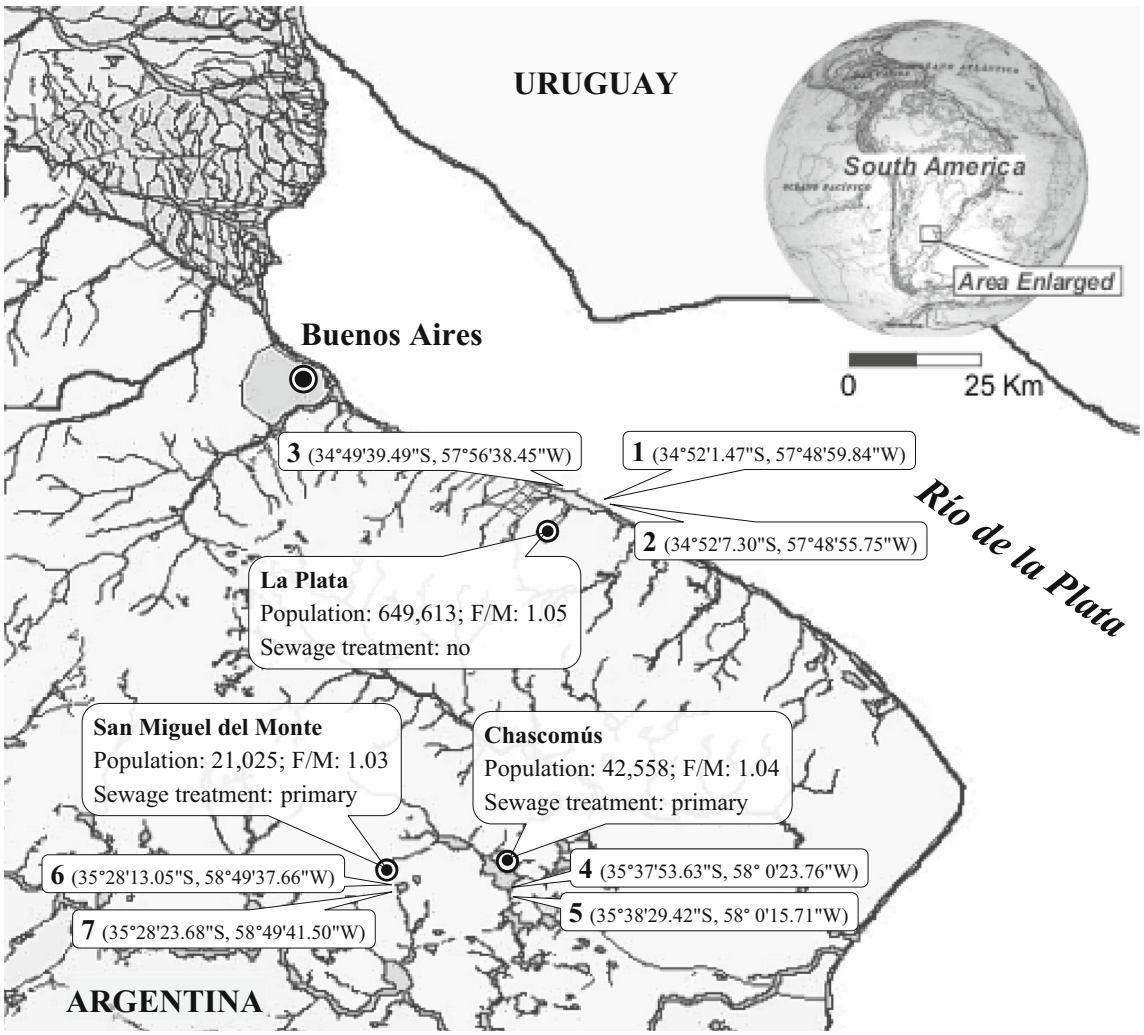

Fig. 1 Location of sewage effluents and surface waters sampling sites. *1* La Plata main raw sewage discharge; *2* Río de la Plata estuary close to the sewage discharge; *3* La Plata drinking water plant intake; *4* Chascomús STP outfall; *5* "Girado" stream (1 km downstream site 4); *6* San Miguel del Monte STP outfall; *7* "Las Perdices" lake (0.5 km from site 6). *F/M* female/male ratio

sewage effluents and the respective receiving waters in the "Río de la Plata" estuary and other streams and shallow lakes of the "Pampas" region of Argentina.

Materials and Methods

Three locations of the Province of Buenos Aires (Argentina) were selected for sampling sewage effluent and surface waters of the corresponding receiving water bodies (Fig. 1) during January 2010 (summer of the south hemisphere). The sewage effluent samples from La Plata city (1), Chascomús (4) and San Miguel del Monte (6) were taken from the inspection chambers at the effluent discharge point. The respective receiving water samples were collected between 200 and 1,000 m from the sewage effluent outfall at the "Río de la Plata" estuary (2), the "Girado" stream in Chascomús (5), and "Las Perdices" shallow lake in San Miguel del Monte (7). An additional sample was collected from the "Río de la Plata" estuary, at the end of the breakwater of the La Plata City drinking water supply station (3). Population data was obtained from the 2010 Argentina National Census (INDEC 2010).

Duplicate samples were collected in 1 L pre-cleaned amber glass bottles. One of the duplicates was fortified with a mixture of the three estrogens at 1 μg/L. Then, samples were stored on ice, transported to the laboratory and processed within 24 h. Temperature, pH and conductivity were determined in situ using a multiparameter Lutron (YK-2001, YK-200PCT, YK-200PATC). Ammonia concentration (NH_4^+) was measured using a commercial kit (Aquaquant® 114423, Merck). Suspended solids were measured gravimetrically after drying samples at 103–105°C (APHA-AWWA-WEF 1998).

Table 1 Estrogen concentration and general physicochemical parameters in sewage effluent and surface waters

		Sewage effluent				Surface water			
		1	4	6	Mean ± SE	2	3	5	7
E1	(ng/L)	<LOD	<LOD	<LOD	–	<LOD	<LOD	<LOD	<LOD
E2	(ng/L)	122	631	170	307.7 ± 198.7	<LOD	<LOD	369	<LOD
EE2	(ng/L)	80	65	187	110.7 ± 47.0	<LOD	<LOD	43	<LOD
T	(°C)	26.3	24.4	26.3	25.7 ± 0.8	28	25.6	31.7	33.4
pH		7.56	7.83	8.21	7.87 ± 0.23	7.6	7.17	8.33	9.08
Cond	(mS/cm)	1.12	2.97	2.62	2.24 ± 0.69	0.80	0.33	3.11	1.49
TSS	(mg/L)	63.9	9.2	58.3	43.8 ± 21.3	61	24.0	95.2	24.0
NH_4^+	(mg/L)	60.0	40.0	12.0	37.3 ± 17.0	8.0	0.20	24.0	0.20

General sample processing was conducted following Pedrouzo et al. (2009) with minor modifications. Samples (400 mL) were filtered (GF/F 0.7 μm, Munktell) and acidified with HCl to pH 3. Solid phase extraction (SPE) was conducted using SampliQ C-18 SPE cartridges (1 g/6 mL, Agilent Technologies) preconditioned with methanol and acidified water (pH 3), loaded, washed and eluted with 5 ml of methanol. The eluates were concentrated under vacuum, dried under gentle nitrogen flow and redissolved in mobile phase. Extracts analysis was performed using a binary pump HPLC (Varian ProStar) with an autosampler (Varian 410) and coupled to a triple quadrupole MS/MS (Varian 1200L) equipped with an electrospray ionization (ESI) source. The injection volume was 20 μL. A pentafluorophenyl column (Kinetex, 150 mm × 4.6 mm × 2.6 μm, Phenomenex) was used for chromatographic separation using a ternary mobile phase, under isocratic conditions (MeOH, ACN and 0.1 % formic acid in ultrapure water, 25:25:50). ESI source was operated in positive mode. Capillary energy was 70 V. Argon was used as collision gas (CE 5 V), while nitrogen was used as both, the nebulizing (58 psi) and drying gas (350°C, 21 psi). The selected reaction monitoring (SRM) mode was chosen for quantitation. The *m/z* of the obtained product ions were 253, 255, 279 for E1, E2 and EE2, respectively, corresponding to the dehydrated product ion, $[M + H - H_20]^+$ (Zhao et al. 2004). Chromatograms were analyzed using the Varian MS Workstation 6.6 software. Limits of detection and quality controls were calculated according to APHA-AWWA-WEF (1998). The limits of detection and quantification of the method (LOD/LOQ) were 16/48, 22/66, 15/45 ng/L, and whole recoveries were 48 %, 41 %, 71 % for E1, E2 and EE2, respectively, in accordance with other studies (Pedrouzo et al. 2009). Final concentrations were calculated using whole recoveries estimated from the fortified samples including losses from sampling, storing, filtering, extracting, and analysis. Solvents used were HPLC grade. Standards of estrogens (purity higher than 98 %) were purchased from Steraloids (Newport, Rhode Island, USA). Data are reported as the mean and the standard error. The strength of the association between population size and sewage effluent estrogen concentration was assessed using the Pearson correlation coefficient.

Results and Discussion

The concentration of E1, E2, and EE2 in sewage effluent and surface waters of the "Pampas" region of Argentina, together with the measured physicochemical parameters, are shown in Table 1. E2 and EE2 were ubiquitous in all studied sewage effluents. In surface waters, these two estrogens were only detected in one of the four analyzed sampling points at levels of 369 and 43 ng/L of E2 and EE2 respectively. Concentrations of E1 were below the LOD in all analyzed samples. The three studied estrogens were undetectable in the La Plata drinking water intake station at the "Río de la Plata" estuary.

According to this findings, the concentration profile of the sewage effluents and surface waters of the "Pampas" region was characterized by the following order: E2 > EE2 > E1. This profile was different from that found in sewages effluents of Asia, Europe, North America and Brazil, where E1 is the predominant estrogen (Table 2). In addition, the concentrations of E2 and EE2 were up to 4 and 30 times higher than those reported for other parts of the world. These could be explained by type and degree of sewage treatment and for elucidating these issues deeper studies will be needed. In surface waters, the profile observed in other countries was not so clear but it was often dominated by EE2 (Table 2). The higher concentrations of E2, and the undetected levels of E1, in the "Pampas" were similar to those reported for US (Kolpin et al. 2002) and Brazil (Montagner and Jardim 2011; Kuster et al. 2009). Deeper studies are being conducted, including the use of stable isotope surrogates, to reinforce the observed patterns.

Unexpectedly, concentrations of estrogens in wastewater were higher in treated effluents than in untreated ones.

Table 2 Concentrations range of estrogens in sewage effluents and surface waters (ng/L) reported for different regions of the world

Location	Sewage effluent			Surface water			References
	E1	E2	EE2	E1	E2	EE2	
Asia	0.30–8.80	<LOD–3.90	<LOD–46.6	1.30–19.8	3.50–5.60	5.00–128	(Sun et al. 2009; Duong et al. 2010)
Europe	<LOD–74.9	<LOD–10.9	<LOD–5.20	<LOD–26.9	<LOD–35.0	<LOD–102	(De Mes et al. 2005; Ribeiro et al. 2009)
North America	6.00–72.0	0.11–10.0	0.11–15.0	0.11–107	0.11–160	0.11–73.0	(Kolpin et al. 2002; De Mes et al. 2005)
Brazil	<LOD–41.6	<LOD–20.8	<LOD–5.83	<LOD	<LOD–62.6	<LOD–63.8	(Ternes et al. 1999; Kuster et al. 2009; Moreira et al. 2011; Brandt et al. 2013)

Although this results could be biased by temporal variations due to grab sampling (Ort et al. 2010), it is also probable that treatment plants were improperly working or even out of service.

The relationship between the estrogens concentration in the sewage effluents and the size of the served population was not significantly correlated (E2 $p = 0.652$ and EE2 $p = 0.726$), indicating that the relationship among the excreted amount of E2 and EE2 and the water volume used at the toilet per person and per day is relatively constant. In consequence, the size of the served population would explain the flow of the effluent and the total estrogen load on the environment better than the expected concentration of these compounds in the effluent.

The concentrations of E2 and EE2 found in the "Girado" stream 1000 m downstream the sewage discharge, were 58 and 66 % of that found in the effluent at the discharge point. Other point or nonpoint sources of estrogens were not identified all along the stream. In addition, the dilution values of the estrogens were consistent with those observed for NH_4^+ (60 %), indicating that under dry periods, as during the sampling, the Chascomús sewage effluent significantly contribute to the flow of the "Girado" stream. In the "Río de La Plata" estuary and "Las Perdices" shallow lake, concentrations of NH_4^+ were, respectively, 13 and 2 % of that measured in the effluent, indicating a greater dilution capacity in these water bodies. The magnitude of dilution in these effluents helped to explain the undetected levels of the studied estrogens in these places. According to this, concentrations of estrogens in surface waters could be estimated from the concentration in the sewage effluent and the dilution factor of the NH_4^+ in the effluent plume. On the other hand conductivity and TSS were not good descriptors of the effluent dilution since, for some places, they showed higher values in the receiving waters than in the effluent.

Estrogen concentrations are able to disrupt the endocrine system of aquatic vertebrates (Sumpter and Jobling 2013). E2 concentrations as low as 50 ng/L were effective impairing the expression of brain aromatase and the sexual behavior of the South American fish *Jenynsia multidentata* (Guyón et al. 2012) and EE2, fifty-time more potent, was able to disrupt the expression of the gonadal aromatase and the sex ratio of *Odontesthes bonariensis* (Pérez et al. 2012), an emblematic species of the "Pampas" streams and shallow lakes. According to this, the measured concentration of the studied estrogens in the receiving waters of the "Pampas" region with low dilution capacity would represent a risk for the reproductive successes of local fish, and probably other aquatic organisms.

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