



Behavior of ionophore antibiotics in aquatic environments in Argentina: The distribution on different scales in water courses and the role of wetlands in depuration



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ABSTRACT

We studied for the first time three ionophore anticoccidial drugs: monensin (MON), lasalocid (LAS), and salinomycin (SAL) as emerging pollutants originating from animal and plant husbandry in surface waters ($n = 89$) in one of the most extensive hydrological basins in South América (Del Plata basin). The soluble fraction of ionophores was pretreated by solid-phase extraction and analyzed by LC-MS/MS at a limit of detection of $1.7 \text{ ng}\cdot\text{L}^{-1}$. A statistical approach noted the need to report parameters calculated by methods based on the number of observations and the censorship percentage over substitution methods for more precise estimations of environmental data with a high percentage of left-censored data. Water collectors adjacent to intensive-husbandry facilities, placed in direct runoffs from animal excreta, or in wastewater emissions contained median concentrations of MON and SAL approximately 70 times higher than those found in regional tributaries and main courses of 5 sub-basins of the pampas and mesopotamic regions, thus exhibiting a relevance to other similar agricultural pollutants widely reported as pesticides. Chemical speciation of these compounds in surface water was characterized especially for MON and SAL, where the pH and chemical oxygen demand of the natural water body was associated with the concentration of the soluble fraction. The concentrations in abundant rivers such as the Gualeguay deliver a contribution to a natural wetland such as the Paraná-River delta, which registered only one sample with a $[\text{MON}] \leq$ the limit of quantification. Since wetlands possess a limited removal capability, these affluent contributions recorded strongly indicate that attention must be paid to the development of guidelines involving quality criteria for assessing the impact of ionophore antibiotics on such ecosystems.

1. Introduction

Among the spectrum of veterinary products, the ionophore antibiotics such as monensin (MON), salinomycin (SAL) and lasalocid (LAS), along with others are widely used in both livestock and poultry production (Novilla, 2011). The ionophores are supplied in animal feed for their growth-promoting and coccidiosis-preventive activities, the latter illness being prevalent in poultry farms where overcrowded conditions—with the average density reaching up to 21 animals/m² (Thaxton et al., 2014)—favor the spread of the disease. Despite having been considered less hazardous than other pharmaceuticals in terms of direct adverse consequences on the environment, ionophores are not exempt from promoting antibiotic resistance in the environmental microbiome (Butaye et al., 2003; Furtula et al., 2010; Watkinson and

Costanzo, 2017).

Animals excrete up to 90% of the antibiotic dosage (Sassman and Lee, 2007) through both the urine and the feces, with quantifiable concentrations being present in the bovine feces. The disposal of those feces is considered a primary source of the antibiotics in the environment, with an increased potential exposure in facilities that involve ineffective (if any) wastewater and solid-waste management (Sarmah et al., 2006; Watanabe et al., 2008). In addition, the replacement of synthetic fertilizers in applications to the land with ionophore-containing liquid manure or poultry litter releases the antibiotics into the soil, from where those agents can leach into groundwater, reach surface water by agricultural runoff, or even become incorporated into food products (Sun et al., 2013).

The presence of ionophores in environmental surface water, soil,

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and sediments has been reported in the USA, the EU, and Asia. MON was detected at levels from 5 to 350 $\mu\text{g}\cdot\text{kg}^{-1}$ in soils from USA and Argentina (Sun et al., 2013; Yoshida et al., 2007). Moreover, up to 2389 $\text{ng}\cdot\text{L}^{-1}$ of MON and 9022 $\text{ng}\cdot\text{L}^{-1}$ of SAL were detected in runoff water from litter-fertilized land, thus underscoring the mobility of ionophores and the role of runoff as a major source of the compounds in surface water bodies. In particular, MON, SAL, and LAS were registered in surface water bodies at concentrations ranging from 1000 $\mu\text{g}\cdot\text{L}^{-1}$ to 1.172 $\mu\text{g}\cdot\text{L}^{-1}$, with maximum concentrations of 40 $\mu\text{g}\cdot\text{L}^{-1}$ of MON being detected in wastewater lagoons (Kim and Carlson, 2006; Lissemore et al., 2006; Sassman and Lee, 2007). Furthermore, these pollutants were detected in bottom-sediment samples at higher concentrations than the levels present in water from the same streams (Kim and Carlson, 2006). MON also was found in groundwater near livestock facilities at maximum concentration levels of 2.350 $\mu\text{g}\cdot\text{L}^{-1}$ (Bartelt-Hunt et al., 2011).

In Argentina, intensive animal-production systems arose > 20 years ago and up to date, still constitute a significant fraction of the market. Consequently, those systems demand large quantities of poultry and cattle feed, with average dosages of ionophores ranging from 3 mg to 150 $\text{mg}\cdot\text{kg}^{-1}$ of feed, depending on the active substance and the target animal (Landoni and Albarellos, 2015; Novilla, 2011). The rolling pampas concentrates > 70% of livestock production, with Buenos Aires and Entre Ríos (Table 1) constituting the highest cattle (34%) and poultry (50%) production in the country (SAI, Argentina).

The study site comprised several major sub-basins—namely, those of the Paraná and Uruguay rivers and their many affluent streams as well as the Paraná-River delta to the south (Fig. 1)—as such constituting the second largest wetland system in South America and the sixth in the world, at a total area of 17,500 km^2 (Kandus et al., 2010). Natural wetlands perform essential functions—indeed, services—in the environment depending on flood-drought cycles, and have the capability of dampening the effects of flooding, reducing erosion, promoting sedimentation, and transforming nutrients and pollutants in both dissolved and particulate form by a combination of physical, chemical and biologic processes (Hammer, 1992; Depetris and Kempe, 1993). Since other agriculture-originating pollutants—e.g., pesticides—have been reported in the affluent courses and tributaries of the Paraná-River delta (Etchegoyen et al., 2017), the input into the wetland of emerging pollutants other than the ionophore antibiotics should also be studied in order to assess the current capability of the ecosystem to depurate those pollutants.

The rise in contaminants emerging from agricultural activities and the influence of those agents on the aquatic environment (Boxall, 2012) has become a matter of global concern. In view of the systematic and extensive use of ionophores in animal husbandry, the application to the fields of organic supplements consisting of bovine manure and poultry litter, and the movement of those compounds into surface waters; we studied the presence and spatiotemporal distribution of the ionophore antibiotics MON, SAL, and LAS in surface water bodies representative of the pampas region and assessed the association between different

sources of those compounds in the aquatic environment, as well as the performance of a natural wetland in their depuration.

2. Materials and methods

2.1. Study area

The monitoring sites (Table 1 and Figs. 1, 2) were divided into the following 3 categories upon considering the influence conferred by the husbandry activities: primary collectors (PCs); main courses and tributaries (MTs), and wetlands (WLs). For the PC samples, we monitored the low-flow water collectors adjacent to production facilities (cattle and poultry) from representative sections of the pampas region since those areas are direct recipients of feces and runoff and, as such, a primary source of those contaminants for other water bodies. In contrast, we sampled 5 locations in the Paraná-river delta (area WL in Fig. 1) without direct influence of ionophore antibiotics. Nevertheless, this area has exhibited subsequent environmental influences (Mesa et al., 2015). Despite the introduction of cattle in recent decades, the management practices have involved grazing without the use of growth promoters. Therefore, *in-situ* inputs of ionophore antibiotics were not expected in the delta. As to the category of main courses and tributaries (MT), surface-water samples were taken at the main course of major rivers, or at the confluence of their principal tributaries, according to accessibility, in order to assess the previous input of ionophores. The MT category includes the following 4 sub-basins (Fig. 2): Gualeguay (GUA), Uruguay (URU), Paraná (PAR), and Saladillo (SAL). This area constitutes the agroproductive core of Argentina, concentrating > 70% of both the crop and the livestock production of the country. In terms of animal production, cattle and poultry facilities are distributed mostly within the Buenos Aires and Entre Ríos provinces. The study sites were selected according to their predominant activity: cattle, poultry, or egg production. According to Castillo et al. (2016), based on animal density (Fig. 1) and the type of aviculture performed, the URU sector there in the eastern region of the province is characterized by meat production, namely broiler chickens; whereas egg production predominates in the western area, whose limit is the Paraná River (PAR). The Gualeguay sub-basin (GUA) constitutes a mixed-use land that includes poultry and cattle production. To assess the productive relevance of GUA and that sector's direct influence on the Paraná-River delta, we studied both hydrological systems in detail in all 4 seasonal sampling periods. The study site as a whole comprised representative water bodies in the mesopotamic and pampas regions, located in the Del-Plata basin. For the mesopotamic region, the northern section of the Paraná River (AP) was taken as a complete section that included tributaries from near the Paraguay border up to the Corrientes province (Argentina).

2.2. Sample collection

The water samples from rivers were collected from the first 50 cm of the water column and filtered *in-situ* through nylon membranes of 0.45-

Table 1
Description of the sampling region and sections monitored.

Province	Entre Ríos			Buenos Aires	–	–	Entre Ríos
Region	Pampas			Pampas	Mesopotamic	Pampas	Pampas
Animals (cattle/poultry)	4,187,378/364,437,857			18.615.0344/	–	–	–
Area	Head of cattle and numbers of poultry			259.677.204			
Subbasin (sampling area)	Main courses and tributaries (MT)*					Primary Collectors (PC)	Wetland (WL)*
Sampling sites	Paraná (PAR)	Gualeguay (GUA)	Uruguay (URU)	Saladillo (BA)	Alto Paraná (AP)	–	–
Prevalent production	7	9	8	6	7	3	5
Sampling period	Poultry + Eggs + Cattle	Mixed	Poultry + Broiler	Cattle	Mixed	Mixed	Cattle (grazing)
	June 2015–March 2017						

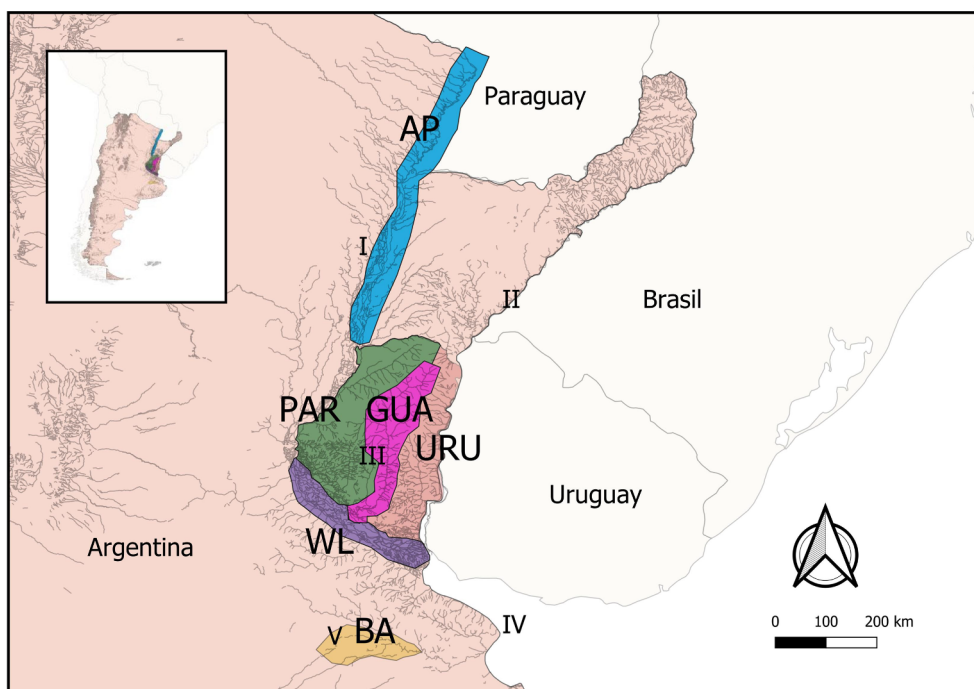


Fig. 1. The study sites within the pampas and mesotimic regions are indicated by the abbreviations AP, GUA, PAR, URU, BA, and WL. In Roman numerals, the main rivers reached by the study are indicated I = Paraná River; II = Uruguay River; III = Gualeguay River; IV = De La Plata River; V = Saladillo River.

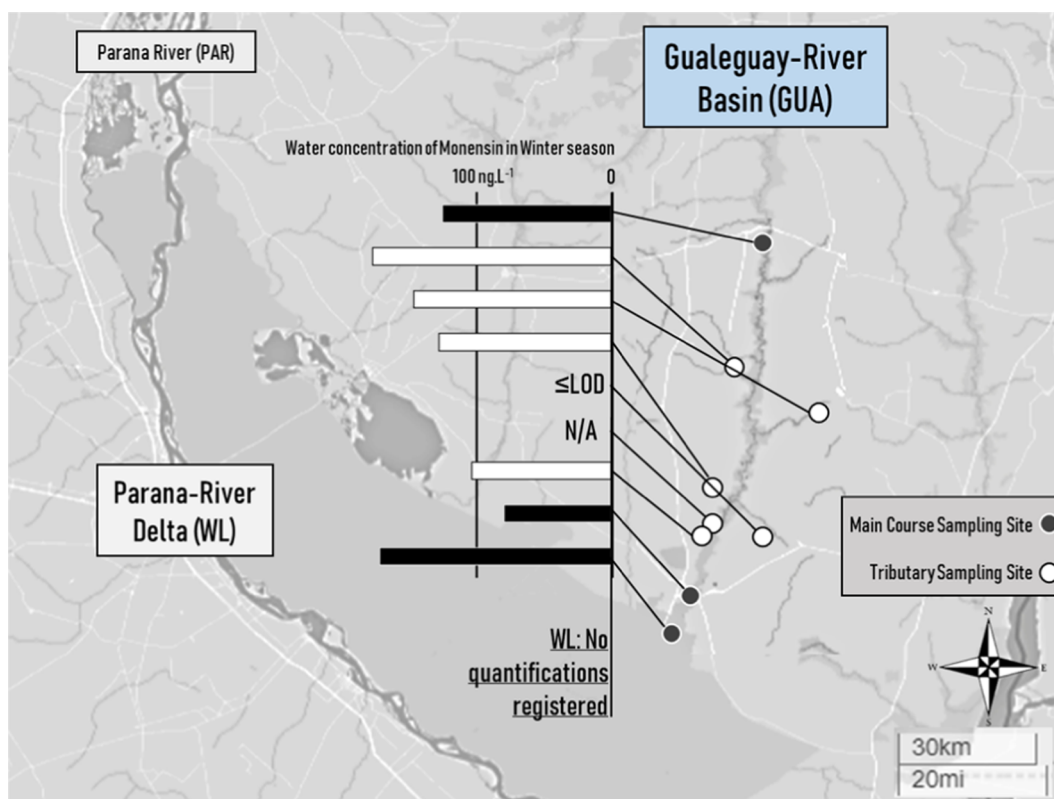


Fig. 2. Winter concentrations of MON in aqueous samples from the Gualeguay-River basin and Parana-River delta. The contribution from the GUA section is especially notable in the main-course samples (black bars) at the river outlet into the wetland.

μm pore size. A volume of 250 mL of filtered water sample was placed in a glass bottle, adding 2.5 ml of aqueous methanol and 50 μL of 5% (w/v) disodium ethylenediaminetetraacetic acid (EDTA) as antibacterial and metal-complexing agents (Tong et al., 2009) and thereafter maintained at 4 °C during transfer to the laboratory. The samples were then stored at

– 20 °C until the time of processing, according to the procedure from the American Public Health Association (APHA, 1998) and Kennedy et al. (1998). A second similarly filtered water sample of 100 mL, was added to another glass bottle without additives to assay chemical oxygen demand (COD) within 3 h from the time of sampling.

Table 2
Summary of UPLC-MS/MS conditions for the analysis of the selected compounds by multiple reaction monitoring.

Compound	Ionization mode	t _R (min)	Inj. volume (μL)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
Monensin	ES+	3.9	20	693.4	675.2	40
					460.9	40
Lasalocid	ES+	4.0	20	613.4	376.9	30
					595.9	30
Salinomycin	ES+	4.2	20	773.4	430.9	40
					531.1	40

2.3. Physical-chemical analysis

The physical and chemical parameters of the water quality from each sampling site—i.e., conductivity, pH, temperature, and dissolved-oxygen concentration—were measured *in situ*. (Multiparameter water quality monitor HORIBA U-52™). The measurements of chemical oxygen demand (COD) from the water samples were made in the laboratory by colorimetry according to the Method 5220-D of APHA (1998).

2.4. Chemicals and reagents

All the solvents used in the chemical and chromatographic analyses were of high-performance-liquid-chromatography (HPLC) grade and all the salts of analytical grade (JT Baker-Mallinckrodt Baker Inc., USA). A Sartorius Arium™ water-purification system (Sartorius AG, Göttingen, Netherlands) was used to obtain ultrapure water. The compounds studied—MON, LAS, and SAL—were purchased from Sigma Aldrich, St. Louis, MO, USA. Individual stock solutions of each ionophore were initially prepared in methanol (1.00 g/L) and then further diluted for the preparation of the mixed working standard solutions. (Zhang and Zhou, 2007).

2.5. Ionophore analysis

2.5.1. Solid-phase extraction (SPE)

The ionophore analysis involved an initial SPE followed by liquid chromatography coupled to tandem quadrupole mass spectrometry because that procedure had been widely reported as a robust method for the extraction and analysis of antibiotics from environmental matrices (Kim and Carlson, 2006, Hernández et al., 2007, Iglesias et al., 2012). Filtered water samples (100 mL) were diluted with 100 mL of a solution of Na₂EDTA 10 mM and then extracted by passage through a Waters® Oasis C18 HLB 60 mg cartridge (Zhang and Zhou, 2007). Before the SPE, the cartridges were first conditioned with 5 mL of methanol (MeOH) and 5 mL of ultrapure water. The samples were then extracted at a flow rate of 5–10 mL/min under vacuum in a Visiprep apparatus (Supelco, Bellefonte, PA). After the extraction, the cartridges were rinsed with 5 mL of nanopure-grade water and the dried under vacuum for 30 min. After elution with 5 mL of MeOH, the extracts were reduced to dryness under a flow of nitrogen and then reconstituted with 0.5 mL of MeOH in amber vials. Before injection, 0.5 mL of 5 mM Na₂EDTA was added to the samples and standards followed by filtration through 0.22-μm nylon membranes.

2.5.2. Instrumental analysis

A system of UPLC-MS/MS (Waters, Milliford, MA), calibrated for positive detection was used for this type of analysis. An Acquity UPLC BEH C18 column (1.7 μm, 100 × 2.1 mm; Waters) was used for analyzing the standards and the samples. Component A of the mobile phase, was of a mixture of HPLC-grade water, 5 mM NH₄ acetate, and 0.01% (v/v) formic acid. Component B of the mobile phase was HPLC-grade methanol, 5 mM NH₄ acetate, and 0.01% (v/v) formic acid. During the run, the gradient was ramped from 100% phase A to 50%

phase A plus 50% phase B in the first min, to 10% phase A and 90% phase B after 1.5 min, and finally to 100% phase B at a flow rate of 0.4 mL·min⁻¹. The sample injection volume was 20 μL. All the samples were analyzed in a Quattro Premier XE Tandem Quadrupole Mass Spectrometer™ (Waters) equipped with an electrospray-ionization source operated in the positive mode. The respective temperatures for the electrospray-source block and the desolvation step were 120 and 450 °C. Nitrogen gas was used for drying and nebulizing. The mass spectrometer was operated for multiple reaction monitoring. The sodium adducts were analyzed for the 3 analytes (Kim and Carlson, 2006; Biswas et al., 2012). Table 2 summarizes the precursor mass, product ion, and optimized tandem-mass-spectrometry parameters.

2.6. Quality control and assurance

The quality-assurance procedures conducted in the study comprised the use of reagent blanks and duplicate samples. In the absence of isotopically labelled starting compounds, parallel blanks and samples from the different matrices were analyzed to assess the analytical recovery and matrix effect. The linearity, reproducibility, detection and quantification limits were accordingly tested. The analytical criteria applied were the relationship of the chromatographic areas of two mass transitions and the retention times, in both the standards and the samples. Confirmation of positive findings was carried out by calculating the peak-area ratios between the quantification (Q) and confirmation transitions (q; SANTE/11813/2017).

2.7. Data analysis

In view of the characteristics of the environmental-data sets for trace-level emerging pollutants, noted for their low detection frequencies (Antweiler, 2015), different tools for data management and statistical-parameter determination of the data sets were assessed and discussed. Six approaches for descriptive statistical calculations were tested: eliminating values below the limit of detection (LOD), substitution techniques replacing values below the LOD with 0.5 LOD and √2/2 LOD, and the following 3 imputation methods: Kaplan-Meier, Regression-Order Statistics, and Maximum-Likelihood Estimation (cf. Supplementary Material, Table 1). A comparison of the sampling sites was performed through the use of contingency tables and the Kruskal-Wallis and Friedman tests according to Helsel (2012) for the censored data that was monitored with only one LOD. Spearman correlation tests were performed to evaluate associations between water-quality parameters and antibiotic concentrations. All the tests were considered statistically significant for p values ≤ 0.05. The analyses were performed with R software (R Core Team, 2018) and NADA-package functions (Lee, 2017).

3. Results and discussion

3.1. Analytical procedures and data interpretation

3.1.1. Performance of the extraction and quantification procedures

The output from the instrumental method used was linear within

Table 3
Recovery data and analytical parameters found.

Compound	IDL	LOD (ng·L ⁻¹)	LOQ (ng·L ⁻¹)	Mean recovery ± RSD (%) ^{a,b}	
				Deionized water (n = 15)	Surface water (n = 12)
LAS	100	1.7	3.4	88 ± 7	85 ± 10
MON	100	1.7	3.4	93 ± 7	96 ± 6
SAL	100	1.7	3.4	96 ± 11	101 ± 9

^a Percent recoveries for a spike level of 100 ng·L⁻¹.

^b IDL, instrument detection limit, LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation.

the interval studied (0.1–500 µg·L⁻¹) for all compounds with an R² of 0.996 (critical value = 0.5132, 95%, n = 23). The limit of detection of the instruments was 50 ng·L⁻¹ and the limit of instrumental quantification 100 ng·L⁻¹. Table 3 lists the detection limits of the method used for each compound. The limits obtained were within the range of values reported for the selected compounds (Forrest et al., 2011; Iglesias et al., 2012; Sandoz et al., 2018). The average recoveries for the surrogate samples at 100 ng·L⁻¹ (Table 3) were consistent with the values reported for these pollutants (Cha et al., 2005; Hao et al., 2005; Song et al., 2007; Zhang and Zhou, 2007). No statistical differences were observed between the surrogate recoveries in the presence of deionized and surface waters, implying a minimal matrix effect (Taylor, 2005). Possibly, the conversion of each of the ionophore antibiotics into a single sodium-adduct and the chelating effect of Na₂EDTA prevented a more complex interaction with other species (Cha et al., 2005; Volmer and Lock, 1998) and also enhanced their water solubility and signal stability (Sun et al., 2016).

3.1.2. Statistical approach to descriptive environmental analyses in terms of left-censored data

More detailed data are presented in the Supplementary Material Table 1. We noted that central-tendency statistics deviated in the order of magnitude (up to 2 in some instances) between the different estimation methods used. To inform the descriptive statistics then, the guidelines proposed by Helsel (2012, Chapter 6) were followed, according to the number of available observations (n) and the percentage of censored data. The result of the Kaplan-Meier estimation was informed for ≤50% censoring (considered as the percentage of data ≤ LOD), while the result of regression-order statistics was presented for censoring between 50 and 80%. For > 80%, only the censored proportion and the LOD were obtained (Tables 4 and 5). The results from each category are discussed below. Even though the literature has incorporated robust imputation approaches (Helsel, 2012; Antweiler, 2015), in view of the discrepancy, clear guidelines should be considered to determine the environmental concentrations of this type of pollutant, since the values used for the estimation of risks could be underestimated when the methods of elimination or data substitution

Table 4

Statistical parameters found for the three main categories. MON concentrations were differentiated between the three campaigns (p ≤ 0.05).

Category	Primary collectors (PC)			Main courses and tributaries (MT)			Wetlands (WL)		
	LAS	MON ^a	SAL	LAS	MON ^b	SAL	LAS	MON ^c	SAL
n	5	5	5	64	64	64	20	20	20
Appropriate estimation method		KM	ROS	ROS	ROS	ROS			
Median	–	288.3	0.62	0.14	4.35	0.35	–	–	–
Mean	–	1221.70	245.77	12.20	51.29	9.83	–	–	–
SD	–	2039.48	506.60	47.87	159.55	29.89	–	–	–
Max	490	4670	1150	337	1233	187	–	–	–
% Cens	80	20	60	76	59	73	100	95	100
Mean estimation with LOD/2 substitution method	98.7	1214.9	246.1	12.6	50.3	10.1	0.9	0.9	0.9

are applied (Antweiler, 2015). The strategy should consider not eliminating information from observations, and avoiding arbitrary substitution values, with an emphasis on the allocation of the most robust method based on the number of observations and the censorship percentage (Helsel, 2012).

3.2. Input and transport of ionophore antibiotics in aquatic environments

3.2.1. Water bodies directly influenced by animal facilities

Residual ionophores were found consistently in the excreta generated in the animal facilities (Arikan et al., 2016; Biswas et al., 2012; Furtula et al., 2009) with reported concentrations from 0.01 to 10 mg·kg⁻¹. Bovine manure and poultry litter are considered the main source of ionophore antibiotics present in environmental water, as those compounds can be transported to water courses by runoff processes (Kim et al., 2010; Sun et al., 2013). Samples from the PC category represent low-flow watercourses with the expected input from direct runoff from adjacent production facilities. MON was present at the maximum concentration in the study (4670 ng·L⁻¹) followed by SAL (1150 ng·L⁻¹), with both values falling within the range reported by other authors for this type of recipient water body (Song et al., 2007; Watanabe et al., 2008). The concentrations of MON in the PC samples were significantly higher than those found in the MT category (discussed in Section 3.2.2), with a median value (288 ng·L⁻¹) over 70 times greater than the median found for the streams within the MT category (4 ng·L⁻¹). Direct runoff from animal excreta loaded with ionophores and wastewater constitute the principal contributions to the concentrations recorded in the PC. Moreover, dilution and degradation influence the lower levels found in courses distant from animal facilities, although other types of processes may influence the input of those antibiotics.

3.2.2. Spatial distribution of ionophore antibiotics in main watercourses and tributaries from the pampas region

From the samples of the MT surface water analyzed (N = 64), 58% of the dataset indicated detections of at least 1 antibiotic, with MON being the most significantly recorded ionophore at 41% of the samples (p ≤ 0.05). SAL and LAS were less frequent at 26% and 23%, respectively. Similar results had been observed in a regional study in Canada—with 23 streams monitored near different degrees of agricultural activities—with MON being the most frequently detected compound at 34% of the samples (Forrest et al., 2011). The diversification of the animal production in the pampas region is consistent with the increasing occurrence of these pharmaceuticals throughout the sampling area. The environmental levels of the 3 ionophore antibiotics analyzed in decreasing order were [MON] < [SAL] < [LAS]. MON and SAL have been reported among the most widely sold antibiotics (Hansen et al., 2009; Sarmah et al., 2006; Yoshida et al., 2007). Concentrations of both compounds were also detected in close association (p = 0.05), possibly because of their combined use in feeding practices (Sarmah et al., 2006). In contrast, the lower occurrence for LAS might

Table 5

The statistical parameters of the different ionophore antibiotics studied, expressed in terms of the most appropriate estimator, are detailed for each area monitored.

Area	Alto Parana (AP)			Saladillo (BA)			Guauguay (GUA)			Parana (PAR)			Uruguay (URU)		
	LAS	MON	SAL	LAS	MON	SAL	LAS	MON	SAL	LAS	MON	SAL	LAS	MON	SAL
n	7	7	7	8	8	8	30	30	30	7	7	7	12	12	12
Appropriate estimation method	ROS			ROS			KM			KM			ROS		
Median	16.43	–	–	–	3.49	7.60	–	22.20	1.10	2.60	1.56	–	0.62	–	–
Mean	NC	–	–	–	184.00	45.75	–	49.90	8.62	8.61	21.16	–	41.50	–	–
SD	24.00	–	–	–	430.41	72.31	–	59.39	15.40	10.70	41.95	–	99.79	–	–
Max	58	–	–	138	1233	187	8	177	50	29	114	–	337	126	10
% Cens ^a	71	100	100	88	75	63	87	37	60	43	57	100	67	83	83
Mean estimation with LOD/2 substitution method	10.3	0.85	0.85	17.9	181.6	43.9	1.3	49.3	8.8	7.9	21.3	0.85	41.8	11.4	1.9

^a Percentage of censored data.

be associated with a lower volume of usage and possibly owing to its enhanced susceptibility to undergo photolysis within relevant solar wavelengths, with a half-life of about 1 h in an aquatic environment (Bak and Björklund, 2014; Bohn et al., 2013).

Table 5 lists the concentrations of the ionophores in the different sectors under investigation. MON exhibited the highest regional mean (\pm SD) and median values at $51 \pm 15 \text{ ng}\cdot\text{L}^{-1}$ and $4 \text{ ng}\cdot\text{L}^{-1}$, respectively followed by LAS and SAL with regional means of $12 \pm 5 \text{ ng}\cdot\text{L}^{-1}$ and $10 \pm 3 \text{ ng}\cdot\text{L}^{-1}$ respectively, with the median values for both compounds being below the LOD. We noted that, in contrast to the environmental data obtained here, MON had been reported at lower concentrations than LAS or SAL in animal excreta, (Section 3.2.1). The estimated mean values for MON in surface water proved to be similar to other environmental studies (Bak and Björklund, 2014; Iglesias et al., 2012; Jaimes-correa et al., 2015; Lissemore et al., 2006; Sandoz et al., 2018), whereas the maximum detected in a tributary creek from the BA category (BA; $1233 \text{ ng}\cdot\text{L}^{-1}$)—with Saladillo being representative of the Province of Buenos Aires—was higher than the maximum values in the fresh water from previous studies. As mentioned before (Section 3.2.1), lower concentrations in the MT category indicated a different source of ionophores than occurring in the PC region. Furthermore, the PC is a source of contaminants for these water-courses, in addition to the agricultural applications of liquid manure and poultry litter to the crop fields for fertilization (Dolliver and Gupta, 2008; Kim et al., 2010; Sun et al., 2013), thus constituting a diffuse source of these compounds. In the absence of previous environmental information on the ionophore antibiotics in Argentine ecosystems, we conducted a comparison with other agricultural pollutants widely investigated as pesticides. Accordingly, the concentration ranges obtained in the present study for the ionophore antibiotics in surface water ($5\text{--}1233 \text{ ng}\cdot\text{L}^{-1}$) exhibited the same order of magnitude as chlorpyrifos, cypermethrin, or the organochlorine pesticides ($10\text{--}6620 \text{ ng}\cdot\text{L}^{-1}$) in the same region (Etchegoyen et al., 2017). The similar concentration levels of the ionophore antibiotics in the aquatic media to other extensively used and well-studied pollutants from agricultural production emphasizes the magnitude of the environmental intake of these compounds.

The detection of ionophore antibiotics among the sampling sites was more frequent in the Guauguay and Paraná sub-basins ($p \leq 0.05$) in the Entre Rios province, which catchments are located in the agroproductive core of Argentina and are responsible for most of the egg and broiler production of the country (SAI, Argentina). A Kruskal-Wallis analysis of the different study areas within the MT categories revealed that the LAS concentrations in the northern section of the AP are higher than those found in the BA and GUA sampling sites. A link between the LAS recorded and the poultry activities is likely since that ionophore was the only compound detected in the northernmost tributaries of the AP section with a low but significant density of poultry facilities, and no further positive detections made until reaching the PAR and URU sectors, where egg and broiler productions increase (Gange et al., 2017). By contrast, the MON concentration in water samples from GUA were

greater than those found in the AP and URU sampling sites. MON in particular is related to multiple animal practices that take place in a mixed-use area such as GUA (Kim and Carlson, 2006). Therefore, higher concentration levels of that compound in several watercourses compared to the other areas studied might be attributed to an input from such multiple sources.

The samples from the MT classification were subgrouped according to the flow rate of the watercourses monitored. Aquatic concentrations of ionophores in the main courses exhibited no significant differences ($p \leq 0.05$) from the creeks or tributaries analyzed. We found especially notable the observation that MON and SAL were detected at quantifiable levels (mean concentrations were $27 \text{ ng}\cdot\text{L}^{-1}$ and $5 \text{ ng}\cdot\text{L}^{-1}$ respectively) in samples from an abundant main river such as the Guauguay—with a mean discharge of $320 \text{ m}^3\cdot\text{s}^{-1}$ (MI Argentina)—representing a major contribution to the Paraná-River delta (Fig. 1).

3.2.3. Relationship between natural-water quality and ionophore concentrations

Table 6 summarizes the physicochemical parameters of the surface water; which data were associated with the ionophore loads. The concentrations of MON and SAL (\geq LOQ) were associated positively with the water COD. The dissolved organic matter enhances antibiotic mobilization within the water column and therefore the reception of compounds in runoff processes from solid matrices (Hussain and Prasher, 2011; Tolls, 2001). Furthermore, the MON and SAL concentrations were correlated with the pH of the water samples. The chemical speciation of ionophore antibiotics is strongly correlated with the pH and cations present in the aquatic medium (Sun et al., 2016), which conditions favor the adsorption equilibria. A decrease in K_d is expected when the pH increases, in the range from 4 to 8.5 (Hussain and Prasher, 2011; Sassman and Lee, 2007). Therefore, parameters such as the pH and the COD of the natural water in these investigations, which former values varied from 7.0 to 8.8 (Table 6), increased the mobility of the ionophore antibiotics in the soluble fraction (Cha et al., 2005). Indeed, the physicochemical characteristics of natural water are an essential determinant of the dynamics of these compounds in the soluble fraction.

3.3. Seasonal patterns of ionophores in a mixed-use basin and effect on wetlands

3.3.1. Temporal distribution of ionophores in the Guauguay basin (GUA)

The Guauguay-River basin was studied in different seasons throughout the year since that catchment is a mixed-use area representative of the pampas region. The detection frequencies followed a pattern with respect to the regions, (Tables 4 and 5; Section 3.2.1) with MON and SAL being the most frequently detected compounds (63.3% and 40.0%, respectively). Although rotational practices of antioccidals have been proposed to prevent long-term effects such as

Table 6
Physicochemical parameters^a from the sampling areas.^b

Section		T (°C)	pH	Cond (µs/cm)	DO (mg·L ⁻¹)	COD (ppm)
AP	Mean ± SD	21.0 ± 0.6	7.7 ± 0.4	521 ± 437	5.8 ± 1.9	129 ± 29
	Min	20.3	7.0	78.1	2.78	99
	Max	21.8	8.4	1237	7.7	158
BA	Mean ± SD	17.5 ± 4.4	7.6 ± 0.5	2407 ± 3248	7.81	339 ± 589
	Min	14	7.05	7.9	7.81	32
	Max	26.9	8.51	9270	7.81	1630
GUA	Mean ± SD	19.2 ± 5.4	7.4 ± 0.5	407 ± 548	6.3 ± 1.0	63 ± 47
	Min	13.2	6.43	101	4.2	1.8
	Max	28.9	8.83	3010	8.4	148.1
PAR	Mean ± SD	18.5 ± 0.9	8 ± 0.3	1782 ± 2756	8.2 ± 0.6	44 ± 20
	Min	17.2	7.7	133.5	7.14	26
	Max	19.6	8.4	7600	8.8	65
URU	Mean ± SD	19.8 ± 3.2	7.4 ± 0.2	192 ± 125	5.9 ± 1.4	36 ± 13
	Min	16.8	7.0	30	4	17
	Max	27.2	7.7	378	8.56	57.2
WL	Mean ± SD	18.8 ± 6	7.6 ± 0.4	370 ± 256	8.2 ± 1.6	29 ± 22
	Min	10.7	7.0	74.4	3.9	3.7
	Max	28.9	8.2	970	10.3	85.5
PC	Mean ± SD	18.5 ± 7.5	7.3 ± 0.5	623 ± 774	4.6 ± 0.7	544 ± 247
	Min	12.4	6.95	93	4.1	256
	Max	26.8	7.6	1512	5.1	873

^a Cond, conductivity; DO, dissolved-oxygen concentration; COD, chemical oxygen demand.

^b AP, Paraná River/Alto Paraná; BA, Saladillo; GUA, Gualeguay; PAR, Paraná; URU, Uruguay; WL, wetlands; PC, primary collector.

bacterial antibiotic resistance (Stanev et al., 2015), MON was used continuously since that ionophore was detected in every season and dominated the total mass of ionophore antibiotics analyzed in each one. The environmental concentration of MON was found to be significantly higher in the winter sampling, compared to the spring and fall. Variations in the seasonal climatic parameters such as a lower radiation and a decrease in the temperature, photolysis rate, and microbial degradation in the winter affect the MON concentration. Unlike other studies where higher concentrations of ionophores were recorded under conditions of high flow and seasonal rainfall associated with a high degree of runoff (Forrest et al., 2011; Jaimes-correa et al., 2015; Kim and Carlson, 2007), the higher concentrations of MON detected in the GUA basin in winter occurred despite the lower rainfall associated with that season—with the average values in the winter months being but half that of the other seasons (Supplementary material). Conversely, in the summer, the rainfall increases resulting in a greater runoff along with a higher discharge in the rivers, thus increasing solute dilution, with such conditions also favoring chemical and microbiologic degradations (Jaimes-correa et al., 2015).

3.3.2. Wetlands as natural depuration systems

After four seasonal monitoring campaigns in 5 different locations (n = 20), no ionophores were detected in the WL samples, with the exception of only one in the Autumn campaign ([MON] ≤ LOQ) in the northwest section of the floodplain. In contrast to the pattern observed with respect to the entire GUA-basin campaign (Section 3.2.3), no changes were observed throughout the four seasons. Although the concentrations of ionophores measured in affluent courses from the PAR and GUA basin—especially those values recorded in an abundant course such as the Gualeguay River—resulted in a transfer of quantifiable loads of ionophore antibiotics to the delta (Fig. 2), the capability of the system—at least at the present time—to dilute and recycle these organic pollutants according to a variety of biogeochemical and ecosystemic processes (Dordio et al., 2008; Verhoeven et al., 2006) is truly remarkable. The ability of wetlands to degrade veterinary pharmaceuticals (Almeida et al., 2013; Li et al., 2014)—and specifically ionophore antibiotics (Hussain et al., 2012; Hussain and Prasher, 2011)—has been studied on a microscale in wetlands constructed specifically for agricultural-wastewater treatment, where removal efficiencies of 20 to 50% were recorded, mostly as consequence of sorption and microbial

degradation.

In view of the concentrations of antibiotics reported in the aqueous phase in the present study, and their low removal efficiency in a pilot-scale wetland (Hussain et al., 2012; Hussain and Prasher, 2011), an awareness that the purification capability of the delta is certainly finite and essential. This wetland waste-removal service is in jeopardy, unless the environmental levels of these compounds in surface waters are not decreased—and will be more so if the concentrations increase. Several consequences of the environmental presence of these veterinary pharmaceutical agents as high-risk compounds have been reported (Hansen et al., 2009). The lack of available information on the long-term chronic effects of such compounds on biota is a matter of great concern, especially in view of the potential effect of those antibiotics on aquatic organisms (Hillis et al., 2007) and the possible promotion of antibiotic resistance in the environmental bacteria (Butaye et al., 2003; Furtula et al., 2010; Pei et al., 2006).

4. Conclusions

We investigated the presence of ionophore antibiotics used in animal husbandry in environmental surface water. Those agents were monitored in several sub-basins from Argentina, which compounds were recorded in different spatial patterns in accordance with the diverse degree and type of animal husbandry. MON proved to be the most frequently detected compound (at 41%), also at greater concentrations than SAL and LAS. We noted that the antibiotics studied exhibited a similar distribution to that of other agriculture-originating pollutants widely reported as pesticides. The chemical speciation for these compounds in surface water bodies is highly relevant, especially with respect to MON and SAL, where the pH and COD of the natural water body are determinants of the concentration in soluble fraction. The maximum values were registered in water collectors adjacent to the husbandry facilities. Because of its greater detection, the following concentration gradient could be observed for MON: [Principal collectors] > [Main courses and tributaries] > [Wetlands], indicating a greater influence of direct manure and/or litter runoff than other potential environmental sources of pollution. The concentrations in the abundant rivers necessarily produce a contribution to the natural wetlands such as the Paraná-river delta. Of all the samples assayed, only one detected MON, with that concentration, moreover, being below the

LOQ. Nevertheless, the limited ionophore removal capability of these wetlands and the ever-increasing contribution of the combined pollutants call for urgent steps to be taken for the preservation of this aquatic ecosystem. The development of quality-control guidelines for assessing the impact of ionophore antibiotics on the environment—as well as on other matrices—are sorely needed, with acute and chronic studies being undertaken involving representative native organisms that are naïve and therefore pollution-sensitive.

The present study constitutes the first investigation of ionophore antibiotics as pollutants in the aquatic environment on a relevant scale in the Del-Plata basin, which catchment after the Amazon-River basin is the second largest hydrological basin in South America.

Declaration of competing interest

The authors declare no conflicts of interest and the research was carried out with funding from national science and technology organizations.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2019.105144>.

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