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Concentration and environmental fate of ivermectin in floodplain wetlands: An ecosystem approach

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HIGHLIGHTS

Presence, concentration and fate of the IVM were assessed in floodplain wetlands subjected to different cattle use and frequency of injection of the drug

Concentration of IVM was detected in cattle manure, sediment, water, macrophytes, invertebrate and vertebrate fauna of wetlands, and the value increased with the number of cows and frequency of injection of IVM

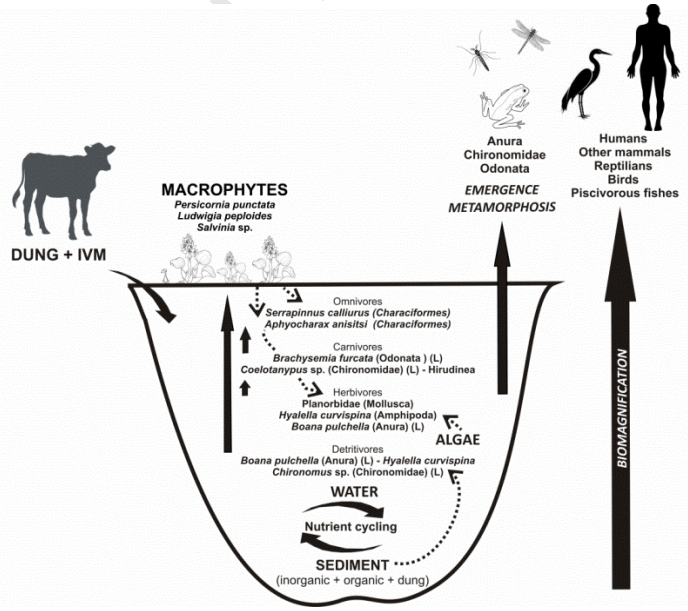
Management strategies should be implemented by farmers that can keep treated animals away from watercourses in order to reduce the introduction, transfer and accumulation of IVM in aquatic systems.

ABSTRACT

Ivermectin (IVM) is commonly used for broad control of endo- and ecto- parasites in cattle. In wetlands of the Paraná Medio River cattle has been treated repeatedly with IVM for years and concerns have been raised on possible presence of the drug in these ecosystems. A field study was conducted to assess concentration of IVM in two wetlands subjected to different cattle use and frequency of IVM injection. Concentration of IVM in roots of macrophytes, *Chironomus* sp., *Coelotanypus* sp., *Brachymesia furcata* (larvae), *Dero* sp., *Hyalella* sp., Hirudinea, Planorbidae, *Boana pulchella* (larvae), *Aphyocharax anisitsi* and *Serrapinnus calliurus* were shown for the first time. Total concentration of IVM in the wetlands, and concentration in cattle manure, sediment, water and macrophytes increased with the number of treated cattle and frequency of IVM injections. Accumulation of IVM in aquatic assemblages is alarming because these organisms fulfill a key role in food webs, constituting a

serious risk to human health. Management strategies should be implemented by farmers to keep recently treated animals away from watercourses to reduce the introduction of IVM into aquatic systems.

GRAPHICAL ABSTRACT



Keywords: Veterinary medical products; Biomagnification; Floodplain systems; Bioaccumulation

1. Introduction

IVM has been widely used since the 1980s due to its high potency and its wide spectrum of activity against endo- and ecto- parasites in livestock. The IVM is a semi-synthetic derivative of avermectin, a macrocyclic lactone produced by the actinomycete *Streptomyces avermitilis*. After use, it is excreted by ruminants through bile and feces as an active drug (Lifschitz et al., 2000), and therefore may reach aquatic ecosystems by excretion in the riverine zone or directly into water bodies (Kovecses and Marcogliese, 2005; EMEA, 2008).

Recently, IVM was pointed out to be locally hazardous for water organisms (van Wezel and Jager, 2002) and of high priority for further environmental monitoring and risk assessment (Boxall et al., 2004; Boxal 2018). Because IVM is a lipophilic compound (Halley et al., 1989), it can bind strongly to organic materials and sediment (Halley et al., 1989; Boxall et al., 2002; Krogh et al., 2008). Once bonded to sediment, it can persist for a long time in the

aquatic systems (Löffler et al., 2005; Sanderson et al., 2007; Prasse et al., 2009), causing long-term exposure to aquatic assemblages (Liebig et al., 2010; Boxal 2018).

Only a few laboratory studies have addressed exposure of aquatic assemblages via dung (Schweitzer et al., 2010; Mesa et al., 2017) and sediment spiked with IVM (Davies et al., 1998; Allen et al., 2007; Egeler et al., 2010). Consistently, these laboratory experiments have documented concentrations of IVM sediment, *Salvinia* sp., and invertebrates such as *Pomacea* sp. and *Lumbriculus variegatus*. In contrast, field studies to assess real concentrations of IVM in wetlands subjected to cattle use are still lacking.

In Argentina, the intensification of agriculture, soybean cropping in particular, has forced the relocation of livestock from rich arable land into to more marginal grazing lands such as floodplain wetlands (PROSAP 2009). This is the case of the Paraná River, the second largest watershed in South America after that of the Amazon River. The Paraná River covers 3.1×10^6 km^2 and throughout most of its course is surrounded by a 10–50 km wide floodplain that extends over 60,000 km^2. It was due to the availability of a forage rich environment that both the density and numbers of cattle in these floodplain systems have raised over 100% just in only one decade (Quintana et al., 2014). Further, to become efficient with grazeable resources, local ranchers have implemented a number of new practices which include the use of heavy stocking rates, planning of rotational grazing and also the systematic and frequent injection of cattle with IVM. The frequent injection of cattle with IVM in absence of a strict veterinarian prescription, followed by contact of cattle with wetlands immediately after injection has raised concerns about the presence and concentration of the drug in these floodplain environments.

A field study was conducted to assess the presence and concentration of IVM in two shallow wetlands subjected to different cattle use. The concentration of this drug in the aquatic system was analyzed in relation to both cattle density and the frequency of injection of

IVM to cattle. We hypothesized that IVM has the potential to concentrate in sediment, macrophytes and aquatic fauna of these wetlands. The second hypothesis was that concentration in sediment and aquatic assemblages was higher in wetlands with greater stocking density and frequency of IVM injection. The biomagnification and potential fate of IVM through trophic webs into the aquatic system was also discussed.

2. Materials and Methods

Study area

The study was conducted in the Middle Paraná River area which extends from the confluence with the Paraguay River to Diamante City (Argentina), and covers an extension of 2,600,000–2,800,000 km^2 (Iriondo and Paira, 2007). This area is under influence of hydro-sedimentological pulses of the Paraná River that integrate the river mainstream with adjacent floodplains in a unique system with constantly exchanges of energy and matter (Junk et al., 1989; Neiff, 1990).

Two shallow wetlands of the Middle Paraná River floodplain included in different paddocks and subject to a rotational management of cattle were sampled: Wetland A (31º 41' 00" S, 60º 31' 24" W) and Wetland B (31º 40' 45"S, 60º 30' 33"W) (Santa Fe City, Argentina, Fig. 1 a, b). Both paddocks were used by cattle as part of a rotational grazing scheme in which cattle were allowed to graze a paddock for one to several months before being rotated to another rested paddock. Sediments of both wetlands were characterized by a high organic matter and rich humic content, laid over a dense horizon rich in clay and fine silt content (Mesa et al., 2015). The prevailing climate in the study area is humid subtropical, the mean

annual temperature is around 19 ºC, and the annual pluvial precipitation is 900-1000 mm, 73% of which is recorded between October and April (Rojas and Saluso, 1987).

The studied wetlands were sampled on October-November 2016 and again on October-November 2018, both during spring and low-water season period (Fig. 2). The mean air temperature in the 2016 and 2018 sampling periods were 19.7ºC and 20.2 ºC, whereas the mean precipitation in the 2016 and 2018 sampling periods were 12.6 mm and 9.2 mm respectively, 75-80% of which were consistently recorded between October and May (Fig. 2). The wetlands become connected with each other and with other adjacent aquatic systems only during the high-water season. During the study period, this connection occurred from December 2015 to July 2016 and from January 2018 to April 2018. During this high-water season hydrometric levels in the main channel of the Middle Paraná River were higher than 4 meters (Fig. 2).

2.1 Environmental variables

Subsurface water samples were collected in triplicate by using plastic bottles, preserved on ice and dark condition for subsequent analysis of nutrients. Water was immediately filtered through Whatman GF/F glass-fiber filters and refrigerated until determination of dissolved components (within 24 h after sampling). The filters (that retain organic and inorganic matter present in water) and filtered materials were kept refrigerated for IVM analyses.

Transparency (Secchi disk), pH (pH-meter), conductivity (Hanna conductivity meter) and water temperature were measured *in situ* in order to determine the prevailing physicochemical characteristics of water during each sampling date. Water soluble reactive phosphorus (SRP) was determined by the ascorbic acid method (Murphy and Riley, 1962),

contents of nitrate+nitrite (NO_3^- +NO_2^-) were determined by reduction of NO_3^- with hydrazine sulphate, followed by subsequent colourimetric determination of NO_2^- (Hilton and Rigg, 1983) and ammonium (NH_4^+) by the indophenol blue method (Koroleff, 1970).

2.2 Cattle management and sampling

One hundred cows were treated with IVM (IVOMEC®; ivermectin 1%) subcutaneously at a dose rate of 0.2 mg kg^{-1} live weight. The first dosing was conducted on October 1, 2016, on 100 beef cows. On October 8, the herd was allocated to graze on the paddock that included the Wetland B (Fig 1 c). On October 21, 2016, the herd was expanded to a total of 200 cows and all received IVM at the same dose rate explained before (Fig 1 c). Consequently, half of the herd (first 100 cows included in the herd) received a second dose of IVM 20 days after they were treated with the first IVM dose. Double dosing of IVM, applied either to mature or growing cattle, enhances the control over most parasite cycles and is therefore suggested as an usual practice in most ranches of the studied region. A second IVM dosing and examination period was initiated on October 16, 2018, with a herd of 100 beef cows that were allowed to graze on the paddock that contained the Wetland A (Fig 1 c).

Sampling of manure, sediment, dominant macrophytes (in coverage), and dominant aquatic invertebrates and vertebrates (in density) was conducted in both wetlands according to cattle stocking densities and frequency of cattle injection with IVM. Sampling of Wetland B was conducted during October and November of 2016 and reinitiated again on October 2018 after a long withdraw period (> six months) of cattle IVM treatments (Fig. 1 c). In Wetland A, background sampling started before cows were injected IVM on October 2018, and was repeated after IVM injection from October through November of 2018 (Fig. 1 c).

Samples of manure and sediment were collected in triplicate with a spoon in the marshy zone of each wetland. Dominant taxa of macrophytes were identified by observation and collected manually. Aquatic invertebrates and Anura (larvae) were sampled with a D net (200 µm mesh). The net was swept horizontally from the margins to the center of the wetland and the epibenthos, nekton and pleuston invertebrates were collected. Zooplankton samples were collected using a 50 µm mesh net from the littoral and planktonic areas of the lake. Fishes were collected manually using a fish net (1 cm mesh size) that was swept carefully through submerged and floating vegetation. Each type of sample was conserved separately in flasks until their processing in the laboratory. In the laboratory, on the same day of collection, roots were washed with tap water and refrigerated for analysis. Zooplankton organisms, including cladocerans, copepods and rotifers were pooled together, rinsed with dechlorinated water and conserved for IVM analysis.

Aquatic invertebrates and Anura (larvae) were hand-picked from samples under a stereoscopic microscope (4x) and separated in taxonomic groups. Dominant invertebrates were separately placed in dechlorinated water for one day to allow the evacuation of gut contents. Taxa identifications were made to the lowest taxonomic level possible using the available keys for invertebrates (Brinkhurst and Marchese, 1992; Lopretto and Tell, 1995; Domínguez and Fernández, 2009; Trivino-Strixino, 2011, among others), Anura (Kehr and Williams, 1990) and fishes (Almirón et al., 2015; Mirande and Koerber, 2015). Planorbidae, Anura (larvae) and fishes were frozen and later dissected. Samples of manure, sediment, filtered water, roots of macrophytes, invertebrates, muscles of snails and fishes, and tails of larvae of Anura were preserved at −20 °C until the extraction and determination of IVM concentration.

2.3 Analyses of IVM concentrations

The extraction of IVM from experimental samples and quantification of IVM by HPLC analysis were carried out following the technique first described by Lifschitz et al. (2000). Samples were weighed, homogenized and combined with the internal standard compound (abamectin). One milliliter of acetonitrile was added to the preparation and mixed (Multi Tube Vortexer, VWR Scientific Products, West Chester, PA, USA) for 15 minutes. The solvent sample mixture was centrifuged at 2000g for 15 minutes. The supernatant was then placed on the appropriate rack of an Aspec XL sample processor (Gilson, Villiers Le Bel, France) to perform the solid-phase extraction. The derivatization of MLs was done with 100 µl of a solution of N-methylimidazole (Sigma Chemical, St Louis, MO, USA) in acetonitrile (1:1) and 150 µl of trifluoroacetic anhydride (Sigma Chemical, St Louis, MO, USA) solution in acetonitrile (1:2). After completion of the reaction (< 30 s), an aliquot (100 µl) of this solution was injected directly into the HPLC system. IVM concentrations were determined by HPLC using a Shimadzu 10A HPLC system with autosampler (Shimadzu Corporation, Kyoto, Japan). HPLC analysis was undertaken using a reverse phase C18 column (Kromasil, Eka Chemicals, Bohus, Sweden, 5 µm, 4.6 mm×250 mm) and an acetic acid 0.2% in water/methanol/acetonitrile (1.6/60/38.4) mobile phase at a flow rate of 1.5 ml/min at 30 °C. IVM was detected with a fluorescence detector (Shimadzu, RF-10 Spectrofluorometric detector, Kyoto, Japan), with readings at 365 nm (excitation) and 475 nm (emission wavelength). Calibration curves were constructed in the range of 0.2 to 80 ng g^{-1}. The linear regression lines showed correlation coefficients >0.99. The limit of quantification for IVM was established at 0.2 ng ml^{-1}. The precision of the analytical procedures obtained after HPLC analysis showed a coefficient of variation of 6%.

2.4 Data analyses

Water content in sediment was determined by weighing 10 grams of sediment and drying it at 65 °C until constant weight. The water content in the sediment was the difference in weight before and after drying and was expressed in percentage units. A paired *t*-test for means was used to compare environmental physicochemical parameters and concentrations of IVM between wetlands, with significance declared at a 5% alpha. SPSS v11.5 was used as statistical software. The total concentration of IVM in wetlands was calculated by adding the concentration for all items collected from each wetland, expressed in a wet weight basis.

3. Results

Environmental variables were not different between wetlands (paired *t*- test, P>0.05). In both wetlands, values of pH and conductivity were almost constant during the studied period. In general, values ranged from 6.5 to 7.9, and from 102 to 144.5 µS cm^{-1} for pH and conductivity, respectively. Water temperature varied from 14.2 to 23.2 °C, whereas dissolved oxygen ranged from 4.6 to 13 ppm, with conditions of anoxia in two sampling days (Table 1). Among dissolved nutrients, maximum values of NH_4^+ (>200 µg N L^{-1}) were observed in two days in both wetlands. The NO_3^-+NO_2^- varied from 1.8 to 180 µg N L^{-1}, with maximum value in one sampling day, whereas the SRP varied from 12 to 48.6 µg L^{-1} (Table 1).

Water content in sediment was 50%, with resulting values of concentration of IVM in sediment between 4 and 32 ng g^{-1} dry wt. Mean concentration of IVM in manure and sediment in days following injections of IVM to cattle were markedly higher in Wetland B than in Wetland A (Table 2). Concentration of IVM in water was only detected in one sampling day in Wetland B. Among macrophytes, IVM was not detected in roots of *Persicaria punctata* in Wetland A, whereas in Wetland B mean value of IVM was ten times higher in days before injection of the drug to cows (Table 2). Similarly, IVM was not detected

in *Ludwigia peploides* in Wetland A, whereas in Wetland B detectable concentrations were observed both for this macrophyte and *Salvinia* sp in days before injection (concentration >0.5 ng g^{-1}) (Table 2). Among invertebrates, concentration of IVM was found in chironomids *Chironomus* sp., *Coelotanypus* sp., *Hyalella curvispina*, Planorbidae, Hirudinea and *Boana pulchella* (larvae) in Wetland A, whereas concentration in *Brachymesia furcata* odonata (larvae) was two times higher in Wetland A than Wetland B. Among fishes, concentrations of IVM was only detected in *Aphyocharax anisitsi* and *Serrapinnus calliurus*, with a mean value higher than 15 ng g^{-1} wet wt for each taxon in Wetland B (Table 2). Concentration of IVM was neither detected in zooplankton nor the oligochaete *Dero* sp.

Total concentration of IVM was significantly higher and about 200% greater in Wetland B than in Wetland A (494.4 and 27.4 ng g^{-1} respectively, paired *t*-test, $P<0.01$, Fig. 3). The maximum value of concentration of the drug was detected in Wetland B one month after injection of cattle with IVM (Fig. 3). Minimum values of the drug were found in both wetlands in days before injection (Fig. 3, Table 2).

4. Discussion

This work is the first to provide real information of concentrations of IVM in floodplain wetlands subjected to different cattle use. In accordance with the first hypothesis, this drug accumulated in manure, sediment, water, macrophytes, and vertebrate and invertebrate fauna of wetlands. Concentrations of IVM in macrophytes including *P. punctata* and *L. peploides*, chironomids *Chironomus* sp., *Coelotanypus* sp., odonata *B. furcata* (larvae), oligochaete *Dero* sp., crustacean *H. curvispina*, Hirudinea, Planorbidae, anura *B. pulchella* (larvae), fishes *A. anisitsi* and *S. calliurus* were shown for the first time.

Following with our second hypothesis, differences in the number of cows and frequency of injection of IVM had a significant influence on the distinct concentration of the drug found in sediment and aquatic assemblages of wetlands. The total concentration of IVM and concentrations found in cattle manure, sediment, water and macrophytes were much higher in Wetland B than Wetland A. The number of cows in Wetland B doubled the number of cows that were allocated to Wetland A, and cattle relative to Wetland B were treated three times, thereby increasing the elimination of the drug via feces and the accumulation of IVM in the aquatic system.

The stability and persistence of IVM was corroborated by Suarez et al. (2003), who examined levels of IVM in dung exposed to environmental conditions for 180 days. In the present study the IVM entered water bodies through excreted dung and consistently with results by Schweitzer et al. (2010) persisted in sediment for a long time period (Fig. 4). Further, the concentration of IVM in the sediment of wetlands were stable during the entire study period, and this result was in accordance with previous works (Kövecses and Marcogliese, 2005; Sanderson et al., 2007; Egeler et al., 2010.; Schweitzer et al., 2010; Mesa et al., 2017). High values of IVM in macrophytes and invertebrates found in the days before injection of cattle with IVM and after a high-water period (more than three months) reinforce this statement. Thus, it appears that high water periods of the magnitude and duration of that registered during this study might not be sufficiently to remove the drug from these wetlands. This statement was also reinforced for the high values of IVM observed in roots of *Persicaria punctata* in Wetland B. The perennial habit of this macrophyte and adaptive capacity to persist fluctuating water levels and flooding (Partridge 2001) may explain the accumulation of the drug in this plant.

The maximum concentration of IVM in sediment in the studied wetlands (Wetland B, 17.1 µg kg^{-1} wet wt) was seven times higher than the values estimated by Liebig et al. (2010) for aquatic systems exposed to cattle dung excreted into surface water (2.4 µg kg^{-1} wet wt). Indeed,

maximum concentrations of IVM in sediment in our study were in the range of the values reported previously in sediment by Mesa et al. (2017) (1.4 and 9.2 μg kg^{-1} wet wt) in a laboratory study conducted with similar concentration of IVM in dung (<458 μg kg^{-1} wet wt). Likewise, Schweitzer et al. (2010) also found markedly lower values of IVM concentration in sediment under experimental conditions (between 1.9 and 4.1 μg kg^{-1} dry wt) in comparison to the high values found in this study (4–32 ng g^{-1}dry wt). In the present study, the high organic matter content of sediment added to the prevailing high temperature and oxygen availability of water would have enabled both, strong sorption of IVM in sediment and high dissipation half lives (Halley et al., 1989).

Additionally, it was plausible that some fraction of the IVM in sediment would have dissolved in the overlying water, with possible implications on nutrient cycling (Fig. 4, Mesa et al 2017). Since IVM is highly hydrophobic, it is rapidly removed from the aqueous phase, and could be accumulated in macrophytes, algae or particulate organic matter present in the water column (Fig. 4, Tišler and KožuhEržen, 2006; Liebig et al., 2010). In the studied wetlands, macrophytes played an important role in the absorption of IVM (Fig. 4). Concentration of IVM for rooted *P. punctata* was higher than those observed for floating *Salvinia* in the study of Mesa et al. (2017), likely because habits of macrophytes apparently play a significant role in the absorption and fate of IVM in wetland ecosystems.

Given the strong binding of IVM to sediment and macrophytes in our study, several authors have pointed out that future studies should address differentially the effect and accumulation of this drug on groups of sediment-macrophytes associated freshwater organisms (Fig. 4, Kövecses and Marcogliese, 2005; Liebig et al., 2010). In the Middle Paraná River system, *Hyalella* and *Chironomus* sp. feed as collector-gatherers, preferably consuming partially decomposed organic matter (Fig. 4, Saigo et al., 2016). Herbivory of Planorbidae is, well known in these floodplain wetlands; this taxon feeds on macrophytes and the organic

matter of sediment (Fig. 4, Saigo et al., 2016). *B. pulchella*, as herbivorous-detritivorous organism, feeds on algae, fungi and particulate organic matter which possibly contain IVM (Lajmanovich, 2000, Fig. 4). Then, ingestion of organic particles present in sediment and herbivory of macrophytes with IVM would represent relevant sources of incorporation of the drug by aquatic assemblages (Fig. 4). Studies examining the concentration of this drug in aquatic fauna are lacking. Mesa et al. (2017) reported concentration of IVM in *Pomacea* sp., finding concentration values that numerically were higher than those reported for Planorbidae in the present study. Undetectable concentrations of this drug in Oligochaete *Dero* sp. was unexpected, considering its wide distribution and abundance in these wetlands, where they burrow into sediment and ingest sediment particles to obtain food (Ding et al., 2001). The relatively large size of the IVM molecule may have limited the uptake via integument and absorption over the gastrointestinal tract of this worm (Opperhuizen et al., 1985).

Accumulation of IVM in tissue could also have sublethal effects on aquatic organisms by reducing their motor activities, with significant effects on survival, reproduction, growth and emergence (Ding et al., 2001; Egeler et al., 2010; Obimakinde et al., 2017). Mesa et al. (2017) found significant effect on survival of *Ceriodaphnia* and *Hyalella*, whereas Schweitzer et al., (2010) found a clear reduction of emergence of *Chironomus* sp. and an extinction of *Daphnia magna* treated with IVM spiked in dung under laboratory conditions. Most of these aquatic organisms, as detritivorous taxa, play a significant role in the decomposition of manure and organic matter in wetlands. Therefore, reduction in abundance and effects on traits of these taxa could delay dung degradation, with possible implications on soil nutrient cycling (Madsen et al., 1990; Sommer and Bibby, 2002, Fig. 4).

Since disruption of development has been recognized as an effect of IVM on soil invertebrates (Strong, 1993; Boxall, 2018), the effect of this drug on the metamorphosis of Odonata, Chironomidae and *B. pulchella* and their translocation to adults need further study (Fig 4).

Therefore, biomagnification of IVM through the food web needs further study, since invertebrates and *B. pulchella* have natural predators like fishes (i.e., *A. anisitsi* and *S. calliurus*), aquatic insects (including Odonata and *Coelotanypus* sp.), hirudinean, and possibly, amphibians and birds (Fig. 4). Accumulation of IVM in fishes could also represent a threat to mammals, reptilians, birds and piscivorous fishes that feed on this resource, and with unknown potential effects for human health (Stehle and Schulz, 2015; Obimakinde et al., 2017) (Fig. 4).

5. Conclusions

The present study determined the presence, accumulation and persistency of IVM in sediment and aquatic fauna of wetlands that were exposed to cattle previously treated with the drug. Further, the concentration of IVM in wetlands increased with the number of treated cows and frequency of IVM treatment. Accumulation of IVM in aquatic organisms is alarming because these assemblages fulfill a key role in food webs, positioned between microbial communities and higher trophic levels, with consequences for several ecosystem functions. Thus, present results call for a greater control over the use of the drug in cattle (dosage, handling and frequency of application), and for management strategies that can keep treated animals away from watercourses in order to reduce the introduction, transfer and accumulation of IVM in aquatic systems. Further, results suggest that differences in growth habits of macrophytes and differential concentrations of the drug in sediment and water are factors that should be considered when phytoremediation studies are conducted or restoration practices are planned.

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Fig.1. A) Map of the Paraná River system, **B)** location of Wetland A (WA) and Wetland B (WB), **C)** dates of injection of IVM and number of cows injected in each period of sampling. The date of entry of cattle in each wetland is also shown.

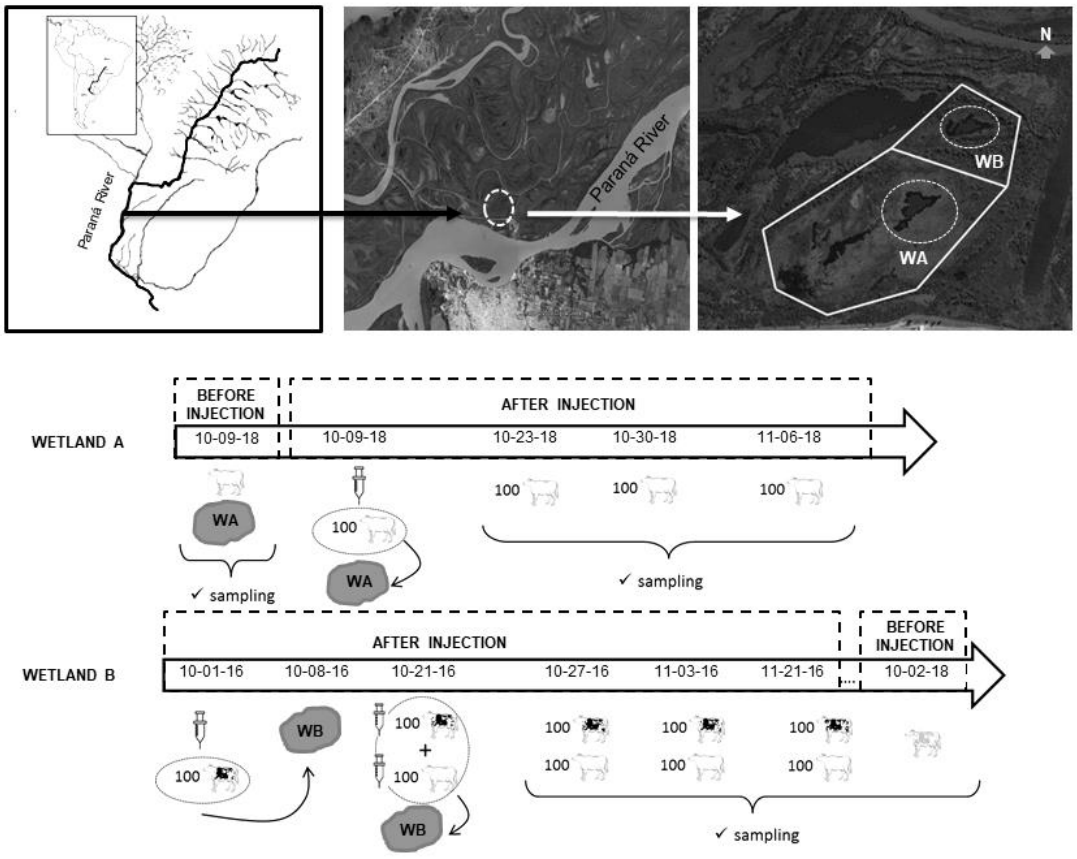

Fig. 2. Monthly values of mean temperature, precipitation and hydrometric levels (2015-2018) of the Paraná River recorded at the Paraná Port gauge scale (Data provided by Centro de Investigaciones Meteorológicas, CIM- FICH-UNL).

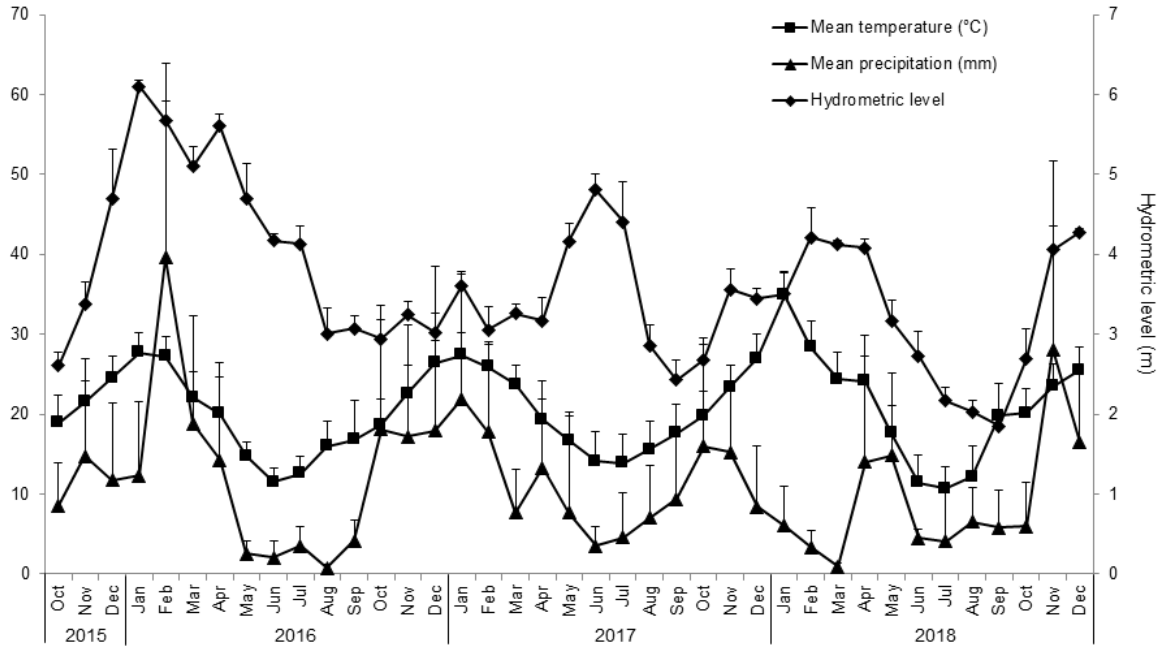

Fig. 3. Total concentration of IVM in both wetlands in days before and after injection of cattle with IVM. Frequency of injection of cattle is also shown.

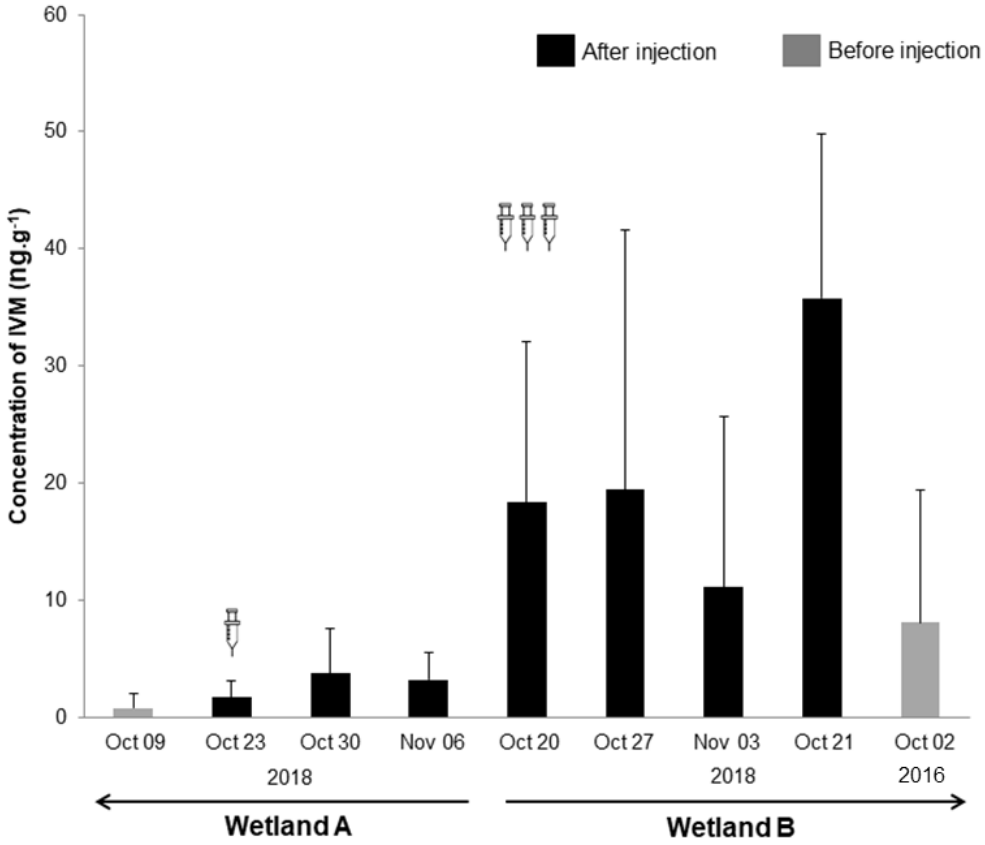

Fig. 4. Hypothetical environmental fate of ivermectin in aquatic food webs of wetlands and its possible biomagnification after its introduction via cattle dung into a water-sediment system. The diagram was constructed based on those taxa that effectively accumulated IVM in the studied lakes.

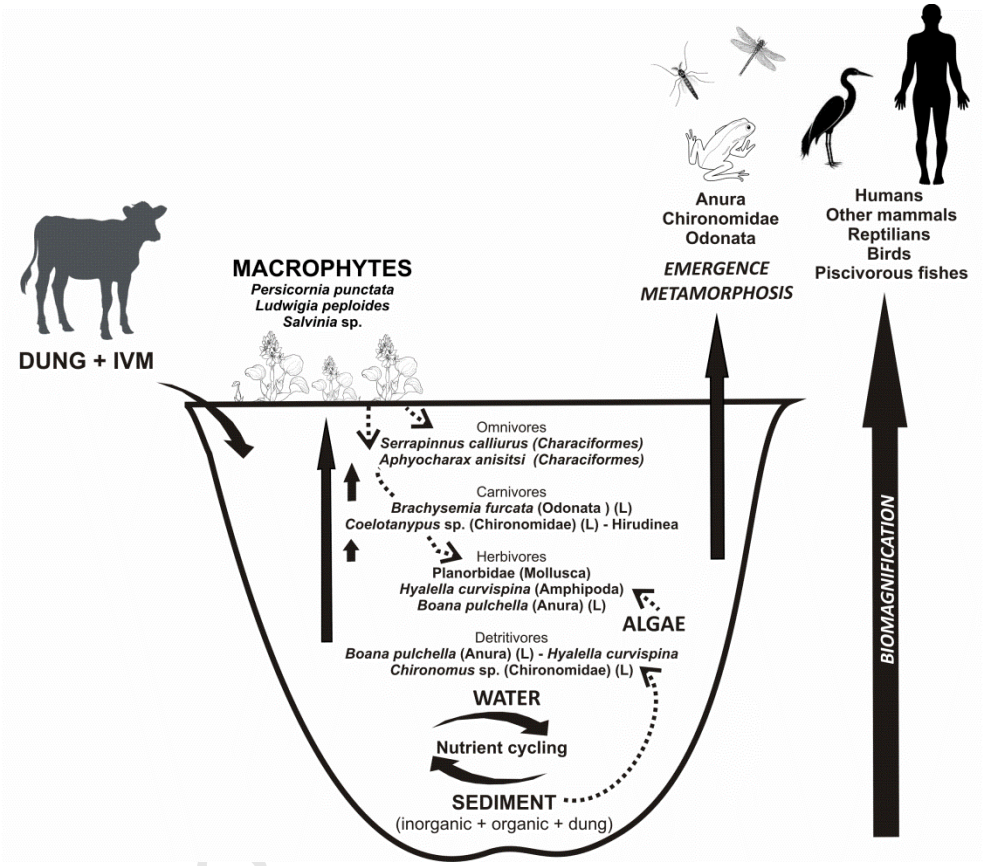

Table 1 Physicochemical variables relative to each sample day and wetlands before and after cattle injection with IVM.

	Wetland A					Wetland B			
	Before injection	After injection				After injection			Before injection
	10/09/18	10/16/2018	10/23/18	10/30/18	11/06/18	10/20/2016	10/27/2016	11/03/2016	10/02/18
pH	6.8	7.5	7.7	6.8	6.5	7.2	9.7	7.6	6.5
Conductivity (µS cm)	113	114	105	102	110	110	113	120	333
Water temperature (°C)	19.5	18.1	14.2	23.8	20.5	19.4	16.0	19.8	15.3
Disolved oxygen (ppm)	4.6	6.3	5.7	0	10.0	9.0	13.0	5.9	0
NH_4^+ (µgN L)	253.9 (0.5)	87.6 (2.1)	112.0 (1.2)	88.3 (2.5)	50.3 (3.1)	441.4 (0.8)	22.8 (2.1)	25.4 (1.2)	116.1 (2.3)
NO_3^- + NO_2^-(µgN L)	1.8 (1.4)	115.2 (2.2)	136.4 (0.9)	58.2 (0.9)	45.5 (2.5)	310.7 (1.3)	12.3 (1.1)	57.3 (0.8)	176.3 (1.2)
SRP (µg L)	31.5 (1.3)	22.3 (2.1)	48.6 (1.4)	22.2 (2.4)	17.9 (2.3)	20.2 (1.3)	12.0 (0.8)	13.6 (0.9)	36.4 (0.7)

Table 2 Mean concentration (±SD) of IVM (ng g^{-1} wet weight) in different compartments: manure, sediment, roots of macrophytes, invertebrates and vertebrates in the days before and after injection of cattle with the drug. Number of injection of cattle is also shown. Empty cells refer to samples not found in the field or not being dominant on this sample date.

	Wetland A				Wetland B			
	Before injection	After injection			 After injection			Before injection
	10/09/2018	10/23/2018	10/30/2018	11/06/2018	10/20/2016	10/27/2016	11/21/2016	10/02/2018
Manure	ND	4.73 (0.3)			31.6 (18.6)	51.4 (68.1)	194.5 (36.1)	ND
Sediment	ND	ND	2.25 (0.5)	1.58 (0.9)	16.2 (11.0)	17.1 (13.9)	13.1 (5.9)	ND
Water	ND	ND	ND	ND	1.24	ND	ND	ND
Roots of *Persicaria punctata*	ND				2.9 (1.1)	3.3 (2.0)	1.4 (1.2)	21.2 (1.4)
Roots of *Ludwigia peploides*	ND	ND	ND	ND			ND	0.57 (0.1)
Roots of *Salvinia sp.*							ND	1.12 (1.5)
Zooplankton	ND	ND	ND	ND	ND	ND	ND	ND
Chironomus sp.	ND	ND	9.5 (1.2)	ND	ND	ND	ND	ND
Coelotanypus sp.					ND	ND	1.9 (1.5)	ND
Dero sp.	ND	ND	ND	ND	ND	ND	ND	ND
Brachymesia furcata (larvae)		ND	ND	4.8 (1.1)				1.9 (1.5)
Hyalella curvispina	0.77 (0.8)	ND	ND	ND			ND	ND
Hirudinea	ND	0.75 (1.6)	1.5 (2.1)	ND				
Planorbidae	ND	2.65	ND	ND				
Boana pulchella (larvae)	ND	ND	1.58 (1.4)	ND				
Aphyocharax anisitsi					27.5 (0.9)		22.3 (1.5)	
Serrapinnus calliurus					30.5 (1.3)	5.9 (0.8)	15.4 (1.4)	
Phallotorynus victoriae								ND
Astyanax sp.	ND	ND						
Cheirodon interruptus		ND	ND	ND				
Hyphessobrycon anisitsi		ND	ND	ND				

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: