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# A one-year follow-up analysis of antidepressants in Portuguese wastewaters: Occurrence and fate, seasonal influence, and risk assessment

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## HIGHLIGHTS

- From the SSRIs studied in Portuguese WWTPs citalopram showed higher frequency/levels.
- Results translate the SSRIs prescription and use between the 5 regions in study.
- Seasonal variations in terms of occurrence and removal efficiencies were observed.
- Most of selected compounds in influents were degraded during the treatment.
- RQs were lower than 1. The species sensitivity was as following algae; fish; daphnids.

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## ABSTRACT

The occurrence, fate, seasonal influence and environmental risk assessment of four selective serotonin re-uptake inhibitors (SSRIs) antidepressants, citalopram, fluoxetine, paroxetine and sertraline, were studied in 15 different wastewater treatment plants (WWTPs) across Portugal. Influent and effluent samples from four sampling campaigns, in 2013, were extracted through Oasis HLB cartridges, and quantified through liquid chromatography with tandem mass spectrometry (LC-MS*n*).Results showed that citalopram was the SSRI most frequently found, both in influents and in effluents, with mean mass loads ranging between 14.56 and 9.51 mg/day/1000 inhabitants, respectively. Fluoxetine and sertraline were only detected in influent samples, in lower mean mass loads (14.60 and 1.36 mg/day/1000 inhab., respectively), whereas paroxetine was found in influent and effluent samples (12.61 and 18.90 mg/day/1000 inhab., respectively). WWTPs were not capable of completely removing these pharmaceuticals; nonetheless, the mean removal efficiency was 82.24%. Removal efficiency was lower in winter (74.21%), summer (72.02%), and autumn (81.19%), when compared to spring (100%).
Our results translate the variations in SSRI prescription and use between the five Portuguese regions in study. Influent contaminated samples were found in WWTPs from Lisbon, Alentejo, Center and North (28.25, 19.01, 16.55 and 6.98 mg/day/1000 inhab., respectively). In the Algarve region no contaminated samples were found. A seasonal pattern in the presence of SSRIs in influent wastewaters was observed. The SSRIs mass loads in influent wastewaters were higher in autumn, followed by spring, winter, and summer.
Finally, the potential ecotoxicological risk posed by SSRIs to different trophic levels of aquatic organisms, exposed to the effluent wastewaters studied was evaluated by means of risk quotients (RQ). Citalopram and paroxetine, the only SSRIs found in these samples, presented RQ lower than 1. According to the results, algae appeared to be the most sensitive followed by fish and daphnids.



## 1. Introduction

The presence of emerging contaminants, such as pharmaceuticals, in the environment is a growing problem that must be tackled to meet the Water Framework Directive (WFD) of the European Union (EU) (Vazquez-Roig et al., 2011). A better knowledge of their environmental occurrence and fate will allow a proper risk assessment (Vazquez-Roig

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et al., 2011). Nowadays, the higher prevalence of psychiatric disorders led to a worldwide increased number of prescriptions for psychiatric pharmaceuticals, namely antidepressants (Silva et al., 2012). According to the latest Eurobarometer of 2010 regarding mental health, 7% of the EU citizens took antidepressants during 2009. The same report claims that the use of antidepressants is highest in Portugal, where the prevalence of use doubles that of the EU average (Eurobarometer, 2010).

Selective serotonin re-uptake inhibitor (SSRI) antidepressants are amongst the most prescribed pharmaceuticals throughout the world. Both their increased consumption and their required chronic administration suggest a higher environmental exposure, dictating an environmental risk evaluation. After intake, these highly active compounds undergo metabolic transformations, with subsequent excretion of significant fractions of the unmetabolized or of active metabolites to raw sewage and wastewater treatment plants (WWTPs) (Schultz and Furlong, 2008).

The physico-chemical characteristics of SSRIs (Table S1, Supporting Information) outline their environmental behaviour. They are basic drugs, with pKa ranging between 9.05 and 10.5, designed to produce a specific pharmacological response, and, in order to reach the specific site of action within the organism, presenting a certain chemical stability. This stability may be later manifested in their insufficient removal during wastewater treatment and by their limited environmental degradation, sometimes resulting in minor structural alteration(s) instead of complete mineralization (Silva et al., 2012). Scientific studies have already demonstrated their incomplete removal by WWTPs, being these facilities considered as the major environmental source since their effluents are discharged to the surrounding water bodies (Schultz et al., 2010).

Consequently, their presence in different environmental matrices is ubiquitous. As far as we know the presence of SSRIs in the environment, specifically fluoxetine, was first reported by Kolpin et al. (2002) in US surface waters, and by Metcalfe et al. (2003) in Canada WWTP effluents. Later on, in 2005, a study reported the presence of two SSRIs and their metabolites (fluoxetine, sertraline, norfluoxetine, and desmethylsertraline) in different fish tissues residing in a municipal effluent-dominated stream (Brooks et al., 2005). Since then, several publications, from different countries, referred the presence of these residues in a wide range of water samples, including wastewaters, in concentrations ranging from 0.15 to 32,228 ng $L^{-1}$, surface and groundwaters, ranging between 0.5 and 8000 ng $L^{-1}$, and drinking waters, from 0.5 to 1400 ng $L^{-1}$ (Silva et al., 2012). Also, in sediments and soils, up to 1033 ng $g^{-1}$ (Schultz et al., 2010; Lajeunesse et al., 2012), and in biota matrices, in concentrations ranging from 0.01 to 73 ng $g^{-1}$ (Chu and Metcalfe, 2007; Ramirez et al., 2007; Schultz et al., 2010, 2011).

These molecules often act by mimicking the effects of the neurotransmitter serotonin, which regulates a wide range of physiological systems in fish, molluscs, and protozoans, and, even at trace levels, have remarkable effects on these and other aquatic organisms (Silva et al., 2012). Alteration of the biological activity of aquatic organisms, reproduction reduction, abnormalities in embryo development, delay in physiological development and sexual maturation were described. Decreased aggressiveness and inhibition of feeding responses were also reported (Mompelat et al., 2009; Demeestere et al., 2010; Santos et al., 2010). Recently, Schultz et al. (2011) demonstrated that exposure of male fathead minnows (*Pimephales promelas*), for 21 days, to sertraline (5.2 ng $L^{-1}$) resulted in mortality. Anatomical alterations were noted within the testes of fish exposed to sertraline and fluoxetine. Additionally, fluoxetine at 28 ng $L^{-1}$ induced vitellogenin in male fish, a common endpoint for estrogenic endocrine disruption.

Heavy contamination pressures from extensive urban activities characterize the Portuguese coast and main rivers that might translate into high aquatic contamination levels and consequent environmental exposure. Although, the concentration of pharmaceuticals, such as SSRIs, in influent and effluent of WWTPs is routinely monitored in many countries, sources of SSRI contamination are geographically diffuse and may be influenced by geographical consumption patterns. Moreover, important fluctuations in concentrations due to seasonal variations might occur. The key driving force of this study was to evaluate, for the first time, the environmental contamination of SSRIs, fluoxetine, paroxetine, sertraline, and citalopram, in WWTP influents and effluents from different Portuguese regions, in order to evaluate geographical contamination patterns. Moreover, we aimed to assess seasonal influence and WWTP removal efficiency. Finally, the potential ecotoxicological risk posed by SSRIs to aquatic organisms, belonging to different trophic levels, when exposed to the studied WWTP effluents was assessed.

## 2. Materials and methods

### 2.1. Sampling site and collection

Influent and effluents of 15 different WWTPs, located in 5 Portuguese regions, North, Center, Lisbon and Tagus Valley, Alentejo and Algarve (Figure S1, Supporting Information), were collected. These WWTPs are designed for 6850 to 756,000 population equivalents, with average loads ranging between 349 and 140,000 $m^3$ per day, having their discharge points in the main Portuguese rivers and Atlantic Ocean. They are designed to treat domestic, hospital and industrial wastewaters, operating with secondary or tertiary treatments, as described in Table 1.

Sampling campaigns, carried out in 2013, were performed during a one year follow-up study, embracing four sampling periods; between 25 February/19 March—winter, 14 May/04 June—spring, 11 July/14 August—summer, and 24 October/7 November—autumn. The characterization of influent and effluent parameters of each WWTP, for the different sampling periods, is shown in Table S2, Supporting Information. For each plant, influent and effluent samples were collected in high-density polyethylene containers previously rinsed with bi-distilled water, as time proportional 24-h composite influent and effluent samples. Samples were kept refrigerated (±4 °C) and during the transport to the laboratory. Upon reception, samples were frozen and stored at −20 °C until analysis.

### 2.2. Standards and chemicals

Reference standards of fluoxetine hydrochloride, sertraline hydrochloride, paroxetine hydrochloride hemihydrate, citalopram hydrobromide, and the labelled surrogate fluoxetine-$d_5$ hydrochloride, all with ≥98% purity, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock and intermediate solutions were prepared in methanol at 5 mg $mL^{-1}$ and 250 μg $mL^{-1}$, respectively, and were stored at −20 °C, for a maximum of 6 months. Mixed standard working solutions, renewed before each analytical run, were prepared at 7.5 and 50 ng $mL^{-1}$, of each SSRI, and used for linearity, accuracy, and repeatability assays. The labelled surrogate fluoxetine-$d_5$ hydrochloride was typically prepared to obtain a final concentration of 50 ng $mL^{-1}$.

HPLC-grade acetonitrile and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Water was prepared from a Millipore Milli Q system (Bedford, MA, USA). Ammonium acetate and formic acid (98%) were obtained from Merck (Darmstadt, Germany); glacial acetic acid was from Panreac (Barcelona, Spain).

### 2.3. Experimental procedure

Samples were acidified with 0.1% formic acid (to a final pH that ranged between 3.0 and 3.2) and vacuum filtered through glass microfiber filters (1.0 μm, 934-AH, from Whatman Schleicher and Schuell, USA), followed by 0.45 and 0.2 μm polyamide membrane filters (from Whatman, Dassel, Germany). As the suspended solids were removed during sample preparation, the measured concentrations of SSRIs correspond to their dissolved fraction.