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RESEARCH ARTICLE

Impacts of cattle management and agricultural practices on water quality through different approaches: physicochemical and ecotoxicological parameters

Julieta Peluso[1,2] · Agostina Martínez Chehda[1,2] · Melisa S. Olivelli[1,2] · Federico M. Ivanic[1,2] · Matías Butler[1,2] · Virginia Aparicio[2,3] · Eduardo De Geronimo[2,3] · Florencia Gonzalez[4] · Lautaro Valenzuela[4] · Roberto J. Candal[1,2] · Carolina M. Aronzon[1,2]

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Abstract

The intensification of livestock farming can pose risks to the environment due to the increased use of veterinary products and the generation of waste in confined areas. The quality of water bodies near livestock establishments (Areco River (A) and Doblado stream (D), San Antonio de Areco, Buenos Aires, Argentina) was studied by physicochemical parameters, metals, pesticides, emerging contaminants, and lethal and sublethal toxicity (neurotoxicity and oxidative stress) in larvae of the native amphibian *Rhinella arenarum*. Six sites were selected: upstream (S1A and S1D), at the level (S2A and S2D), and downstream (S3A and S3D) from the establishments. A low concentration of dissolved oxygen was observed in Doblado stream (< 2.34 mg/L). Cu, Mn, V, and Zn exceeded the limits for the protection of aquatic life at various sites. Between 24 and 34 pesticides were detected in all sites, with 2,4-D, atrazine, and metolachlor being the most recurrent. In water and sediment, the concentrations of ivermectin (S2A, 1.32 µg/L and 58.18 µg/kg; S2D, 0.8 µg/L and 85.22 µg/kg) and oxytetracycline (S2A, < 1 mg/L and < 1 mg/kg; S2D, 11.8 mg/L and 39 mg/kg) were higher at sites near the establishments. All sites caused between 30 and 38.3% of lethality and produced neurotoxicity and alterations in the reduced glutathione content. Moreover, larvae exposed to samples from all sites incorporated ivermectin. These results demonstrate the degradation of the studied sites in relation to the agricultural activities of the area, highlighting the need to take measures to protect and preserve aquatic ecosystems.

Keywords Cattle breeding · Agriculture · Emerging contaminants · Amphibians

Introduction

Agricultural activities constitute one of the pillars in Argentine economy. In particular, the rapid growth of meat industry and the expansion of intensive crop cultivation have resulted in the displacement of cattle breeding to smaller and confined regions (feedlots) (Rearte & Pordomingo 2014). One of the main issues related to this intensification of cattle breeding is the production of large volumes of waste (manure, urine) concentrated in reduced areas, becoming one of the primary sources of metal and nutrient contamination. Physicochemical studies conducted on water bodies nearby, runoff water, and pore water in feedlots have found high levels of contamination by nitrogen, particularly nitrate in groundwater and ammonium in surface waters (Uusi-Kämppä et al. 2007). Additionally, elevated values of phosphorus, chlorine, potassium, and metals such as copper,

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✉ Carolina M. Aronzon
caronzon@unsam.edu.ar

1 IIIA-UNSAM-CONICET, Instituto de Investigación e Ingeniería Ambiental, Escuela de Hábitat y Sostenibilidad (EHyS), Universidad Nacional de San Martín (UNSAM), Campus Miguelete, 25 de Mayo y Francia, 1650-San Martín, Provincia de Buenos Aires, Argentina

2 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

3 Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria, Balcarce, Buenos Aires, Argentina

4 Laboratorio de Fluorescencia de Rayos X, Gerencia Química, Comisión Nacional de Energía Atómica, Buenos Aires, Argentina

zinc, and iron have been reported (Andriulo et al. 2003; Míguez et al. 2019). Some compounds, such as those containing arsenic, can be applied as antimicrobial agents (Mittapally et al. 2018). Furthermore, the overcrowding leads to a higher percentage of sick animals, resulting in an increased use of veterinary drugs, which are excreted unchanged and/or as metabolites along with feces and urine (Manyi-Loh et al. 2018). Consequently, these drugs can reach aquatic ecosystems either through excretion near or in water bodies, or through runoff, causing adverse effects on aquatic organisms (Manyi-Loh et al. 2018). Intensive livestock operations, due to the concentration of animals in confined spaces, can indeed lead to soil compaction. Soil compaction can result in soil degradation and reduced water permeability (Mielke & Mazurak 1976), which may lead to increased runoff of certain elements, such as metals and veterinary drugs.

In livestock production, thousands of tons of antibiotics are employed worldwide each year, with an increasing tendency (Van Boeckel et al. 2015). Some veterinary antibiotics are employed as growth promoters and incorporated in animal food to enhance the performance and improve the animal husbandry (Ronquillo & Hernandez 2017). The principal antibiotics that are utilized are streptomycin, bacitracin, and oxytetracycline, among others (Koch et al. 2021). Oxytetracycline (OTC), a member of the family of tetracyclines, is one of the most consumed in agriculture and medicine. As a result of its low bioavailability, approximately 70–80% of OTC is released directly into aquatic environments in its active form (Xu et al. 2021). Nowadays, OTC is frequently detected in the aquatic environment, with concentration in the range of ng/L or µg/L (Ashfaq et al. 2019). However, other pharmaceuticals, such as antiparasitics, are commonly applied to cattle (Di Guardo & Finizio 2017). Ivermectin (IVM) is primarily one of the most widely used antiparasitics worldwide (Lorente et al. 2023), administered to livestock such as cattle, sheep, horses, and pigs, functioning as both an endo- and ectoparasiticide. Up to 80–90% of the administered dose is excreted unaltered in the dung of the treated animal (Lozano et al. 2021). Information about the presence of veterinary pharmaceutical in the environment is scarce (Ramirez-Morales et al. 2021). There are just a few previous studies that report pharmaceutical pollution from livestock production in Argentina. However, comprehensive studies that integrate physicochemical parameters and ecotoxicological endpoints are lacking. To determine the impacts of agricultural activities and livestock production, it is essential to have knowledge on how production system affect aquatic ecosystems and wild populations.

Complementary to physicochemical analyses, toxicity bioassays assess the potential risk of organisms from exposure to matrices of various origin. Within the species used for bioassays, amphibians cover a large percentage. Amphibians fulfill crucial ecological roles in both terrestrial and aquatic ecosystems, including nutrient cycling and circulation (West 2018). They act as general predators, helping control agricultural pests, and play a vital role in disease vector management by regulating populations of mosquitoes and flies, thus contributing to public and veterinarian health (West 2018). However, Blaustein et al. (2003) suggest that amphibians, especially during the larval phase when their organs are still forming and they rely on the aquatic environment, are more susceptible to contaminants. Negative impacts during early developmental stages may have serious consequences in the long term, such as impeding their progression to adult phases, limiting their ability to find reproductive partners, and affecting their egg-laying (Blaustein et al. 2003; Curi et al. 2021). Also, amphibians' populations are in decline and a high frequency of malformations was reported in populations of agricultural areas of Argentina (Peltzer et al. 2011). The AMPHITOX standardized test uses larvae of a native amphibian from Argentina, *Rhinella arenarum*, for the evaluation of the toxicity of different substances (Pérez Coll et al. 2017).

Biomarkers play an important role in toxicity testing since they provide relevant information about early sublethal effects. Oxidative stress biomarkers have been widely employed as indicators of toxicity and risk assessment of environmental pollutants (Xu et al. 2021). Catalase (CAT) is a key antioxidant enzyme while reduced glutathione (GSH) is a molecular antioxidant. Thiobarbituric acid reactive substances (TBARS) are an important biomarker for lipid peroxidation, whose contents demonstrate the degree of oxidative damage by pollutants (Elizalde-Velázquez et al. 2017). On the other hand, glutathione S-transferase (GST) is an enzyme that plays an important role in detoxifying xenobiotics and lipid peroxidation products (Sun et al. 2020). Studies suggested that pesticides, antibiotics, and heavy metals can trigger oxidative stress and induce antioxidant responses (Nunes et al. 2021; Sule et al. 2022; Wang et al. 2020). Other useful biomarkers indicative of neurotoxicity and neuroprotection are the activities of cholinesterases. Initially, the measurement of the activity of acetylcholinesterase (AChE) and/or butyrylcholinesterase (BChE) was used as a biomarker of exposure to organophosphate and carbamate insecticides (Fukuto 1990). However, in the last times, it was discovered that not only these groups of insecticides cause neurotoxicity, but also some metals and various contaminants can alter the activity of this enzyme (Frasco et al. 2005).

Since there is little information about the impact of cattle breeding activities on water quality, the aim of the present study was to evaluate the quality of two water bodies near two cattle establishments with different animal load through physicochemical indicators, metals, pesticides, emerging contaminants (OTC and IVM), and ecotoxicological parameters with *R. arenarum* larvae (lethality bioassays,

the incorporation of IVM and biomarkers of oxidative stress and neurotoxicity).

Material and methods

Selection of study area

The proposed study area is located in the district of San Antonio de Areco, in the northwest of Buenos Aires province (Fig. 1), an area where there are intensive cattle breeding establishments (feedlots). In particular, the Doblado stream (D) was selected, which passes about 220 m from a large feedlot (34°02′10.3″S 59°19′40.1″W), and the Areco River (A), which passes about 700 m from another feedlot. Three sites were selected on each water body: upstream from the feedlot (S1D and S1A), at the height of the feedlot (S2D and S2A), and downstream of the feedlot (S3D and S3A). Due to the difficulty of finding a pristine site in the area, S1 of each water body was considered a reference site without the effect of the feedlot, in addition to the laboratory control.

Water and sediment samples

A sampling campaign was performed during spring 2022. The water samples were taken according to Alberro et al. (2011). Electrical conductivity, pH, dissolved oxygen (DO), total solids, and turbidity were measured in situ in triplicate with a multiparameter probe (HORIBA U-50). At each site, a 2-L water sample was taken for the determination of physicochemical parameters, metal, pesticides, and emerging contaminants and a 5-L for the performance of toxicity bioassays in clean plastic bottles. Also, a 2-kg sediment sample was taken at each site according to ASTM (2014) for the performance of toxicity bioassays and the measurement of metals and pesticides.

Fig. 1 Study area and the sampling sites from Areco River (S1A, S2A, and S3A) and Doblado Grande stream (S1D, S2D, and S3D). The image was personally created based on maps from Instituto Geográfico Nacional (https://mapa.ign.gob.ar)

Physicochemical parameters, metals, and pesticides

In the laboratory, the following were measured: total suspended solids (TSS) by filtering water samples with glass fiber filters (0.7 µm pore diameter). Phosphate, nitrate, and ammonium were measured in the filtered water samples while chemical oxygen demand (COD), biological oxygen demand (BOD5), nitrite, chloride, and sulfate were determined in unfiltered water samples. Phosphate, nitrate, ammonium, nitrite, chloride, and sulfate were evaluated following the HACH protocols using a HACH DR 1900 spectrophotometer. Dissolved organic carbon was analyzed using a Shimadzu analyzer TOC-5000A by SM 5310B and BOD5 following the recommendations of APHA (2012).

The analysis of metals in water and sediment samples was conducted using a fluorescence spectrometer of X-ray by total reflection (TXRF): S2 Picofox model by Bruker, with a molybdenum tube, as previously outlined in the study by Peluso et al. (2022a).

Ultra-high-performance liquid chromatography-MS/MS (UHPLC-MS/MS) using Waters equipment was employed to measure pesticides (51 residues). The sediment and water samples were divided into two separate aliquots for analysis. The first aliquot followed the methodology outlined by Aparicio et al. (2013) to determine glyphosate and AMPA. The second aliquot underwent analysis for multiple pesticide residues using the procedure reported by (De Gerónimo et al. 2015). The limit of detection (LOD) and the limit of quantification (LOQ) for each measured molecule are presented in the results section (Table 4).

Table 1 Parameters employed for the calculation and their environmental objectives

Parameter	Environmental objective	References
Ammonium	< 1.29 mg/L	Ávila Pérez et al. (2011)
As	< 50 µg/L	Law 24,051; decree 831/93
BOD5	< 11.63 mg/L	Ávila Pérez et al. (2011)
Conductivity	< 1.25 mS/cm	Ávila Pérez et al. (2011)
Cu	< 2 µg/L	Law 24,051; decree 831/93
DO	> 4.7 mg/L	Ávila Pérez et al. (2011)
Mn	< 100 µg/L	Law 24,051; decree 831/93
Nitrate	< 17 mg/L	Ávila Pérez et al. (2011)
Nitrite	< 0.06 mg/L	Law 24,051; decree 831/93
Pb	< 1 µg/L	Law 24,051; decree 831/93
pH	> 5– < 9	Ávila Pérez et al. (2011)
Phosphate	< 0.4 mg/L	Ávila Pérez et al. (2011)
TSS	< 81.25 mg/L	Ávila Pérez et al. (2011)
Vn	< 100 µg/L	Law 24,051; decree 831/93
Zn	< 30 µg/L	Law 24,051; decree 831/93

Emerging contaminants

Ivermectin and OTC were determined in water and sediment samples by HPLC–MS (Thermo Scientific Ultimate 3000 equipped with an autosampler), following the methodology detailed in Peluso et al. (2023). For IVM, a reverse phase C18 column was used (Hypersil Gold, USA, 1.9 m, 2.1 mm 50 mm).

For OTC determination in water samples, 5 mL was firstly diluted 1:1 by adding Na_2EDTA (13 mM, Anedra), doxycycline (Sigma, USA) as internal standard (10 µM), and MilliQ water. No changes in pH from the original water sample

Table 2 Physicochemical parameters from all sampling sites measured in situ and laboratory in water. "*nd*" not detected, "*DO*" dissolved oxygen, "*BOD5*" biological oxygen demand 5, "*DOC*" dissolved organic carbon, "*TSS*" total suspended solids

Parameters	Units	S1A	S2A	S3A	S1D	S2D	S3D
In situ							
Conductivity	mS/cm	1.48 ± 0.01	1.48 ± 0.02	1.4 ± 0.1	1 ± 3	1.01 ± 0.01	1.51 ± 0.05
DO	mg/L	6.7 ± 0.4	6.58 ± 0.03	4.5 ± 0.2	2.34 ± 0.07	0.6 ± 0.8	1 ± 2
pH		8.36 ± 0.01	8.13 ± 0.05	8.04 ± 0.01	7.70 ± 0.01	7.51 ± 0.04	7.53 ± 0.05
Temperature	°C	18.98 ± 0.04	19.7 ± 0.1	21.1 ± 0.1	22.6 ± 0.3	18.5 ± 0.7	19 ± 2
Turbidity	NTU	42 ± 7	35 ± 2	132 ± 12	66 ± 10	61 ± 6	312 ± 30
In laboratory							
Ammonium	mg/L	0.23	0.17	0.13	0.09	0.09	0.17
BOD5	mg/L	3.53	3.67	3.58	7.21	8.09	7.85
COD	mg/L	20.00	18.00	7.00	32.00	38.00	119.00
Chlorides	mg/L	5.00	3.90	1.20	0.40	1.00	3.00
DOC	mg/L	9	34	7.5	13.6	15	46.5
Nitrates	mg/L	2.00	1.60	1.60	0.30	0.30	0.10
Nitrites	mg/L	0.09	0.08	0.03	nd	0.01	0.01
Phosphates	mg/L	3.52	3.12	1.90	0.93	1.07	2.54
Sulfates	mg/L	94	98	102	nd	1	35
TSS	mg/L	29.75	9.60	38.40	16.22	25.43	73.33

Table 3 Metal concentrations in water (µg/L) and sediment (mg/kg) samples from all sites. "*nd*" not detected, "*na*" not available. *Exceeded the limit for protection of aquatic life (Argentine law 24,051, decree 831/93)

	S1A	S2A	S3A	S1D	S2D	S3D
Water (µg/L)						
As	42	39	42	34	32	31
Br	161	182	228	77	112	258
Cu	nd	15*	29*	17*	nd	25*
Fe	1238	877	3430	1621	1109	1118
Mn	96	76	108*	481*	375*	14,357*
Pb	nd	nd	nd	nd	nd	16
Sr	497	461	469	291	321	488
Ti	154	94	416	129	145	127
V	108*	103*	100*	45	nd	nd
Zn	21	18	33*	40*	36*	80*
Sediments (mg/kg)						
As	8.8	10.2	14.7	2	4.6	5
Ba	200.7	223.1	247.2	388	295.5	317.3
Br	5.4	6	6.4	2.4	1.7	6.2
Cr	12.8	13.6	32	32.6	16	31.8
Cu	15.5	17.1	26.8	20.8	13	21.3
Fe	12,212.1	16,595.4	26,460.1	21,569.2	15,457	22,959
Mn	967.9	1402.8	1314.3	189.4	190.5	479.4
Ni	6.2	7.4	12.6	6.8	5.1	7.5
Pb	5.4	8.6	22.3	nd	11.4	30.9
Rb	40.3	54.5	78.6	70.4	57	72
Sr	275.3	247.7	552.3	205.9	172.9	182.5
Ti	1463	1617.6	2223.9	2712.9	2122.4	2619.5
V	41.1	39.9	45.1	176.8	50.9	46
Zn	41	50.3	63.9	47.8	37.2	63.3

were detected after dilution. Samples were then concentrated by using tandem SPE cartridges (Strata-SAX followed by Strata-X, Phenomenex), using methanol with 1% formic acid as eluent, which was later evaporated, redissolving the sample in 0.5 mL of water. Oxytetracycline determination in sediment samples (starting from 2 g of wet sediment) and chromatographic analysis were performed as reported in previous publications (Bracco et al. 2023; Peluso et al. 2023; Ivanic et al. 2023). External calibration curves for oxytetracycline and doxycycline were performed, ranging from 0.5 to 50 ppm and 0.5 to 10 µM, respectively.

Biological material and toxicity bioassays

The AMPHITOX protocol was followed (Pérez Coll et al. 2017). Briefly, ovulation of adult *Rhinella arenarum* females was induced by intraperitoneal injection of human chorionic gonadotropin. The oocytes were fertilized with a 10% testicular macerate prepared in AMPHITOX solution (AS, composition: KCl 0.5 mg/L, NaCl 36 mg/L, $NaHCO_3$ 2 mg/L, and $CaCl_2$ 1 mg/L in deionized water) (Pérez Coll et al. 2017). Organisms were maintained in AS until they reached the larval stage, operculum stage (S.25) (Gosner 1960). The photoperiod (14/10 light darkness) and the temperature (20 ± 2 °C) were kept constant. The media were renewed every other day to ensure oxygen levels.

For the lethality toxicity bioassays, 20 larvae were exposed to water and sediment samples (approximately 200 mL of water sample in order to form a 2-cm layer and 200 mL of sediment) from each site for 504 h according to Peluso et al. (2022a). Simultaneously, a control group was performed using 200 mL of a silica inert substrate and 200 mL of AS. Larvae were fed with TetraColor fish food every other day, and the media were partially renewed (100 mL) every other day.

Ivermectin incorporation in *Rhinella arenarum* larvae

At the end of the lethality bioassay, the remaining larvae were collected, washed with deionized water, dried, weighted, and frozen until determination. The concentration of IVM accumulated in the larvae was determined by HPLC–MS using the same analysis employed for water and sediment samples. A Thermo Scientific Ultimate 3000 was equipped with an autosampler and a reverse phase

Table 4 Concentration of pesticides in water (µg/L) and sediment (µg/kg) samples of the different sites. *LOD* limit of detection, *LOQ* limit of quantification, "*nd*" not detected

	S1A	S2A	S3A	S1D	S2D	S3D	LOD	LOQ
Water (µg/L)								
2,4-D	0.0162	0.0124	0.0197	0.0081	0.0098	0.0205	0.0001	0.0003
2,4-DB	0.0011	nd	< 0.0004	< 0.0004	nd	nd	0.0001	0.0004
Allethrin	nd	nd	< 0.0004	nd	nd	< 0.0004	0.0001	0.0004
Ametrine	< 0.0003	< 0.0003	< 0.0003	0.0012	0.001	< 0.0003	0.0001	0.0003
AMPA	2.58	2.65	2.01	nd	nd	< 0.15	0.08	0.15
Atrazine	0.0182	0.0194	0.0169	0.0096	0.0108	0.0165	0.0001	0.0004
Atz-desethyl	0.0042	0.0039	0.0033	0.001	0.0013	0.0042	0.0003	0.0008
Atz-OH	0.0038	0.0041	0.0046	0.0328	0.0386	0.123	0.0003	0.0009
Carbaryl	nd	nd	nd	< 0.001	< 0.001	nd	0.0003	0.001
Carbofuran	nd	nd	< 0.0006	< 0.0006	< 0.0006	nd	0.0002	0.0006
Chlorpyrifos	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0004	0.001
Diazinon	nd	< 0.0004	< 0.0004	nd	nd	nd	0.0001	0.0004
Dicamba	< 0.009	< 0.009	< 0.009	nd	nd	0.0093	0.003	0.009
Diclosulam	nd	nd	0.004	nd	0.007	nd	0.0002	0.0006
Epoxiconazole	< 0.0008	nd	nd	< 0.0008	< 0.0008	< 0.0008	0.0002	0.0008
Ethyl chlorimuron	0.0011	< 0.0007	< 0.0007	nd	< 0.0007	< 0.0007	0.0003	0.0007
Fipronil	0.0005	0.0004	0.0005	< 0.0003	nd	< 0.0003	0.0001	0.0003
Fomesafen	< 0.003	< 0.003	< 0.003	< 0.003	nd	nd	0.001	0.003
Glufosinate	nd	nd	nd	nd	< 0.1	nd	0.05	0.1
Glyphosate	< 0.1	< 0.1	< 0.1	nd	< 0.1	nd	0.05	0.1
Halauxifen-methyl	nd	nd	< 0.0003	nd	< 0.0003	0.0008	0.0001	0.0003
Imazapyr	nd	nd	0.0003	nd	0.0018	nd	0.0001	0.0004
Imazaquin	nd	nd	< 0.0008	nd	nd	nd	0.0003	0.0008
Imazethapyr	< 0.0004	nd	nd	0.0132	0.011	0.0126	0.0001	0.0004
Imidacloprid	0.007	0.0044	0.0069	nd	nd	nd	0.0003	0.0008
Metalaxyl	nd	nd	< 0.0004	nd	< 0.0004	nd	0.0001	0.0004
Metconazole	< 0.0006	nd	< 0.0006	< 0.0006	< 0.0006	nd	0.0002	0.0006
Methomyl	< 0.001	< 0.001	nd	nd	nd	nd	0.0004	0.001
Metolachlor	0.1925	0.1801	0.1298	0.2903	0.0892	0.1087	0.0001	0.0004
Metsulfuron methyl	< 0.0006	< 0.0006	< 0.0006	nd	nd	< 0.0006	0.0002	0.0006
Pendimethalin	nd	nd	nd	nd	nd	< 0.0008	0.0002	0.0008
Piperonyl butoxide	0.0206	0.0232	0.0021	< 0.0008	0.0026	0.0015	0.0002	0.0008
Pirimicarb	< 0.0003	< 0.0003	< 0.0003	< 0.0003	0.0021	nd	0.0001	0.0003
Pirimiphos methyl	nd	nd	nd	< 0.0008	nd	< 0.0008	0.0002	0.0008
Simazine	nd	< 0.001	nd	nd	nd	nd	0.0007	0.001
Sulfentrazone	0.0031	nd	0.0086	0.0163	0.02	0.0239	0.0002	0.0005
Tebuconazole	0.0011	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	0.0002	0.0005
Tetramethrin	nd	nd	nd	nd	< 0.0008	nd	0.0002	0.0008
Sediment (µg/kg)								
Atz-OH	nd	< 0.5	< 0.5	1.3	1.4	0.6	0.1	0.5
2,4-D	< 1.6	nd	nd	nd	< 1.6	nd	0.6	1.6
Ametrine	nd	nd	nd	< 0.3	< 0.3	nd	0.1	0.3
AMPA	163.95	40.35	55.15	30.85	6.95	9.95	0.4	1.4
Atrazine	nd	< 0.4	< 0.4	< 0.4	nd	< 0.4	0.1	0.4
Epoxiconazole	nd	nd	< 0.6	< 0.6	nd	nd	0.2	0.6
Glyphosate	17.15	15.9	11.65	8.55	6.9	2.25	0.3	0.8
Metolachlor	< 1.4	< 1.4	< 1.4	nd	< 1.4	nd	0.5	1.4
Piperonyl butoxide	nd	nd	nd	< 0.6	nd	nd	0.2	0.6

Table 4 (continued)

	S1A	S2A	S3A	S1D	S2D	S3D	LOD	LOQ
Total detected elements	28	24	34	27	29	25		

Table 5 Ivermectin and oxytetracycline concentrations in water and sediment samples from all sites. "*nd*" not detected

	Ivermectin		Oxytetracycline	
	Water (µg/L)	Sediment (µg/kg)	Water (mg/L)	Sediment (mg/kg)
S1A	0.62	4.83	nd	nd
S2A	1.32	58.18	< 1	< 1
S3A	< 0.30	15.72	nd	nd
S1D	< 0.30	29.6	nd	nd
S2D	0.80	85.22	11.8 ± 0.2	39 ± 8
S3D	< 0.30	42.19	< 1	nd

C18 column (Hypersil Gold, USA, 1.9 m, 2.1 mm 50 mm). Extraction was performed as follows: 2 g of larvae (approximately 20 larvae) was shock-frozen with liquid nitrogen and homogenized with 2 mL of acetonitrile and the internal standard compound (1 ppm abamectin, PESTANAL®) using a mortar and pestle. The homogenate was transferred to a beaker with 20 mL of acetonitrile. The preparation was incubated in agitation for 15 min and sonicated for 20 min at 20 °C. The solvent-sample mixture was centrifuged at 3500 *g* for 10 min. The supernatant was separated to perform the solid-phase extraction (SPE): the solvent was evaporated, and 10 mL of double-distilled water was added. The aqueous solution was passed through C18 SPE cartridges and eluted with 100% acetonitrile (HPLC–MS quality). Desorbed analytes were diluted with a solution of 5 mM NH4AcO and 0.05% acetic acid in acetonitrile (25/75).

Biomarkers of oxidative stress and neurotoxicity

For the determination of biomarkers of oxidative stress and neurotoxicity, groups of 50 larvae were exposed for 96 h, in triplicate, to the water and sediment samples (approximately 200 mL of water sample in order to form a 2-cm layer and 200 mL of sediment). A control group was performed simultaneously with 200 mL of a silica inert substrate and 200 mL of AS (Peluso et al. 2022a). The employed protocols were previously adapted for *R. arenarum* larvae (Peluso et al. 2020). Once the exposure ended, organisms were washed, dried, and frozen. Then, samples were homogenized with a 0.154 M KCl solution (with inhibitors of proteases) and centrifuged at 10,000 *g* for 20 min. Biomarker measurements were made on the supernatant. Proteins were determined according to Bradford (1976) and the values were used to relativize the biomarkers. Catalase (CAT) was measured according to Aebi (1974) (units of CAT (mmol*min)/mg protein using a molar extinction coefficient of 200 40/M × cm) and glutathione S-transferase (GST) following the Habig et al. (1974) method (units of GST mg (mmol*min)/protein using a molar extinction coefficient of 9.6/mM × cm). Reduced glutathione (GSH) was evaluated according to Anderson (1985) (nmol GSH/mg protein) and lipoperoxidation was estimated by the thiobarbituric acid reactive substances (TBARS) following the technique from Buege and Aust (1978), with slight modifications (nmol TBARS/mg protein). Butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) activities were measured according to the technique of Ellman et al. (1961). The calculation of BChE and AChE activities utilized molar extinction coefficients of 13.6/mM × cm and 14.15/mM × cm, respectively. The activities were expressed as units of BChE (nmolmin)/mg protein and units of AChE (nmolmin)/mg protein.

Data analyses

Physicochemical parameters measured in situ are presented as mean ± standard deviation (SD). Statistical comparisons of lethality and biomarkers were made using one-way ANOVA followed by Tukey's test if the requirements were fulfilled. In the case requirements have not been met, non-parametric tests were performed. All comparisons were performed using GraphPad Prism 8.

A water quality index (WQI) was calculated based on the proposal by Neary et al. (2001). Briefly, environmental objectives (EO) for the protection of aquatic life were established according to the Argentine law 24051, decree 831/93, and (Ávila Pérez et al. 2011). In Table 1, the parameters employed for the calculation and their corresponding limits are presented. The WQI categorization was very poor (0–29), poor (30–44), marginal (45–64), bad (65–79), good (80–94), and excellent (95–100).

Ethical statement

The handling of the animals was carried out in accordance with the regulations for the use of live amphibians and reptiles in the field and laboratory research (Beaupre et al. 2004). They were controlled and approved by the Institutional Committee for the Care and Use of Experimental Animals (CICUAE Res. 01/2022) of the University National of

San Martin (UNSAM) and the Dirección de Flora y Fauna (Res. 01133069).

Results

Physicochemical parameters, metals, pesticides, and water quality index

Table 2 details the physicochemical parameters analyzed both *in situ* and in the laboratory. In all the sites from the Doblado stream, and mainly in S2D, the dissolved oxygen concentration was lower than the environmental objective, varying between 2.34 and 0.59 mg/L. In S2D, DOC, BOD5, and COD concentrations were higher in comparison to the other sites. Conductivity was higher than the EO in all sites from the Areco River and S3D. Likewise, in the Areco River, the concentration of sulfates and ammonium were greater than in the Doblado stream.

Some metals exceeded the limit for protection of aquatic life (Argentine Law 24,051 decree 831/93) in all sites (Table 3). In particular, V exceeded its limit in water samples from all sites of the Areco River, while Mn exceeded its limit in all water samples from Doblado stream and water samples from S3A. The Cu levels exceeded their limit in S2A, S3A, S1D, and S3D, and Zn levels exceeded their limit in S3A and in all water samples from Doblado stream. The Pb concentration was higher than the limit for protection of aquatic life in water sample from S3D. On the other hand, in sediments, most of the elements were concentrated between 1.07 and 67 times, which may increase the risk of exposure to organisms.

According to the WQI, the quality of all sites varied from bad to poor, except for S1A, where the quality was good. Along the Areco River, the quality progressively deteriorated: it was good at S1A (88.3), bad at S2A (74.4), and marginal at S3A (59). Conversely, in the Doblado stream, the quality showed a variation from marginal at S1D (64) to bad at S2D (70.4) and poor at S3D (36.1).

On the other hand, a wide variety of pesticides were detected in water samples, while in sediments, fewer pesticides were present but in higher concentrations (Table 4). The insecticide imidacloprid was detected in all samples from the Areco River (0.0044–0.007 µg/L) while atrazine (0.0096–0.0194 µg/L) and its degradation metabolites (Atz-OH (0.0038–0.123 µg/L) and Atz-desethyl (0.001–0.0042 µg/L)) were detected in all samples from both water bodies. The herbicide sulfentrazone was detected in almost all samples (0.0163–0.0239 µg/L), with the exception of S2A. The herbicides ametrin (0.0003–0.0012 µg/L), metolachlor (0.0892–0.2903 µg/L), and 2,4-D (0.0081–0.0205 µg/L) were detected in water samples from all sites. On the other hand, the adjuvant piperonyl butoxide (0.0008–0.0232 µg/L) and the fungicide tebuconazole (0.0005–0.0011 µg/L) were also detected in water samples from all sites. The glyphosate degradation metabolite (AMPA) was present in high concentrations in all water samples from Areco River (2.01–2.65 µg/L) and in sediments (6.95–163.95 µg/kg) from all sites. In general terms, all the sites presented between 24 and 34 pesticide residues, and S3A was the one that presented the greatest variety with concentrations varying between 0.0021 and 2.01 µg/L. The residues were mainly found on water samples. However, in sediment samples from all sites, glyphosate and AMPA were concentrated between approximately 69 and 171 times for glyphosate and 27 and 63 times for AMPA.

Emerging contaminants related to animal breeding activities

The antiparasitic widely used in cattle breeding, IVM, was found in all sites (Table 5). In particular, IVM concentrations in water and sediments were higher in the sites close to the cattle breeding establishments: S2A (1.32 µg/L and 58.18 µg/kg) and S2D (0.8 µg/L and 85.22 µg/kg). On the other hand, OTC was found in water and sediment samples near the cattle breeding establishments. In particular, it was detected in the water (< 1 mg/L) and sediment (< 1 mg/kg) samples from S2A. However, higher concentrations of OTC were found in water (11.8 mg/L) and sediment samples (39 mg/kg) from S2D. Finally, OTC was detected (< 1 mg/L) in the water sample from S3D.

Chronic toxicity bioassay

All exposures caused significant lethality (Fig. 2). Particularly, S2A and S3A caused significant ($p < 0.05$) mortality since the 48 h of exposure. At the 288 h of exposure, S2D and S3D also caused significant ($p < 0.05$) mortality while S1A and S1D caused significant mortality ($p < 0.05$) from the 336 h of exposure. At the end of the bioassay (504 h), S2A and S3A caused the highest lethality percentage (38.3 ± 5.8 and 38.3 ± 12.6%, respectively), followed by S3D (35 ± 5%), and finally S1D, S2D, and S1A (30 ± 5, 30 ± 0, and 30 ± 18%, respectively). Lethality in the control group (6.7 ± 2.9%) was lower than the 10% accepted for the AMPHITOX protocol.

Ivermectin incorporation bioassay

In the surviving larvae of each treatment, ivermectin concentrations were measured (Table 6). Larvae exposed to samples from all sites incorporated ivermectin. In particular, those exposed to samples from S2A and S1D exhibited the highest concentration (0.35 ± 0.10 and 0.35 ± 0.17 µg/g, respectively).

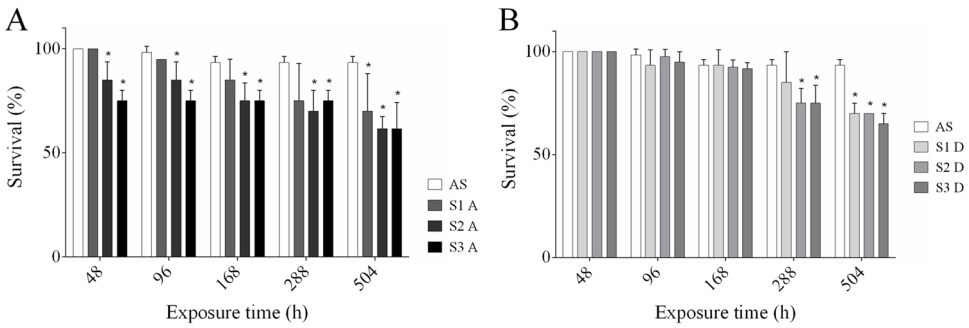

Fig. 2 Mean survival ± SD (%) of larvae exposed to water and sediment samples from **A** the Areco River (S1A, S2A, and S3A) and **B** Doblado Grande stream (S1D, S2D, and S3D) and the control group (AS)

Table 6 Mean (± standard deviation, SD) ivermectin concentration in µg/g in the surviving larvae from lethality bioassays exposed to the control group (AS) and water and sediment samples from all sites

	Mean	SD
AS	0.00	0.00
S1A	0.26	0.21
S2A	0.35	0.10
S3A	0.27	0.09
S1D	0.35	0.17
S2D	0.19	0.11
S3D	0.24	0.26

Oxidative stress and neurotoxicity biomarkers

According to the oxidative stress biomarkers measured, only the GSH levels were lower ($p < 0.05$) in larvae exposed to all water and sediment samples in comparison to the control group (Fig. 3A–C). On the other hand, larvae exposed to all water and sediment samples presented lower AChE and BChE activities ($p < 0.05$) in comparison to the control group (Fig. 3D, E).

Discussion

Existing research about the impact of cattle breeding on water quality is limited, and there is a need for more extensive studies to provide a comprehensive understanding of the effects of these practices on water resources. Such analyses are crucial for assessing potential contaminants, identifying risks to aquatic ecosystems, and developing effective management strategies to ensure the sustainable coexistence of agriculture and water quality preservation.

In the three evaluated sites of the Doblado stream and in S3A of Areco River, near the cattle breeding establishment, the DO was lower than the limit for protection of aquatic life (5.5 mg/L) (Ossana et al. 2016). This might be a consequence of the high organic matter content found in these sites. The decline in DO levels can be attributed to various factors, including human activities. Pollution from agricultural runoff, excessive nutrient inputs, high organic matter content, and the release of pollutants, as was the case for all water samples, can contribute to oxygen depletion in water bodies. Low DO levels in aquatic habitats pose a significant threat to aquatic life, especially amphibians (Cohen et al. 2012). These low levels impact their survival, growth, reproduction, behavior, and overall population dynamics. Moreover, DOC was high in all sites from Doblado stream. In water samples from S2A of the Areco River and all samples from the Doblado stream, the DOC concentrations were higher than the average concentration of riverine DOC (10.4 mg/L) across the global rivers (Liu & Wang 2022). The concentration of DOC in water bodies can have significant implications for the health and functioning of aquatic life. High concentrations of DOC can lead to imbalanced nutrient dynamics, potentially causing shifts in ecosystem productivity and composition (Creed et al. 2018). The decomposition of DOC by microorganisms consumes DO in water bodies, which can potentially lead to reduced DO levels. Moreover, DOC can act as a carrier for contaminants and pollutants in aquatic environments (Bhateria & Jain 2016). Organic compounds present in DOC can form complexes with metals and other contaminants, affecting their bioavailability and transport (Yang et al. 2021). Consequently, high DOC concentrations can facilitate the spread and persistence of pollutants, potentially increasing their toxic effects on aquatic organisms. On the other hand, nitrate levels were higher in all sites from Areco River and can enter the environment through natural and anthropogenic sources such as animal farming, urban and agricultural runoff, industrial wastes (Camargo et al. 2005). The normal concentration of nitrates in freshwater ecosystems can vary depending on a range of factors such as geographical location, surrounding land use, hydrological conditions, and local nitrogen inputs. In water samples from the Areco River, the nitrate concentrations were similar to the previously reported in areas impacted by agricultural activities from Argentina (Andrade et al. 2021; Lutri et al. 2020). On the other hand, phosphate plays a vital role in aquatic ecosystems. However, when present in elevated concentrations, it can have significant impacts on freshwater ecosystems (Correll 1998). In pristine rivers, annual loads of phosphorus to the river system are extremely low, since phosphorus is not abundantly available from the

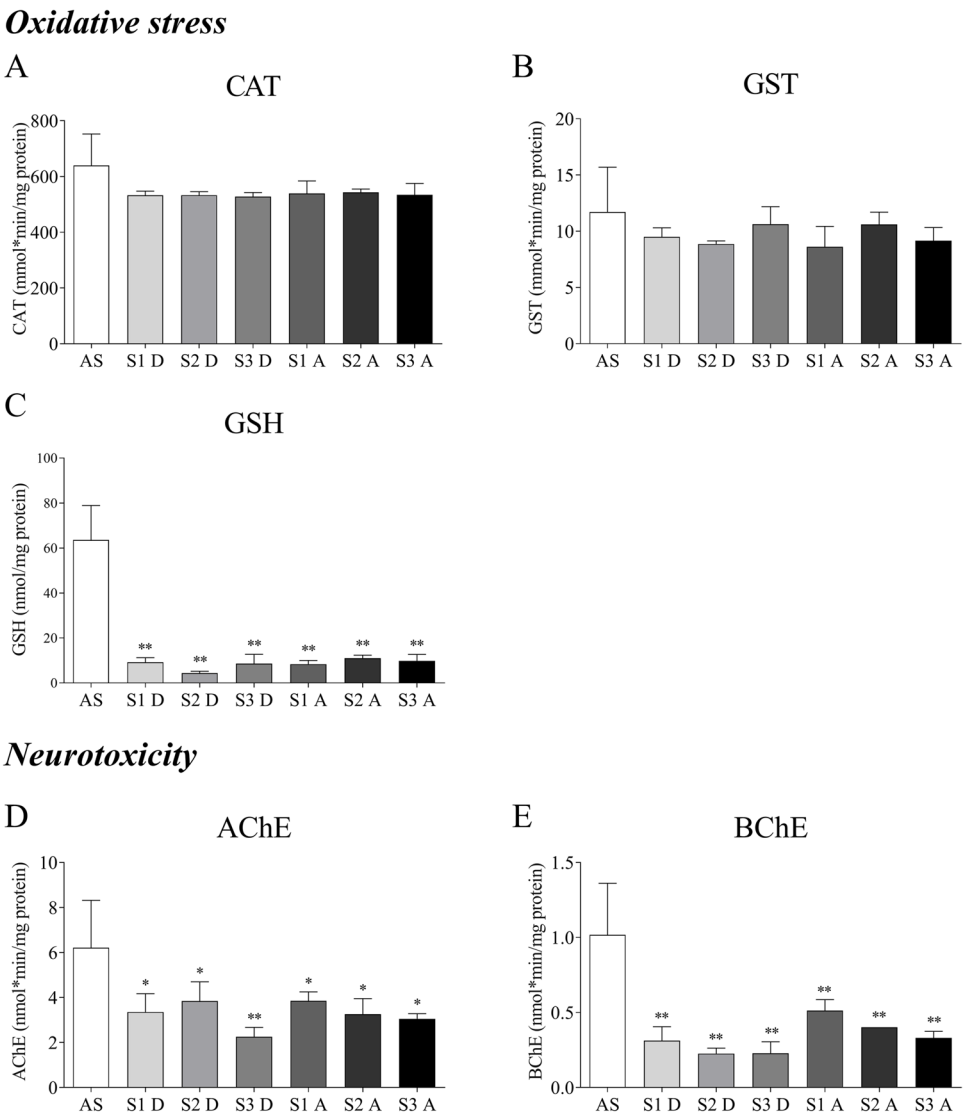

Fig. 3 Mean (±SD) oxidative stress and neurotoxicity biomarkers in larvae exposed to water and sediment samples from the Areco River (S1A, S2A, and S3A) and Doblado Grande stream (S1D, S2D, and S3D). **A** Catalase activity (CAT). **B** Glutathione S-transferase activity (GST). **C** Reduced glutathione levels (GSH). **D** Acetylcholinesterase activity (AChE). **E** Butyrylcholinesterase activity (BChE)

majority of natural geologies (Mainstone & Parr 2002). In this case, in all water samples, the levels of phosphate were similar or even higher than the reported in other agricultural areas from Argentina (Arreghini et al. 2005). Elevated nitrate and phosphate concentrations in freshwater ecosystems can lead to eutrophication, a process characterized by excessive nutrient enrichment and subsequent algal blooms and low DO levels (Correll 1998).

The presence of metals in water bodies can have significant implications for aquatic life and ecosystem health. Metals are naturally occurring elements that can be found in water bodies through various processes, including the natural weathering of rocks, volcanic activity; however, there are some anthropogenic sources, such as the use of fertilizers and livestock feeds with metals in agricultural practices (Carnelo et al. 1997). In this study case, some metals exceeded the limit for protection of aquatic life, such as Cu in water samples from almost all sites (S2A, S3A, S1D, and S3D). It can derive from human activities, such as industrial discharges, agricultural practices through the use of copper-based pesticides and fertilizers (Zhang et al. 2018). Furthermore, metal compounds, such as copper (Cu) and zinc (Zn), are extensively utilized in livestock production as essential supplements, playing crucial roles as trace elements necessary for the nutritional requirements of numerous animal species (Hejna et al. 2018). Additionally, these compounds are deliberately added to animal feed in higher concentrations to achieve supplementary beneficial effects (Rensing et al. 2018). The presence of Cu in aquatic ecosystems can have significant ecological implications. Particularly, the concentrations in water samples were similar or even higher than the 168 h-LC50 (19.5 µg/L) for *R. arenarum* embryos (Aronzon et al. 2011). Finally, Zn was higher than its limit (30 µg/L) in most sites from the Doblado stream. Zinc is naturally present in rocks and minerals, including sphalerite (zinc sulfide). It can also enter the environment

through the use of zinc-containing fertilizers, pesticides, dietary supplements for animals and animal manure in agricultural activities (Mortvedt & Gilkes 1993). On the other hand, Mn exceeded its corresponding limit mainly in the sites from the Doblado stream. Manganese can derive from natural sources and/or through human activities, such as its use in manganese-based fertilizers (Kheirkhah et al. 2016). Vanadium was higher than the limit for protection of aquatic life in all sites from the Areco River. It may also come from natural sources. However, some industrial processes, such as metal smelting, and fossil fuel combustion can contribute to the release of V into the environment (Barceloux & Barceloux 1999).

Pesticides, including herbicides, insecticides, and fungicides, can enter water bodies through various pathways such as runoff from agricultural fields, irrigation practices, and accidental spills (Akhtar et al. 2021). This contamination poses a significant threat to aquatic ecosystems. Pesticides can accumulate in sediments and persist for long periods, leading to chronic exposure for aquatic organisms. In this case, pesticides were detected in all sites, mainly in water samples rather than in sediments. Site 3A presented the highest variability. In particular, 2,4-D was detected in water samples from all sites. The presence of 2,4-D in water bodies can have detrimental effects on aquatic ecosystems. Fish, amphibians, and aquatic plants may be directly exposed to the herbicide, leading to acute toxicity or sublethal effects (de Castro Marcato et al. 2017). Atrazine and its metabolites were detected in all water samples. Atrazine is known to be a highly toxic endocrine disruptor to a wide range of aquatic organisms, including fish, amphibians, and invertebrates (Yang et al. 2021). Piperonyl butoxide was detected in all water samples. It is a common pesticide synergist widely used in combination with insecticides to enhance their effectiveness and it is known to be toxic to aquatic organisms, including fish, invertebrates, and amphibians (Khan & Law 2005). Tebuconazole, a commonly used systemic fungicide, was detected in all water samples. Tebuconazole is also known to be toxic to aquatic organisms, including fish, invertebrates, and algae (Tofan et al. 2023). On the other hand, glyphosate, a widely used herbicide, and aminomethylphosphonic acid (AMPA), glyphosate's degradation metabolite, were detected in all sediment samples. Both compounds can negatively affect sediment-dwelling organisms, such as amphibians' larvae, which play essential roles in sediment health and nutrient cycling (Tresnakova et al. 2021).

Ivermectin was detected in all water and sediment samples. Studies have shown that IVM can remain active for extended periods in aquatic environments, leading to potential accumulation (Liebig et al. 2010; Suárez et al. 2022). Furthermore, due to its physicochemical characteristics, it exhibits a strong attraction to sediment and organic matter, along with a limited ability to desorb and leach (Krogh et al. 2008), resulting in its quick transfer and long-term presence in the environment (Liebig et al. 2010). Its high persistence can result in adverse effects on aquatic organisms such as fish, invertebrates, and amphibians. A previous study reported a concentration of IVM of 1.24 µg/L in water from wetlands and a maximum concentration of 17.1 µg/kg in sediment (Mesa et al. 2020). Our study showed similar concentrations in water samples. However, in sediment samples, our results were between 1.73 and 4.98 times higher. On the other hand, OTC, a commonly employed antibiotic in animal breeding, was detected in water and sediment samples near the cattle breeding establishments (S2A and S2D). Once in water bodies, OTC can persist for extended periods due to its low degradation rate (Lee et al. 2022). This persistence increases the likelihood of exposure for aquatic organisms. Oxytetracycline is known to have adverse effects on aquatic ecosystems, particularly on the microbial communities, algae, and aquatic invertebrates (Siedlewicz et al. 2020). Even though at higher concentrations than those reported in the present study, it has been shown that OTC can have negative effects on amphibians, particularly in *R. arenarum* larvae (Lourido et al. 2022). In a previous study (Peluso et al. 2023), we reported the first environmental concentrations of OTC in Argentina. However, in the current study, concentrations in both sediment and water were higher. This could be linked to an accumulation or a continuous input into the environment over time. Nevertheless, it could also be associated with a specific discharge or with changes in livestock load at the livestock facility.

Sediment testing serves as an initial indication of the toxicity of substances within this environmental compartment (Chapman 2007). Field and laboratory studies are fundamental in identifying factors contributing to toxic effects. However, there is limited evidence regarding contamination in aquatic systems that integrates both water and sediment components as a complex entity. The exposed *R. arenarum* larvae exhibited lethal effects in all sites, during acute, subchronic, and chronic exposure periods. Although some of the drugs, pesticides, and metals detected in the water samples were found at concentrations below the known lethal thresholds for *R. arenarum* larvae, as documented by previous studies (Brodeur et al. 2009; Sztrum et al. 2011), the toxic effects of most pesticides and metals on this particular species remain uncertain. It should be noted that the concentrations of Cu in some sites were near the 168 h-LC50 reported for *R. arenarum* embryos (Aronzon et al. 2011). It is important to note that the observed lethality could result from complex interactions among multiple identified substances, including synergistic interactions such as between As and glyphosate (Lajmanovich et al. 2019), as well as 2,4-D and glyphosate (Peluso et al. 2022b). Moreover, it is crucial to highlight that the majority of the study sites caused lethality in organisms during extended exposure

times. This finding emphasizes the significance of extending the duration of exposure when assessing the potential toxic effects of environmental samples. By extending the exposure times, a more comprehensive understanding of the cumulative and chronic impacts of the contaminants present on the samples is achieved (Luan et al. 2020). This approach enables a more accurate assessment of the long-term risks and helps to identify any delayed or sublethal effects that may not be apparent during shorter exposure periods.

The rise in trace element concentrations has become a significant concern in terms of environmental pollution, as these elements can be absorbed and incorporated by wildlife (Stankovic et al. 2014). Indeed, due to the high persistence and resistance to degradation of IVM, it tends to accumulate in aquatic environments (Mesa et al. 2020). Consequently, this persistence in aquatic systems can lead to prolonged exposure of amphibian larvae, potentially exacerbating its effects on them. The incorporation of IVM by amphibian larvae poses a great concern about its impacts on their development, survival, and overall fitness. Aquatic organisms can be exposed and incorporate IVM through the consumption of sediment particulate matter and the bioaccumulation of dissolved IVM (Mesa et al. 2020). In a previous study, it was observed that IVM was accumulated in aquatic communities within wetlands subjected to various cattle activities and differing frequencies of drug injection (Mesa et al. 2020). The larvae that survived the toxicity bioassays demonstrated evidence of IVM incorporation during the 504 h of exposure. However, no bioconcentration of this drug was observed at this exposure time, as the IVM concentrations in larvae were lower than those found in water samples. Nonetheless, due to their transition from aquatic to terrestrial environments, amphibians have the ability to transfer contaminants across diverse habitats. This capacity allows them to introduce these substances into the trophic chain, potentially leading to their incorporation and subsequent biomagnification throughout food webs. Consequently, this process has the potential to exert significant influences on the functionality of ecosystems (Mesa et al. 2020).

Exposure to all sediment and water samples only caused reduced GSH levels. Reduced glutathione (GSH) is a crucial antioxidant that protects cells from oxidative stress and maintains redox homeostasis (Ali et al. 2020). Exposure to stressors like habitat degradation, pollution, and diseases increases reactive oxygen species (ROS), causing oxidative stress. GSH serves as a first-line defense, neutralizing ROS and detoxifying harmful molecules. In amphibians, GSH levels serve as an important biomarker for overall health and response to stressors (Tsukada et al. 2023). Exposure to contaminants lowers GSH levels in amphibians, weakening their antioxidant defenses and increasing susceptibility to oxidative harm. In polluted habitats, amphibians frequently display reduced GSH levels, potentially compromising their ability to withstand oxidative stress and survive. This depletion of GSH due to contaminant exposure heightens amphibians' vulnerability to oxidative damage, potentially leading to negative impacts on their survival (Costa et al. 2008).

In recent years, researchers have noted a concerning decline in AChE and BChE activity in amphibians, raising questions about the potential ecological implications (Relyea 2003). Pesticides, such as organophosphates and carbamates, are known to inhibit AChE and BChE activity, leading to neurotransmitter imbalances and impaired nervous system function (Nunes 2011). However, in recent years, it has been reported that other compounds, such as metals and antibiotics, may alter AChE and/or BChE activity (Frasco et al. 2005; Lourido et al. 2022). Agricultural runoff and direct applications of pesticides are major sources of contamination in amphibian habitats. A decrease in AChE and BChE activity disrupts the breakdown of acetylcholine, the primary neurotransmitter involved in neuromuscular function. This impairment can lead to muscle weakness, coordination deficits, and compromised motor control in amphibian (López et al. 2015). These effects may hinder their ability to forage, evade predators, and reproduce successfully. Consequently, their fitness may be reduced, which can lead to population decline and local extinctions.

Conclusion

The contamination of water bodies with pesticides, metals, and veterinary products and the alteration of physicochemical parameters are frequently associated with neighboring agricultural activities. While these activities contribute with nutrients and pesticides to water bodies, they also may introduce emerging contaminants, such as ivermectin or oxytetracycline. The observed negative effects on amphibian larvae exposed to water and sediment samples from these water bodies serve as a warning sign for the negative impacts on organisms. These findings underscore the importance of robust monitoring and effective management practices in agriculture to minimize risks to aquatic ecosystems and organisms. By implementing appropriate measures, we can safeguard water quality and the health of both wildlife and human populations.

Author contribution All authors contributed to the development of this study. Conceptualization, methodology, validation, formal analysis, investigation, data curation, writing—original draft, and writing—review and editing were performed by Julieta Peluso. On the other hand, Agostina Martínez Chehda contributed to the formal analysis of data and investigation. Melisa Olivelli, Federico M. Ivanic, Florencia Gonzalez, Lautaro Valenzuela, Virginia Aparicio, Eduardo De Geronimo, Matías Butler, and Roberto J. Candal performed the determination of different contaminants. Carolina M. Aronzon played a role in contributing resources, offering visualization and conceptualization,

supervision, overseeing project administration, and reviewing and editing the manuscript. All authors whose names appear on the submission (1) made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; (2) drafted the work or revised it critically for important intellectual content; (3) approved the version to be published; and (4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data availability Data will be available upon request to the corresponding author.

Declarations

Ethical approval The handling of the animals was carried out in accordance with the regulations for the use of live amphibians and reptiles in the field and laboratory research. They were controlled and approved by the Institutional Committee for the Care and Use of Experimental Animals (CICUAE Res. 01/2022) of the University National of San Martin (UNSAM) and the Dirección de Flora y Fauna (Res. 01133069).

Consent to participate All authors consent to participate in this study.

Competing interests The authors declare no competing interests.

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