Human pharmaceuticals surface water pollution across urban gradients in the Buenos Aires Metropolitan Region, Argentina

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Data availability statement

Raw data available on request

Author contributions

Daniela Perez: Conceptualization, Investigation, Methodology, Formal analysis, Data curation, Writing - original draft. Macarena Rojo: Supervision, Conceptualization, Methodology, Writing – review & editing, Diego Cristos: Methodology, Formal analysis, Data curation. Pedro Carriquiriborde: Supervision, Conceptualization, Data curation, Visualization, Writing – review & editing, Funding acquisition.

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HUMAN PHARMACEUTICALS SURFACE WATER POLLUTION ACROSS URBAN GRADIENTS IN THE BUENOS AIRES METROPOLITAN REGION, ARGENTINA

ABSTRACT

Human active pharmaceutical ingredients (HAPIs) are micropollutants of emerging concern for aquatic ecosystems. Adequate access to health systems combined with poor sewerage connectivity and inadequate wastewater treatment plants has placed developing countries among the most vulnerable scenarios regarding HAPIs water pollution. However, field data to better understand this phenomenon is still insufficient for large metropolises of South America. This study aimed to evaluate, in two contrasting seasons of the year, the occurrence of 16 highly prescribed HAPIs in 26 sites distributed along urban gradients of 7 watersheds of the Rio de la Plata estuary southern plain within the Buenos Aires Metropolitan Region. Owing to their average annual concentrations and frequencies, acetaminophen, ibuprofen, carbamazepine, atenolol, enalapril, metformin, and sildenafil were the dominant HAPIs. Total HAPIs concentrations and numbers significantly increased as the watercourses ran into more intensely populated areas. Sampling sites within urban conglomerates receiving WWTP discharges displayed higher HAPIs concentrations and numbers, demonstrating the inefficacy of current plants in eliminating these substances. Sampling sites within urban conglomerates without official discharges also showed high HAPIs concentrations and numbers, pointing out “straight-piping” and septic tank leaking as significant pollution sources. Detecting some HAPIs in rural sites would indicate that other minor sources (e.g., veterinary uses or atmospheric deposition) are also contributing. Shifts in HAPIs frequencies and

concentrations around the overall medians were a better indicator of seasonal variations than the absolute values affected by hydrological conditions. This study enhances understanding of the relationship between urbanization and HAPIs surface water pollution in a major metropolitan area of South America, offering valuable data for future HAPIs aquatic ecological risk assessments in the region.

KEYWORDS

South America, emerging contaminants, hydrological basins, unplanned urbanization, seasonal variations.

1. INTRODUCTION

Human active pharmaceutical ingredients (HAPIs) are currently considered micropollutants of emerging concern in aquatic systems (Patel et al., 2019). Besides manufacturing processes and inappropriate disposal, the primary sources of environmental HAPIs are human excreta that reach surface waters through wastewater (Boxall et al., 2012). Demographics, access to health systems, sewerage connectivity, and sewage treatment systems have been proposed as crucial factors influencing levels of HAPIs pollution (Kookana et al., 2014). Consequently, important differences in HAPIs occurrence frequencies and concentrations have been observed among countries on a global scale (Wilkinson et al., 2022). Higher HAPIs surface water pollution levels have usually been detected in lower-middle-income countries, a fact that has been linked to adequate access to health systems coupled with poor sewerage connectivity and inefficient or absent municipal

wastewater treatment. Differences in the HAPIs' surface water occurrence patterns among regions have also been linked to a broad list of environmental and human factors (e.g., prescription and use).

Human active pharmaceutical ingredients' environmental issues have been mainly linked to the urban water cycle (Brooks, 2018). The relationship between the occurrence of these micropollutants along water courses and the urbanization intensity within their watersheds has been specifically addressed in other regions of the world (Csaba-Kondor et al., 2022; Hong et al., 2018; Ke et al., 2024), but no previous studies have focused on urbanization gradients within metropolises of South America. The Buenos Aires Metropolitan Region (BAMR), in Argentina, includes Buenos Aires City and 43 districts of the Buenos Aires Province with a total surface of 13,975 km^2, including 2,440 km^2 of urban area (DPOUyT, 2007). The region holds about 15 million people and is the most densely populated area of the country (INDEC, 2024). According to the last definitive National Census, on average 57% of the residences of the BAMR were connected to the sewage system, though in some districts the percentage is as low as 8.1% (INDEC, 2024). The worst scenario is within informal neighborhoods usually settled along the watercourses' floodplains.

In earlier studies, the occurrence of a small number of HAPIs in raw or treated municipal wastewater effluents (point sources) was assessed in cities of different sizes, together with their impact on receiving waters (Elorriaga et al., 2013a; Elorriaga et al., 2013b). Later, a bigger group of HAPIs was analyzed in the Matanza-Riachuelo River, a representative river within the BAMR,

compared with other world rivers (Wilkinson et al., 2022). In the present study, the objective was to assess the occurrence of highly prescribed HAPIs in selected BAMR watercourses related to different urbanization intensities within the watershed and the presence or absence of official wastewater treatment plants (WWTPs) discharges, comparing contrasting seasons of the year.

2. MATERIALS AND METHODS

Study area and sampling sites. The BAMR is the most populated area of Argentina, and hydrological basins within this region belong to the Rio de La Plata South Slope (MIySPBA, 2020). For this study, seven water courses were selected, with different population distributions and density patterns within their hydrological basins (Figure 1). According to their length, two to five sampling sites were chosen from the source to the end, resulting in 26 total sampling sites. Selected rivers and sampling sites were: 1) The Lujan River, which flows across a rural area with four medium-sized cities and a neighborhood nucleus along its course before its confluence with the Reconquista River in a conglomerate urban area previous to end in the Río de la Plata estuary; five sampling sites L1, L2, L3, L4, and L5 were selected along it; 2) the Reconquista River, which runs 36 km from its source across a rural area, and then it goes into an urban conglomerate until its confluence with the Rio Lujan, six official WWTPs discharge their effluents into it (AySA, 2023), and the river flows across the largest landfill in Argentina, the CEAMSE, four sampling points were selected along this river: R1, R2, R3, and R4; 3) the Matanza-Riachuelo River, which runs the first sector from the source across a rural area, then goes along a urban conglomerate mainly developed in the north margin, and finally goes through a densely urbanized sector until

ending in the Rio de la Plata estuary, the river receives the discharges of four official WWTPs (AySA, 2023), and five sampling sites were selected along it: MR1, MR2, MR3, MR4, and MR5; 4) the Del Gato Stream, which has it source located within a semi-rural area with horticulture activity, then goes across the northern suburbs of La Plata city, receiving the discharge of the Ringuelet WWTP (ABSA 2018), and finally runs a non-urbanized flood plain with a small hamlet just before its mouth, four sampling sites were selected: G1, G2, G3, and G4; 5) the Maldonado Stream, which is only 8 km long, the first half is within a semi-rural area with horticulture activity, and the second one goes across La Plata city's southeastern suburbs, ending in the Maldonado wetland, currently channelized to the Río de La Plata estuary, no official WWTP discharges alongside its course, and three sites were selected along its course: M1, M2, and M3; 6) The El Pescado, which has a hydrologic basin mostly rural dominated by low-density cattle ranching, though, lately intensive horticulture has increased in the stream head and populated spots has developed in the northern margin of the middle sector without official WWTP discharges alongside its course, three sampling points were selected along its course: P1, P2, and P3; 7) The Espinillo Stream, which has a hydrological basin within a rural area dominated by low-density cattle ranching without official WWTP discharges, and considered as a "reference" watercourse, two sampling points were chosen here: E1 and E2. A fully detailed description of each watercourse and sampling site is given in the Supplementary Material (Doc_SI-1).

Surface water sampling. Two sampling campaigns were conducted, one in summer and the other in winter. Samples were collected from bridges using a stainless-steel bucket attached to a cord following a previous study (Wilkinson et al., 2022), but using larger water volumes. A watercourse

water pre-rinsed 50 ml syringe, bound to a 47 mm filter holder, was used to in situ filter 60 ml of water through a 0.45 µl nitrocellulose filter into an amber glass bottle, pre-loaded with 0.6 µg of each isotope standard, that was conserved in ice until extraction the next day. Complementarily, dissolved oxygen (DO), ammonium (NH_4^+), temperature, electrical conductivity (EC), and pH were also measured in situ. The VISOCOLOR ECO 931-088 and VISOCOLOR ECO 931-010 kits were used to analyze DO and NH_4^+, respectively. Temperature, pH, and EC were measured using a field multiparameter instrument, Lutron WA-2015.

Chemicals. Analyzed pharmaceuticals were selected according to the number of units sold in Argentina (Elorriaga et al., 2013a). Active ingredients of acetaminophen (ACE), alprazolam (ALP), atenolol (ATE), bromazepam (BRO), carbamazepine (CBZ), carvedilol (CRV), clonazepam (CLO), diphenhydramine (DIP), enalapril (ENA), fluoxetine (FLX), ibuprofen (IBU), lorazepam (LOR), metformin (MET), salbutamol (SAL), sertraline (SER), and sildenafil (SIL) were Parafarm® from Drogueria Saporiti, Argentina. The isotope-labeled standard carbamazepine (carboxiamide-$^{13}C^{15}N$) was Sigma-Aldrich, and atenolol-d_7 was Supelco. HPLC grade methanol and acetonitrile were JT Baker, and formic acid and ammonium formate were Sigma-Aldrich.

Water sample extraction. Filtered water samples were processed by solid phase extraction (SPE) using Oasis® HLB cartridges (3 cc, 60 mg) Waters Corporation, according to previous studies (Elorriaga et al., 2013a). The procedure was done according to vendor instructions; the cartridge was preconditioned with methanol and ultrapure water (Type I), loaded with the sample, washed with methanol 5% in ultrapure water, and eluted with 5 ml of methanol. The eluates were

evaporated to dryness in a thermostatic bath at 40 ˚C under a gentle nitrogen stream and resuspended in 0.6 ml (100X concentration factor) of a 50:50 methanol and acetonitrile solution.

Instrumental analysis. HAPIs were analyzed using an ultra-high-performance liquid chromatograph equipped with an autosampler (ACQUITY® UPLC, Waters Corp.) attached to an electrospray ionization (ESI) source coupled to a mass spectrometer (ACQUITY® SQD, Waters Corp.). The sample injection volume was 20 µL. The instrument was run according to a previous study (Rojo et al., 2021). A ternary mobile phase consisting of ACN/MeOH (50:50) and ultrapure water, with 0.1% formic acid and 5 mM ammonium formate as modifiers, was used for chromatography, based on the information in Table 2 of the United States Environmental Protection Agency (USEPA) Method 1694 (USEPA, 2007). Separation was performed using an XSelect CSH C18 column (3.5 µm particle size, 4.6 mm i.d., and 75 mm length), running a gradient program of 11 min starting from 80:20 to 0:100 (aqueous: organic) at a flow rate of 0.3 ml/min and a column temperature of 50 ˚C. ESI was carried out in single ion monitoring (SIM) mode under positive or negative ionization mode for quantification and confirmation of the selected ions of each pharmaceutical. ESI source parameters were set as follows: cone gas flow 1.1 ml/s, desolvation gas flow 193 ml/s, capillary voltage 3240 V, cone voltage 35 V, source temperature 148 ˚C, and desolvation temperature 350 ˚C. Control and data acquisition were performed using the MassLynx™ software (Waters-Corporation, 2021). Quantification and confirmation ions used for each HAPI, besides the cone voltage, are given in Table SI-1.

Quality assurance. The recovery for the entire method was calculated in triplicate at two spiking levels of the selected pharmaceuticals using pooled aliquots of rural samples from each season. Recoveries were calculated by spiking the field samples and SPE extracts to overcome matrix effects as in Trufelli et al. (2011). Briefly, rural sample pools were divided into three aliquots, two were spiked before SPE at concentrations of 0.1 and 1 µg/L. Then the three aliquots were extracted by SPE, and the extract of the non-spiked aliquot was split into three sub-aliquots, two spiked at concentrations of 10 and 100 µg/L (equivalent to the 100X sample concentration during the SPE). Then, the areas of each pharmaceutical standard in the pre-SPE spiked aliquot (A1) were compared to the respective ones in the post-SPE spiked extract (A2), for both spiking levels, after the subtraction of the respective areas in the no-spiked aliquot (A0), using the equation: $Recovery_{(li)} = (A1_{(li)} - A0)/(A2_{(li)} - A0) \times 100$, where li denotes the spiking level. The recoveries of each pharmaceutical obtained for every spiking level and season were averaged.

The method detection (MDL) and quantification (MQL) limits for each analyte were calculated according to the definition given in section 1010 C Terminology in the APHA-AWWA-WEF (2017). Briefly, a five-point calibration curve for each pharmaceutical (0.1, 1, 10, 100, 1000 ug/L) was built on pooled aliquots of rural sample extracts from each season and run in triplicate. Linearity was maintained throughout the whole interval. The MDL and MQL were calculated as three and ten times the standard deviation of the lowest standard significantly different from the blank, then corrected by recovery percentage.

All pharmaceuticals were quantified using the "Standard Addition Method" (APHA-AWWA-WEF, 2017) to overcome the matrix effect on the ESI source. Briefly, each sample extract was split into three aliquots, one run without spiking and the others spiked at 10 and 100 µg/L of the mix of pharmaceutical standards. The concentration of each drug in the samples was obtained as the modulus of the regression line intercept on the x-axis, corrected by the recovery. Complementarily, the sample-spiked isotope-labeled standards of ATE and CBZ were quantified by isotope dilution mass spectrometry as quality assurance controls. In addition, a matrix control fortified with the lowest standards mix and a solvent blank was intercalated every five samples to check for potential instrumental drift and column carryover.

Data analysis. For the statistical analyses, censored data <MDL and <MQL were respectively replaced by MDL/2 and (MDL+MQD)/2 according to the "Simple Substitution Method" (Helsel and Hirsch, 2002).

Site classification was based on the census radio population data of the National Institute of Statistics and Census of Argentina (INDEC, 2024) and the short-terms classification for the degree of urbanization proposed by the EUROSTAT (2021). Classes were: i) Urban conglomerate for densely contiguous populated areas, distinguishing between those receiving (UCR) or not (UCNR) WWTP discharges, ii) Isolated Urban Nucleuses for spotted urban areas (IUN), and iii) Rural Areas for very low density or unpopulated areas (RA), considering a 2 km radius of influence upstream of the sampling point. Additionally, a second classification criterion was used based on the river sector: source (S), middle (M), or end (E). Sites classified as S were close to the

watercourse head, placed in a first or second-order stream. Sites classified as E were situated near the watercourse mouth, a few kilometers before ending into the Rio de la Plata Estuary and under its tidal influence. Sites classified as M were third or fourth-order watercourses, located between S and E. To compare the hydrological conditions between summer and winter campaigns, rainfall data during the sampling months were obtained from the National Meteorological Service of Argentina, and the river flow of the Matanza-Riachuelo River at site MR3 was obtained from the River Basin Authority (ACUMAR).

One-way analysis of variance (ANOVA), followed by the post-hoc Fisher's Least Significant Difference (LSD) Test, was used to test differences in the average of total concentrations (THAPIs) and physicochemical parameters among sites and sampling campaigns. In those cases where ANOVA assumptions were not met, the non-parametric Kruskal-Wallis ANOVA was used followed, by a means-rank multiple comparison. Pearson correlation was used to evaluate the relationship between NH_4^+ concentration and THAPIs (Zar, 2010).

Occurrence frequency (%OF) for each HAPI was calculated as the number of detections over the total number of samples analyzed. HAPIs were plotted according to their occurrence frequency and the average concentration using an Olmstead-Tukey diagram (Fisher, 1983), and classified as dominant, frequent, occasional, and rare (Ernst et al., 2018), if the average concentration and frequency of each HAPI were above or below the median concentration or frequency of all HAPIs. Principal component multivariate analysis (PCA) was performed to assess associations among

HAPIs and with physicochemical parameters, and to analyze how such associations cluster sampling sites within different populated sectors (Harlow, 2005).

3. RESULTS AND DISCUSSION

This study reports the occurrence of 16 highly prescribed HAPIs in 26 sites distributed along urban gradients of 7 hydrological basins of the Rio de la Plata estuary southern plain within the BAMR for two contrasting seasons of the year. The information regarding sites classification, concentrations, and frequencies of the selected HAPIs, as well as the detection and quantification limits, method recoveries, and values of the water physicochemical parameters measured in the two sampling campaigns, is available in the supplemental material (Table SI-2). The DO and IBU data for the summer campaign were unavailable due to instrumental and analytical drawbacks. Additionally, the E1 winter sample was lost during processing.

The average total concentration (THAPIs) and detected numbers (NHAPIs) of human active pharmaceutical ingredients of the two sampling campaigns for each site are shown in Figure 2. This figure illustrates how NHAPIs and THAPIs increased as the water courses entered densely urbanized areas. The two-campaign average THAPIs showed the Reconquista River middle sector (R2 18.8 µg/L and R3 18.6 µg/L) as the highest polluted watercourse within the BAMR. On the other hand, the Espinillo stream (E2 0.5 µg/L) was the least impacted. During the winter campaign, the watercourse with the highest THAPIs was the Reconquista River (R2: 33.3 µg/L, R3: 31.7 µg/L, R4: 19.7 µg/L) followed by the Maldonado Stream (M3: 20 µg/L and M1: 14.0 µg/L), then the Del Gato Stream (G3: 19.9 and G4 14.9 µg/L), and only after them the Matanza-Riachuelo

River (MR4: 12.7 µg/L and MR3: 9.3 µg/L), which had been previously classified among the 15th most polluted rivers in the world by these emerging pollutants (Wilkinson et al., 2022). In the summer campaign the most polluted watercourse was the Matanza-Riachuelo River (MR5: 18 µg/L, MR4: 3.7 µg/L, and MR: 2.6 µg/L), followed by the Maldonado Stream (M1: 6.6 µg/L and M3: 3.7 µg/L), the Reconquista River (R3: 5.6 µg/L, R4: 5.2 µg/L, and R2: 4.4 µg/L), and the Del Gato Stream (G3: 5.1 µg/L and G3: 2.9 µg/L, and G2: 2.8 µg/L).

A statistically significant effect was found on the THAPIs, NHAPIs, and NH_4^+, but not on the EC, associated with the urban-intensity classes, increasing from RA to IUN, then UCNR, and finally UCR (Figure 3). Results show that THAPIs, similarly to the well-known NH_4^+, are a consistent indicator of water quality impairment associated with urban intensification. Among urban-impacted sites, those urban conglomerates receiving WWTP discharges (UCR: R2-4, MR3-4, L5, and G3-4) presented the worst conditions regarding HAPIs and NH_4^+ concentrations, empirically corroborating that current WWTPs in the region are inefficient at eliminating HAPIs collected by the existing sewerage system. Those sites within the urban conglomerate not receiving WWTP discharges (UCNR: M1-3, MR2, and G1-2) were in second place, demonstrating that informal straight-piping to the water courses and septic tank leaks into the water table would be important sources of HAPIs in the region. Those sites placed downstream WWTP discharges from isolated urbanization nuclei (IUN: L1-4) were in third place, showing how the impact of HAPIs on the water quality of that kind of urbanization is extended to downstream unpopulated sectors. The unpopulated sites (RA: R1, MR1, P1-3, E1-2) showed the lowest values, but unexpectedly,

up to 7 HAPIs were detected, though concentrations were markedly lower than in populated sectors.

The relationship between HAPIs surface water pollution and urban developments concerning the type of wastewater treatments has been previously studied in North America (Schaider et al., 2017; Scott et al., 2019), Europe (Csaba-Kondor et al., 2022; Wilschnack et al., 2024), and Asia (Hong et al., 2018), but not in South America. Our results contribute, with field data, to understanding the high impact of urbanization on HAPIs surface water pollution in upper-middle income countries linked to both the existence of inadequate WWTPs for HAPIs elimination and diffuse sources due to low sewerage connectivity coverage, previously identified based on a bibliographic data review (Kookana et al., 2014). Despite efficiency depending on treatment types and chemicals, it is known that conventional centralized (WWTPs) or less-efficient on-site treatment systems (OSTS), such as septic tanks (ST), are unable to reduce HAPIs completely (Du et al., 2014). Therefore, the observed HAPIs impact on urbanized watersheds may be partially attributed to the inability of those treatment systems to remove these chemicals. However, as discussed below, ACE detection in high concentrations, a drug usually well-reduced by the conventional treatment systems, would indicate that straight-pipelines and raw sewage leaking from cesspools would also be important pollution sources. On the other hand, the occurrence of HAPIs in rural unpopulated basins agrees with the HAPIs surface water contamination recently found in English National Parks (Boxall et al., 2024), and it points to atmospheric transport and deposition (Ferrey et al., 2018) or veterinary uses (Abdallah et al., 2024) as other sources to be considered.

When the above-assessed variables were related to the watercourse sectors (Figure 4), increasing values from the source to the mouth were observed, but in this case, differences were not statistically significant. Additionally, NH_4^+ concentrations were higher in the middle sector than in the end, a fact that could be explained because NH_4^+ is usually linked to other pollution sources, such as slaughterhouses and food processing facilities outfalls, which represent 16 and 26 % of all industries within the Matanza-Riachuelo basin. (ACUMAR, 2025). Similarly, in a study conducted in the USA, the elevated nitrogen concentrations found in reference sampling sites were linked to additional non-household discharges (Guyader et al., 2018). Therefore, according to this site classification, HAPIs occurrence resulted in a more specific indicator of urbanization impact on water quality than NH_4^+ concentration.

All the above-assessed variables were significantly different between seasons (Figure 5). The EC, NH_4^+, and THAPIs were higher in winter than summer, while the opposite was observed for the NHAPIs. The average THAPI concentrations in winter and summer were 8.83 and 2.99 µg/L, while the average frequencies at each season were 26 % and 40 %. The average rainfall during the month before the sampling campaign and the river flow at R3 were 45.3 mm and 47,812 m^3/s in winter and 142.7 mm and 92,842 m^3/s in summer. The higher THAPIs concentration in winter could be thought of as a higher drug prescription in the cold season. However, if this is linked with the lower conductivity and NH_4^+ values and higher rainfalls and flow observed in summer, it could better be associated with a dilution process. An inverse relationship between the ACE concentration and the river flow was found in the Cancela-Tamandaí catchment, south Brazil (Ghesti Pivetta and Do Carmo Cauduro Gastaldini, 2019). Likewise, higher concentrations of

HAPIs in the dry season than in the wet season were reported in the Jiulong River watershed, southeastern China (Hong et al., 2018). On the other hand, the higher detection frequencies and NHAPIs in summer would be mainly explained by the lower MDLs obtained for samples collected in that season due to a lower matrix effect linked to more diluted river water.

The average concentrations and frequencies obtained for each HAPI, together or separated by sampling campaigns, are shown in Figure 6. The sixteen studied HAPIs were detected between 2% (e.g., SER) and 88 % (e.g., CBZ) of the samples, with average concentrations ranging from 0.025 µg/L (ALP) to 3.56 µg/L (ACE). The annual median for the detection frequency of all the compounds was 55 %, with wide differences between summer (81 %) and winter (42 %), mainly explained by the lower MDLs obtained in summer. The median for the HAPIs concentration was 0.17, 0.07, and 0.12 µg/L for the annual average, and the summer and winter campaigns, respectively. The three HAPIs showing the higher concentration were: ACE (max: 30 µg/L, mean: 10.1 ± 9.9 µg/L), CBZ (max: 15 µg/L; mean: 1.1 ± 3.3 µg/L), and IBU (max: 8 µg/L; mean: 3.8 ± 2.3 µg/L), during the winter campaign. Seven HAPIs were overall classified as dominant (ACE, IBU, CBZ, ATE, ENA, MET, and SIL), three as frequent (CLO, BRO, and LOR), two as occasional (CRV and SAL), and four as rare (SER, DIP, FLX, and ALP). Frequencies and concentrations of ACE, CBZ, and ATE were in the same order of magnitude as those previously found in the Matanza-Riachuelo river (Wilkinson et al., 2022), not only confirming the result obtained in that sampling, but also placing the BAMR watercourses among the most polluted with HAPIs worldwide. Additionally, ACE, IBU, ATE, MET, and CBZ values were like those reported for the Suquía River, in the Cordoba Province, Argentina (Valdés et al., 2014; Valdés et al., 2023),

differing mainly in the concentrations of CBZ and the concentration and frequency of ENA that were higher in the BAMR. Concentrations of ACE, IBU, ATE, and CBZ were like those recently reported in the Reconquista and Lujan Rivers (Mastrángelo et al., 2022). ACE, IBU, CBZ, and ATE were also among the most relevant pharmaceuticals detected in different Brazilian urban rivers (de Jesus Silva Chaves et al., 2020; Ribeiro de Sousa et al., 2018). Little information is still available globally for SIL, which appears in our study as a dominant HAPI.

Particularly interesting is the comparison between ACE and CBZ, as the former has been reported to be more efficiently removed by WWTP and STS than the latter, which is more persistent (Du et al., 2014). The average ACE/CBZ ratio for UCR and UCNR sites was 28.7 ± 20.1 and 14.9 ± 20.3, respectively, indicating higher concentrations of ACE in both site classes. Contrary to expectations, no significantly higher ratios were observed in UCNR compared to UCR sites, suggesting that raw or poorly treated sewage reached the watercourses regardless of whether WWTPs were present in the area.

Regarding occurrence patterns between sampling seasons, some HAPIs did not shift from classes while others did. For example, SIL and BRO were dominant in summer but rare in winter, while the opposite was observed for SAL, ENA, and MET, which were dominant in summer but frequent in winter, and SER and DIP, which were rare or occasional in the same seasons. Among the HAPIs that didn't shift between seasons, ACE, ATE, and CBZ were dominant, CLO was frequent, and FLX, CRV, ALP, and LOR were rare, independently of the season. The shift of HAPIs between classes would reflect changes in prescriptions and uses among seasons, since the median was used

as a reference value to neutralize seasonal variations in the occurrence frequencies due to the differences in the detection limits. For example, the erectile dysfunction drug SIL was classified as dominant in the summer and rare in winter, consistent with the season when sexual activity has been reported to be higher (Wellings et al., 1999).

According to the multivariate analysis, 56.8 % of the total variance was explained by the first three factors. In Figure 7, variables (left panel) and sites (right panel) factor scores are plotted for the first two factors. Factor 1 (23.3 %) was mainly explained (factor loading >0.7) by occasional and rare HAPIs (DIP, FLX, CRV, and SER), which discriminated against site M1 from the winter campaign, indicating a particular situation for this sampling point. Factor 2 (18.6%) was mainly explained by dominant HAPIs (ATE, ACE, MET), together with THAPIs and NH_4^+, and the site-score values on this factor significantly discriminated against urbanized (conglomerate and isolate nucleus) and rural sites (Kruskal-Wallis; $p<0.05$). Factor 3 (14.9 %) was mainly explained by temperature and SIL (not plotted), consistent with the water thermic differences between winter and summer and the shift in the detection frequencies of this HAPI among seasons. Those HAPIs classified as frequent did not significantly contribute to any of the three main factors. SIL and SAL overlap dominant and occasional/rare polygons since they were two major season-swinging HAPIs.

4. CONCLUSIONS

The study empirically measured, for a major metropolitan area of a South American upper-middle income country, the impact of urbanization on HAPIs surface water pollution. It was

concluded that the number, concentration, and frequency of HAPIs were directly linked with the degree of urbanization and closely associated with the water quality parameter NH_4^+, regardless of whether sewage connectivity was served and WWTPs existed. This conclusion evidences the importance of providing sewerage connectivity systems in current urban areas whenever linked to suitable WWTPs. Additionally, regarding water quality and freshwater ecosystem health, it remarks on the environmental cost of urban growth without sewage connectivity and WWTPs for future urban planning.

Additionally, the study provides valuable data regarding concentrations and frequencies of highly prescribed HAPIs in the region, concluding that ACE, IBU, CBZ, ATE, ENA, MET, and SIL are dominant, BRO, CLO, and LOR are frequent, SAL and CRV are occasional, and ALP, DIP, FLX, and SER are rare. Also, the study concluded that shifts in the HAPIs frequencies and concentrations around the overall medians were a better indicator of seasonal variations than the absolute values, identifying SIL and SAL among the most sensitive HAPIs to these changes. These conclusions will be useful for further aquatic ecological risk assessments of HAPIs and prioritization for establishing regional water pollution control policies for these substances.

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Legends to figures

Figure 1. Satellite image of the Buenos Aires Metropolitan Region (BAMR) showing the studied watercourses (clear blue lines), sampling sites (blue circles), wastewater treatment plants (red triangles), and populated (gray areas) and unpopulated areas (green areas). L1, L2, L3, L4, and L5: Lujan River sites 1 to 5, respectively. R1, R2, R3, and R4: Reconquista River sites 1 to 4, respectively. MR1, MR2, MR3, MR4, and MR5: Matanza-Riachuelo River sites 1 to 5, respectively. G1, G2, G3, and G4: El Gato Stream sites 1 to 4, respectively. M1, M2, and M3: Maldonado Stream sites 1 to 3, respectively. P1, P2, and P3: El Pescado Stream sites 1 to 3, respectively. E1 and E2: Espinillo Stream sites 1 and 2, respectively.

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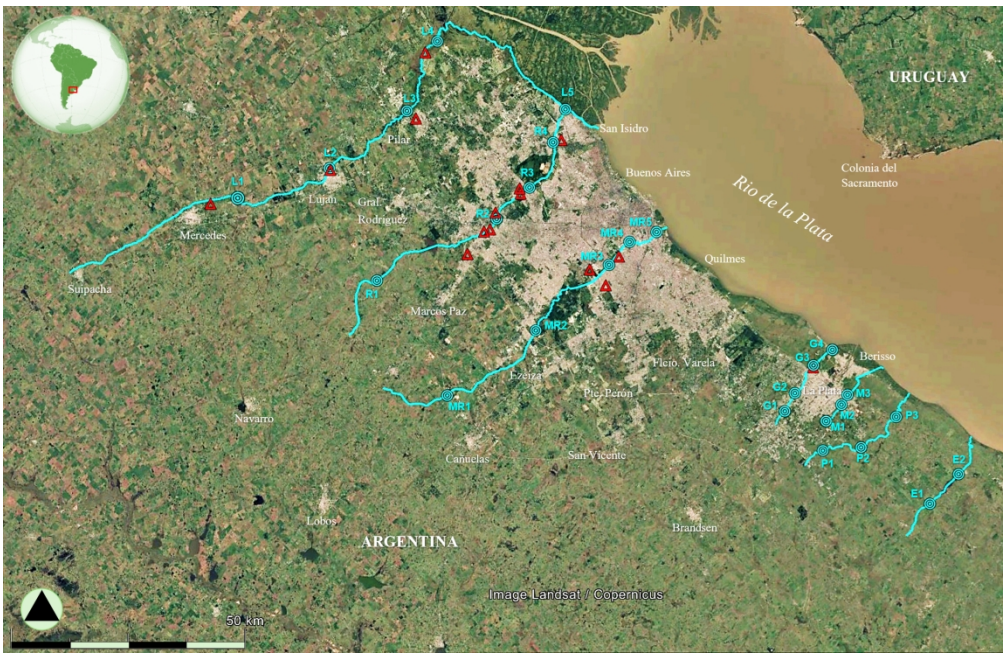

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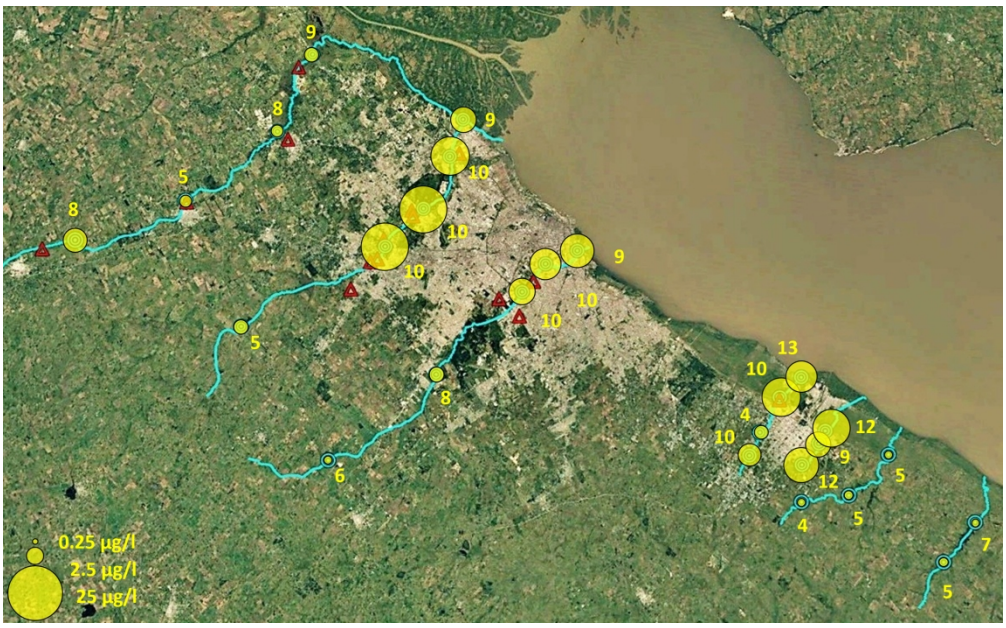

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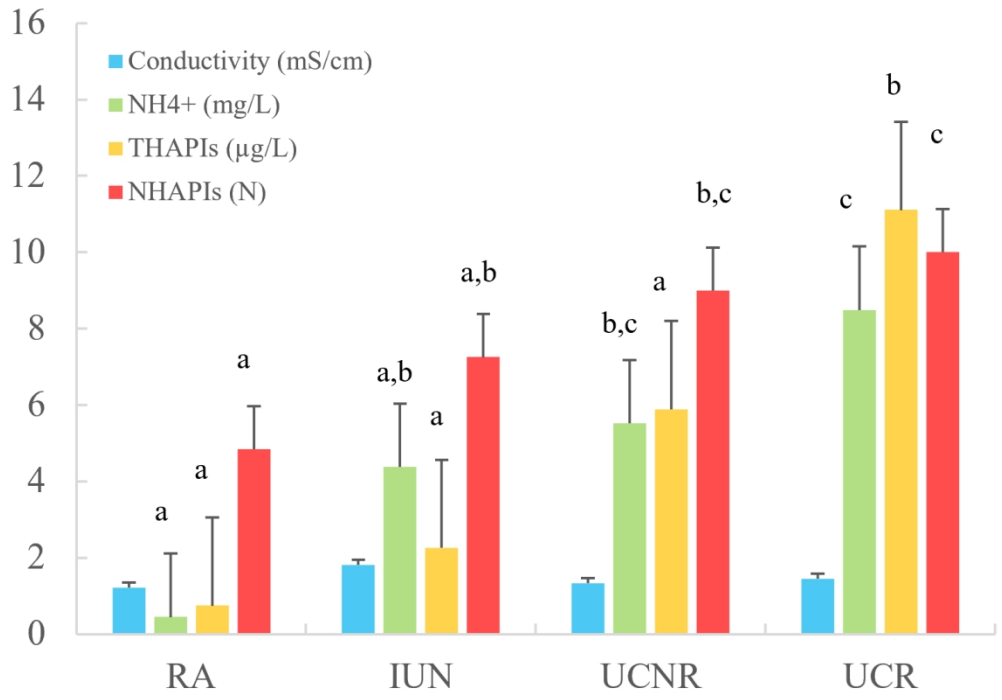

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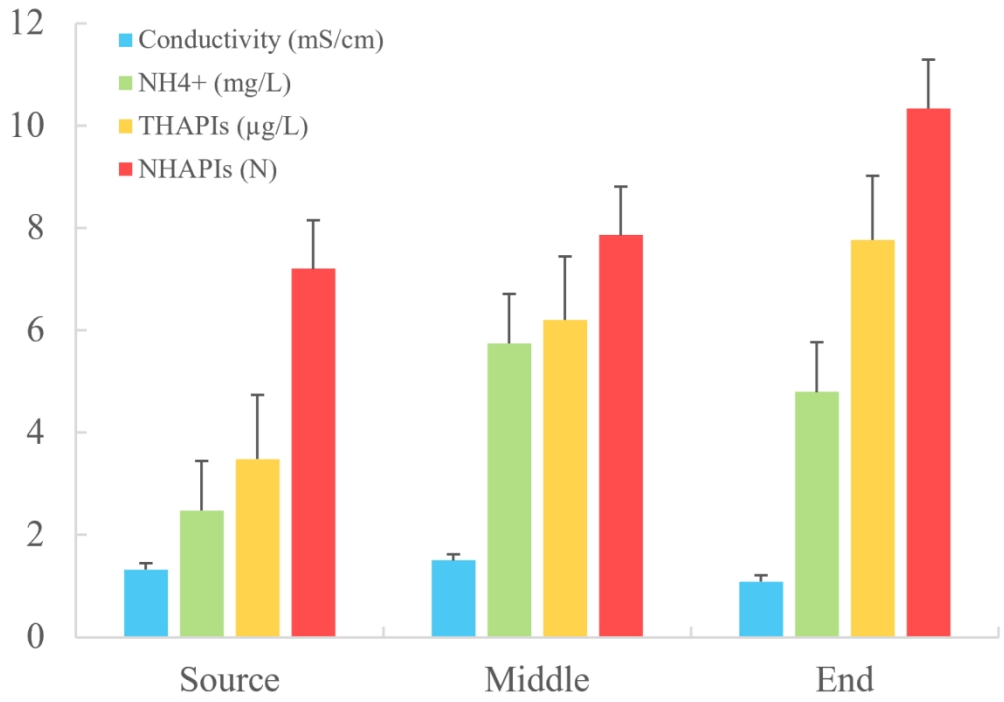

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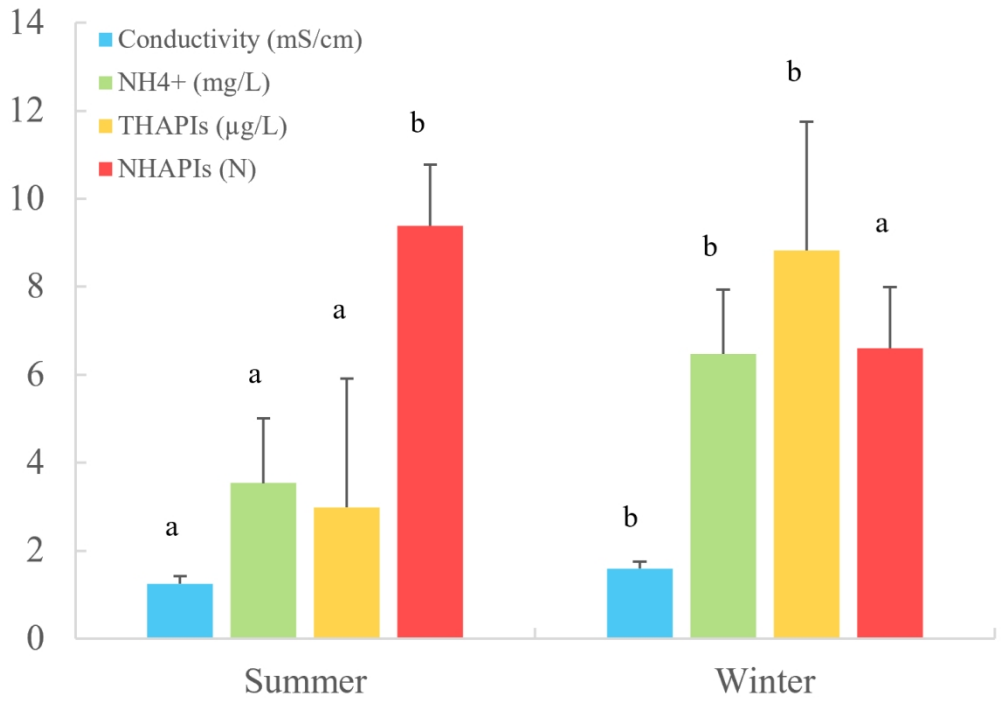

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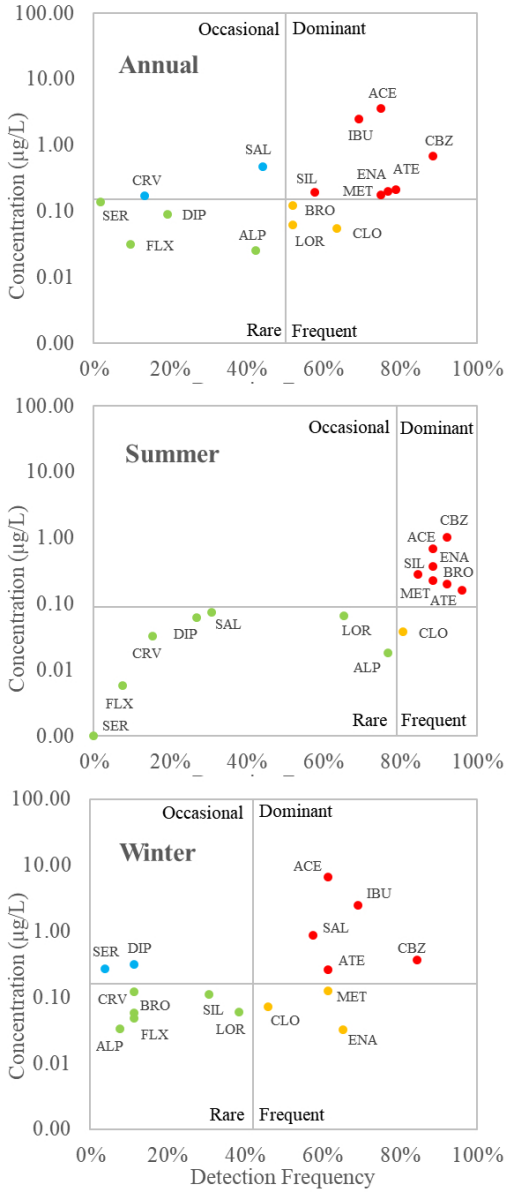

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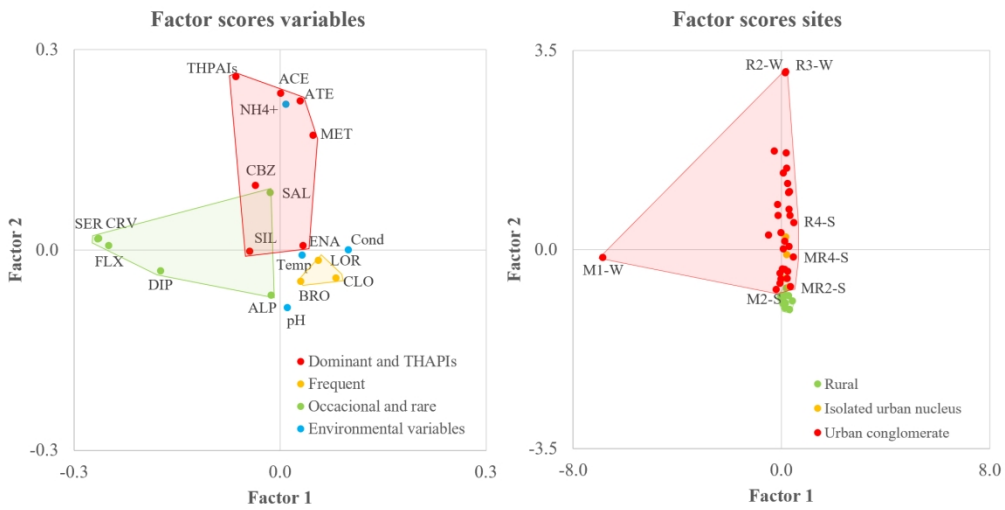

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