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**ORIGINAL ARTICLE**

# Environmental Investigation of Psychiatric Pharmaceuticals: Guandu River, Rio De Janeiro State, Southeast Brazil

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**KEYWORDS**

Bromazepam

Clonazepam Diazepam

Environment

Water supply

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**ABSTRACT:** Pharmaceuticals are considered to be important environmental contaminants. The aim of this study was to evaluate their presence in water intended for public supply to Municipally of Rio de Janeiro, Brazil. The regional environmental assessment was done from the analysis of surface water and distribution for the presence of psychiatric drugs. The study reveals the presence of benzodiazepine derivatives in all samples of surface water samples of the Guandu River, with the amounts of 42 ng $L^{-1}$, 198 ng $L^{-1}$ and 335 ng $L^{-1}$ for bromazepam, clonazepam, and diazepam, respectively. The population served by Companhia Estadual de Águas e Esgotos (CEDAE) is the order of ten million. Psychiatric pharmaceuticals, in particular, are thought to impose significant ecological risks. A better understanding of the real impact of these pollutants implies a comprehensive evaluation of their persistence and fate in environmental matrices. The studies presented in this article are intended to contribute to improve the knowledge about the occurrence of some psychiatric drugs in the water supply.

## INTRODUCTION

The occurrence and fate of trace-level contamination of pharmaceuticals in the aquatic environment has been recognized as one of the emerging issues in environmental chemistry [1]. Notwithstanding, a great and diverse group of organic compounds are used in very high amounts throughout the world [2, 3], developed with the aim of applying a biological effect [4, 5]. Thus, principally due to the valuable effect produced in humans, and also to the increase of life expectancy, the consumption of pharmaceuticals is expected to become systematically in growth [6].

Benzodiazepines are one of the greatest consumed pharmaceuticals [7]. Diazepam, clonazepam, and bromazepam are considered relevant substances of this

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group used in Brazil, corresponding to 37.04, 25.93, and 18.52% of the total manufactured amount consumed, respectively, in special, from fifty years old [8]. They all have sedative/hypnotic, anxiolytic, and amnesic, muscular relaxant and anticonvulsant actions with minor differences in the relative potency of these effects. Among all the pharmaceutical groups, psychiatric drugs (mainly comprising anxiolytics, sedatives, hypnotics, antidepressants and anticonvulsants) are one of the most commonly prescribed [9].

Nowadays, 35 benzodiazepines are under international control for therapeutic use [10]. The basic chemical structure of benzodiazepines consists of a seven-membered ring fused to an aromatic ring. The aromatic ring has four main substituent groups that can be modified without loss of activity (Figure 1).

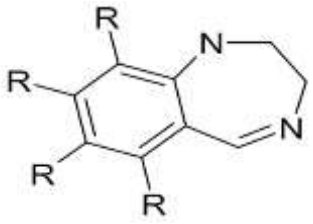



**Figure 1**. Fundamental chemical structure of benzodiazepine

Pharmaceuticals ingested by humans are almost always not completely metabolized, resulting in the excretion of variable percentages of the active compound along with several metabolites and conjugates in urine and feces [11]. Excretion by patients is considered to be the main pathway for the entrance of pharmaceuticals into the environment due to the inadequacy of removal methods of Sludge treatment in wastewater treatment plants (WWTPs) [12], and thus, human metabolism and excretion rates of psychiatric drugs should be addressed. In a WWTP effluents and a Water treatment plant (WTP) treated water, many pharmaceuticals compounds do not get removed to a sufficient degree. This is because of the configurations of the current WWTP and WTP that are not efficient enough to remove these micropollutants. Consequently they are present in the effluents of WWTPs and WTP treated water; enter the surface water where they may pose effects onto aquatic life and public health [3] (Figure 2).

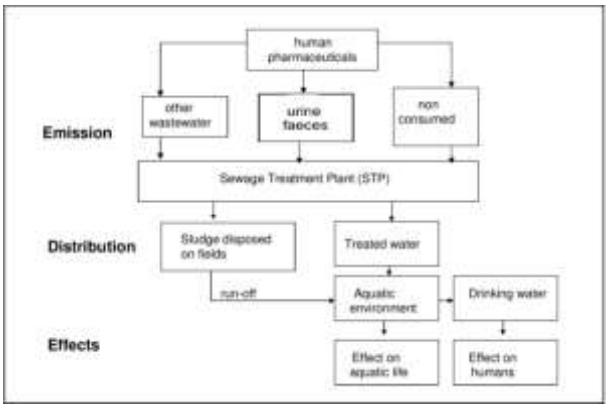



**Figure 2**. Exposure routes of human pharmaceuticals in the environment

Amongst the vast universe of pharmaceuticals, psychiatric drugs are thought to pose significant risk to ecosystems and, for this reason, should be made object of special attention [13]. The extremely high rates of consumption of psychiatric pharmaceuticals, in conjunction with their mode of action, reinforce the need to assess contamination levels and better understand their real ecological impact. According to Environmental Protection Agency [14] the mean period of stability of pharmaceuticals, personal care products, steroids, and hormones in aqueous samples, publicly owned treatment works (POTW) effluents, and biosolids, not exceed 30 days. In the past, the water was considered a perennial and wrongly associating itself the idea of infinite capacity for self-purification of water bodies. However, the growing population has changed this concept to a reality where the water came to be treated as a finite resource. The water quality of Brazilian municipalities is monitored with reference to legislation dealing potability [15] or the quality of its surface waters [16].

In this context, the focal objective of the present study was to determine the contamination of benzodiazepines

(bromazepam, clonazepam, and diazepam) in surface water samples from the Guandu River on its way through the Rio de Janeiro Region (southeast Brazil).

## MATERIALS AND METHODS

### *Study area*

The Guandu River is a planned system with a watershed area of 1,430 $km^2$. It is formed by the confluence of the Lajes and Santana streams, with an extension of *ca.* 108.5 km and an average flow of 156 $m^3$ $s^{-1}$. The main contribution of water to the river is made by an interbasin transfer from the Paraíba do Sul River basin since 1952. An electric company pumps water for hydropower purposes, releasing a water discharge of *ca.* 160 $m^3$ $s^{-1}$ into Lajes stream [17]. The river is impounded 30 km upstream from the estuary at 11.8 m above sea level. From this impoundment, *ca.* 47 $m^3$ $s^{-1}$ are withdrawn to supply tap water for the municipality of Rio de Janeiro and nearby areas, thus decreasing the river flow in the mid-lower segment [18]. The river is channelized above the estuarine zone.

### *Sampling*

Five river stretches surveying three stretches upstream from the WTP and two downstream It were sampled (Figure 3). During the 12 h scheduled for sample collection, occurred between 8 and 20 h, it was gathered two samples per site during the collection period. Surface waters were sampled directly into one litre polyethene bottles at approximately 0.5 m depth. The collections in the Guandu River were performed in December 2013-March 2014, and the results express the consolidated obtained from each analysed site.

### *Preparation of standard solutions and quality control samples*

The standard solutions of diazepam, clonazepam, and bromazepan (Sigma®, Rio de Janeiro, Brazil, min. 98%) were prepared by dissolving each drug in methanol (EM Science®, HPLC grade) to obtain a concentration of 1 mg $ml^{-1}$, and stored at -20 °C. From these, working solutions were prepared by serial dilutions to reach the following final concentrations with acetonitrile: 20 mg $L^{-1}$. HPLC water was obtained from a Millipore's Milli-Q System and used throughout the analysis

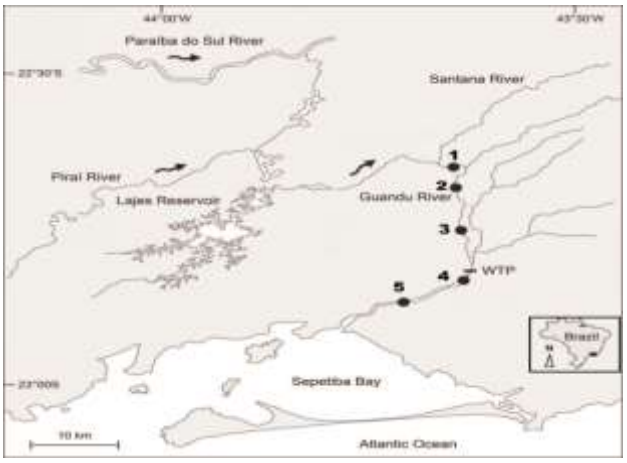



**Figure 3.** Localization of study area: Guandu River

### *Sample preparation*

All water samples (100 mL) were filtered through a 0.45 µm membrane filter (MF, Milllipore, Rio de Janeiro, Brazil) and acidified to pH 3.0 using sulphuric acid. Then 50 ng of each of the isotopically labelled pharmaceuticals used as internal and surrogate standards were added to each sample. Solid phase extraction (SPE) columns (Waters Corporation, Milford, MA, USA) were pre-conditioned and equilibrated with 5.0 mL of methanol and 5.0 mL of de-ionized water. Samples were applied to the SPE columns at a flow rate of 5 mL $min^{-1}$. Water with 5 % methanol was used to wash the SPE column before eluting with 5 mL of methanol. Eluates were collected in 10 mL vials, evaporated to 20 µL under a gentle air stream, and dissolved in 5 % acetonitrile in water to a final volume of 1.0 mL.

### *Instrumentations*

Chromatographic analysis was carried out on a Shimadzu liquid chromatograph, using LC-10ADVP solvent delivery system with low-pressure gradient flow control valve FCV-10ALVP, SCL-10AVP system

controller, SPD-M10AVP photodiode array detector and DGU-14A degasser. The injections were performed by a Shimadzu SIL-10ADVP automatic injector and the analyses were performed using Shimadzu CLASS-VP software (Version 6.12) [19, 21].

***Chromatographic conditions***

The mobile phase used for the chromatographic separation was composed of 20 mM sodium dihydrogen phosphate monohydrate – methanol (20:80, v/v, apparent pH 8.8). The mobile phase was filtered before using and it was delivered isocratically at a flow rate of 1.0 mL/min. The analysis was carried out at room temperature using Shimadzu Shim-pack HPLC column (150 mm x 4.6 mm i.d.) packed with 5 µm ODS stationary phase, protected by Waters Novapak guard column packed with 4 µm RP18 material. The autosampler was set to inject 150 µL sample aliquots and the analyte was monitored using a photodiode array detector at 257 nm.

***Validation procedures***

The method was validated for the parameters like Specificity, range and linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision. In addition, system suitability parameters were also calculated. Precision was calculated as relative standard deviation (RSD) of the experimental concentrations and accuracy as the comparison between the experimental and nominal samples concentration. The criteria for acceptability of the data included accuracy within ± 15% deviation from the nominal values and precision within ±15% RSD, except for the lower limit of quantification (LLOQ), where it should not exceed 20% of RSD [22].

**RESULTS**

Regarding the optimization of the chromatographic conditions, the most important aspects in the development of a chromatographic method are separation, obtaining satisfactory resolution, and a reasonable analysis time. The best results for separation and simultaneous quantification of the three benzodiazepines studied involved a constant composition of the mobile phase consisting of 35% acetonitrile, 60% ammonium formate buffer 2 mmol $L^{-1}$ and 5% methanol. The Figure 4 shows the chromatogram obtained under optimal conditions. The detection limits for the benzodiazepines varied among 2-5 ng $L^{-1}$.

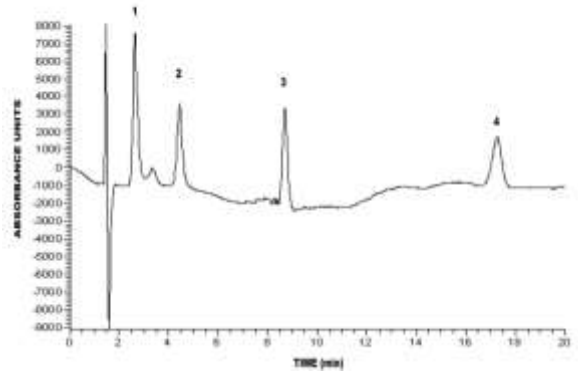



**Figure 4.** Chromatogram of benzodiazepines (100 ng $L^{-1}$). Peaks: 1 clordiazepoxide; 2 bromazepam; 3 clonazepam and 4 diazepam

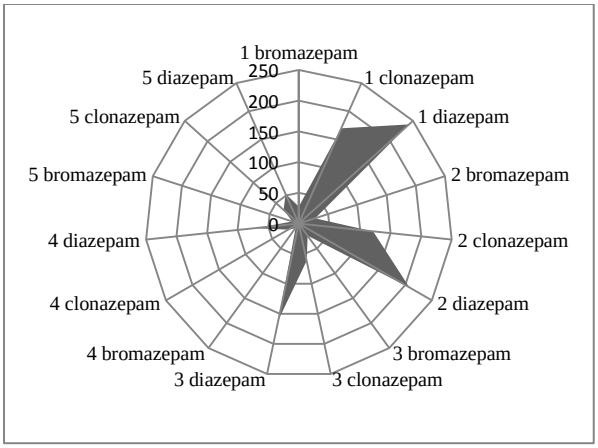
In Table 1 shows the frequencies of detection and the measured concentrations of benzodiazepines (bromazepam, clonazepam and diazepam) detected in surface water samples of the Guandu River.

**Table 1.** Bromazepam, clonazepam, and diazepam concentration (ng $L^{-1}$) in surface water samples of the Guandu River, Rio de Janeiro, Brazil

| Guandu River | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Sites** | **BZD** | **Statistical analyses** | | | | | | | |
| | | **X** | **Sd(yEr±)** | **Se(yEr±)** | **Min** | **Q1** | **Median** | **Q3** | **Max** |
| **1** | Bromazepam | 27.75 | 13.64 | 6.82 | 11 | 17 | 29 | 38.5 | 42 |
| | Clonazepam | 169.75 | 13.57 | 6.78 | 154 | 159.5 | 169.5 | 180 | 186 |
| | Diazepam | 241.25 | 65.14 | 32.57 | 187 | 199 | 221.5 | 283.5 | 335 |
| **2** | Bromazepam | 27.75 | 7.67 | 3.83 | 18 | 22 | 28.5 | 33.5 | 36 |
| | Clonazepam | 121.5 | 54.79 | 27.39 | 69 | 85.5 | 109.5 | 157.5 | 198 |
| | Diazepam | 207.75 | 75.02 | 37.51 | 118 | 158 | 206 | 257.5 | 301 |
| **3** | Bromazepam | 24.25 | 6.5 | 3.25 | 18 | 19.5 | 23 | 29 | 33 |
| | Clonazepam | 61.5 | 29.49 | 14.74 | 34 | 40.5 | 55 | 82.5 | 102 |
| | Diazepam | 160.5 | 69.69 | 34.84 | 99 | 105 | 147 | 216 | 249 |
| **4** | Bromazepam | 9 | 2.82 | 1.41 | 5 | 7 | 10 | 11 | 11 |
| | Clonazepam | 19.5 | 3.87 | 1.93 | 15 | 16.5 | 19.5 | 22.5 | 24 |
| | Diazepam | 71.75 | 27.41 | 13.7 | 43 | 49 | 71 | 94.5 | 102 |
| **5** | Bromazepam | 11.25 | 2.98 | 1.49 | 8 | 9 | 11 | 13.5 | 15 |
| | Clonazepam | 33.5 | 6.13 | 3.06 | 26 | 29.5 | 33.5 | 37.5 | 41 |
| | Diazepam | 52.75 | 18.6 | 9.3 | 26 | 40.5 | 58.5 | 65 | 68 |

X – mean; Sd – standard deviation; Se – standard error; Min – minimum; Q1 – lower quartile, Q3 – upper quartile, Max - maximum

As shown in Figure 5 there were the major concentrations of all pharmaceuticals researched on the three stretches upstream from the WTP, and the minor ones at the two downstream, denoting that although the proceeding done at WTP was satisfactory in sanitary terms, yet is not sufficient to remove them from the water supply.




Sites: 1, 2, 3 – upstream WTP 4, 5 – downstream WTP

Figure 5. Pharmaceuticals concentration in Guandu river before and after Water treatment plant

## DISCUSSION

One of the main challenges in monitoring the occurrence of psychiatric pharmaceuticals in waste, surface and ground waters (as for all pharmaceuticals, in general), is the lack of simple, sensitive and low cost analytical methods to quantify pharmacologically active substances (and their metabolites) in the concentration range of ng $L^{-1}$ to μg $L^{-1}$ [23, 25]. However, in the last decade, major advances have been achieved with the development of new analytical methods that allow the quantification of trace amounts of pharmaceuticals [26]. The first efforts in the field of pharmaceutical trace analysis often resulted in single-analyte methodologies but, nowadays, the development of multi-analyte methodologies, capable of quantifying up to several dozens of pharmaceuticals is becoming more and more common [27, 28]. The development of robust analytical methodologies is of crucial importance taking into account that all steps involved in a proper environmental risk assessment would depend, inevitably, on the existence of reliable analytical data.

In environmental research, benzodiazepines are among those pharmaceuticals less commonly addressed [25-29]. Diazepam has been detected in WWTP originating from hospitals as well as in effluents from municipal WWTP plants. Ternes et al. [29] and Martínez Bueno et al. [30] found levels of diazepam as high as 53 ng $L^{-1}$ and 87 ng $L^{-1}$, respectively, in WWTP effluent. Diazepam was further found at 33 ng $L^{-1}$ in German rivers [29], up to 21 ng $L^{-1}$ in rivers in the region of Madrid [30] and Zuccato et al. [31] found up to 23.5 ng $L^{-1}$ diazepam in drinking water. Benotti et al. [6] found 0.49 ng $L^{-1}$ in untreated drinking water in the U.S. In a WWTP in Belgium was found 1.18 μg $L^{-1}$ [7]. In addition, bromazepam and diazepam were determined in the Llobregat River (north eastern Spain), a source of potable water, were found mean concentrations of 7 ng $L^{-1}$, 20 ng $L^{-1}$ and 3 ng $L^{-1}$, respectively [32].

Actually, WWTPs were not specifically intended to remove bioactive xenobiotics and removal efficiencies can range from zero to almost complete removal, depending on specific treatments used in more or less sophisticated WWTP facilities [3]. Biological degradation and sorption are the most common mechanisms applied in WWTP; though, as it will be shown below, the removal efficiencies are not always satisfactory and more advanced techniques should be applied. However, even with the present knowledge, municipal WWTPs have been hardly focused on the removal of trace organic pollutants, such as pharmaceuticals. Even in the case of more sophisticated WWTPs, the large diversity of trace organic compounds and the difficulty of predicting individual responses to more advanced treatments illustrate the complexity of this issue [12, 33]. It must also be emphasized that following the disappearance of a pharmaceutical in the liquid phase is not sufficient to conclude that it was completely removed as it may pass into the solid phase, or exist in a different form of the parent compound due to chemical transformations [3].

Final treated effluents are commonly discharged in surface waters; this can lead to indirect reuse of wastewaters in areas where these surface waters are a source of potable water [4]. Besides, WWTPs effluents are increasingly being used for irrigation of crops and reuse in several countries throughout the world [34, 35]. All these performs have the obvious advantage of reducing the demand for water supplies, which is a focal concern due to the scarcity of potable water, but constitute viable pathways for the introduction of pharmaceuticals in drinking waters through runoff and infiltration.

## CONCLUSION

It is clear that accessible data regarding the presence, cycling and destiny of benzodiazepine derivatives in the environment are insufficient. These compounds are being introduced into the environment on a systematic basis, mainly through WWTPs, as a result of the insufficiency of the treatment processes applied in general. The occurrence of these widely consumed compounds in a large variety of environmental matrices has indicating their high persistence and toxicity to non-target organisms substantiate the growing concern about these relatively recent environmental pollutants. However, there is still a considerable lack of knowledge about the environmental effects, persistence of these compounds, and the impact on public health due to water supply.

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