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Water Quality and Toxicological Impact Assessment Using the Nematode *Caenorhabditis elegans* Bioassay in a Long-Term Intensive Agricultural Area

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Abstract Due to intensive agricultural activities to meet the growing needs for food, large volumes of water are consumed and an increasing amount of agrochemicals are released into the environment threatening the aquatic ecosystem. In order to ensure a sustainable agricultural management, it is crucial to develop an integrated water assessment plan that includes not only water quantity and quality but also toxicological assessments. The Pergamino River basin (province of Buenos Aires, Argentina) was selected as a representative case of study to monitor and assess the impact of both the long-term intensification of soybean production and fast-growing urban development on surface and groundwater sources. Physicochemical analyses and a Water Quality Index were determined and showed that water quality falls into the marginal category, compromising the irrigation purposes and threatening aquatic life. Glyphosate and aminomethylphosphonic acid were detected at least once in all sites. *Caenorhabditis elegans* toxic bioassays were performed and a toxicological ranking was developed. This analysis proved to be useful to detect toxicity even when water parameters met regulatory requirements and water quality seemed to be satisfactory. This research constitutes a valuable model to be replicated in other river basins that have been impacted by intensive agriculture and growing urban development in order to assess water quality conditions and ensure sound water resources management.

Keywords Environmental toxicology · Water characterization · Glyphosate · Agriculture · Pergamino

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1 Introduction

In order to meet the demand for food for the growing world population, it is necessary to increase production.

In the case of agriculture, this can be achieved in two different ways: by expanding the cultivated areas or by increasing crop yields. Currently, in Argentina, as well as worldwide, these two alternatives occur simultaneously leading to big changes in the rural landscape. This transformation is due to multiple interrelated factors such as increasing use of water resources for irrigation, soil exhaustion by intensive monocultures, rising use of agrochemicals associated to transgenic crops, and expansion of cultivated frontier to urban areas (Flores and Sarandón 2006; Viglizzo et al. 2006; Cabrini and Calcaterra 2016).

In particular, soybeans produce significantly more protein per acre than any other crops (Lee et al. 2013). This is the main reason why glyphosate-resistant transgenic soybean technologies were developed in the early 1990s. This technology increases the soybean cultivated area worldwide and guarantees an economic success of the soybean producers (both communities and countries). Since every year an increasing amount of glyphosate is released into the environment, concerns have been raised about ecotoxicological safety and human health (Battaglin 2016). Thus, one of the greatest challenges of the agriculture sector is to protect water quantity and quality to ensure sustainable development and provision of ecological services in aquatic and terrestrial system (Palmer et al. 2014). It is then crucial to implement robust monitoring and evaluation of water quality. However, it is difficult to obtain information on the overall quality status because the selection of parameters that have to be evaluated in the monitoring depends on the water use (i.e., drinking water, recreation, irrigation, aquatic life protection). Besides, the scientific community agrees that the simple determination of parameters only offers a partial view of the water quality status. Other analyses should be included in order to complement or synthesized traditional water monitoring such as mathematical modeling or water quality indices (WQI) calculation. The calculation of this index is composed by different parameters that meet regulatory standards and summarizes a large number of water quality data into a single value for each site (Bhutiani et al. 2016; Bharti and Katyal 2011). The use of WQI makes it possible to categorize water and provide governmental planners and stakeholders with a quick overview of the ecological status of the environment (Kannel et al. 2007; Khalil et al. 2014).

Nonetheless, neither the physicochemical parameters nor the WQI help to characterize the toxicological properties of water samples. To overcome this limitation, several ecotoxicological bioassays have been developed and included into water monitoring plans (De Castro-Català et al. 2015; Kuzmanovic et al. 2015; Palácio et al. 2016; Wernersson et al. 2015), but they still lack direct indicators on the regulatory level to verify the toxicological status of water bodies. This situation calls for the need to link research with water management. For this reason, great efforts are being made to include the bioassays into the regulatory framework for water quality testing (Vidal et al. 2012).

Species adopted as a bioindicators should meet certain criteria: they should be sensitive to toxicant, easy to manage in the laboratory, and be available throughout the year (Martínez-Sales et al. 2015; Wah Chu and Chow 2002). The free-living soil nematode *Caenorhabditis elegans* (Rhabditidae, Nematoda) is a valuable bioindicator organism for aquatic environment analysis such as interstitial waters from soil, aqueous extracts from river sediments, and superficial river water, as many authors have demonstrated (Clavijo et al. 2016; Höss et al. 2001; Leung et al. 2008; Tejeda-Benitez et al. 2016). Moreover, the bioassay using the nematode *C. elegans* has been recognized and ratified by international standards as a model for Environmental Impact Assessment (ASTM 2014; Höss et al. 2009, 2012; ISO 2010).

The aim of this work is to study the quality and the toxicological status on surface and groundwater sources in areas where both long-term intensive agricultural and fast-growing urban development have an impact on the environment. To address this issue, we selected the Pergamino River basin in Buenos Aires Province (Argentina) located in one of the most productive region worldwide as a representative case of study of intensive transgenic soybean crop production. This region experienced the green revolution that drove to an accelerated economic development with a concomitant increasing population. Since different farming and urban areas have been developed there, water quality is threatened by agricultural activities and by the discharge of urban effluents. Twenty sampling sites were selected including groundwater for drinking or irrigation purposes and surface water flowing across cultivated fields and through the city. Physicochemical parameters as well as glyphosate and AMPA concentration were determined and WQI was built to integrate all these data. In order to obtain a comprehensive view of the water quality conditions, a bioassay using the nematode

C. elegans was conducted to determine toxicity in water samples. Furthermore, a toxicity ranking was proposed based on the effects on nematode growth to better interpret the toxicological data.

Altogether, this paper provides an integrated environmental assessment approach that addresses the impacts of intensive agricultural and urban activities on surface and ground waters. These data may contribute to improve water management, support its conservation, and ensure the freshwater ecosystem services.

2 Materials and Methods

2.1 Study Area and Sampling

The district of Pergamino located in the north of the province of Buenos Aires (Fig. 1) belongs to the subhumid central Pampas region. The climate is temperate-humid with an average annual rainfall ranging from 800 to 1000 mm per year (Cabrini and Calcaterra 2016). Because of the extremely fertile conditions of the soil, it is the most productive region in Argentina with high agricultural intensification (Auge 2006; Deluchi et al. 2010).

Water monitoring was carried out in the Pergamino River basin. Twenty georeferenced water sampling sites were chosen for rural groundwater (RGW), rural surface water (RSW), and urban surface water (USW) (Fig. 1, Table A1 and Fig. A1). RSW sampling sites N° 6 and 12 were located at the Botija stream, a main tributary of the Pergamino River. RSW site N° 7 was established at the confluence of the Botija stream and Pergamino River. RSW sites N° 8 and 15 were located at the Pergamino River. RSW site N° 14 was located in a small stream that flow through an out-of-use dumping point. Sampling site N° 20 was located at an artificial pound. All these monitoring sites were upstream from the city of Pergamino. On the other hand, USW sites N° 16 to 19 were established on the Pergamino River that flows through the city of Pergamino (Fig. 1 and Fig. A1). Surface water samples were collected from the middle of the river in sterile recipients in accordance to standardized techniques. Flow turbulence at the monitoring sites ensured samples representativeness.

RGW samples were obtained from selected sites (N° 1, 2, 3, 4, 5, 9, 10, 11, and 13) in the Pergamino River basin. These sites are north-east of the Botija stream located in depressed areas with deficient soils drainage (INTA 1972; Sainato et al. 2000). For further references, site N° 2, 3, and 11 were used for drinking water. RGW sampling sites N° 1 to 3, 11, and 13 were collected from windmill wells, while RGW sites N° 4 and 5 were taken from a pivot irrigation system. In all cases, the samples were taken after water had been flowing for a few minutes. RGW sites N° 9 and 10 were collected

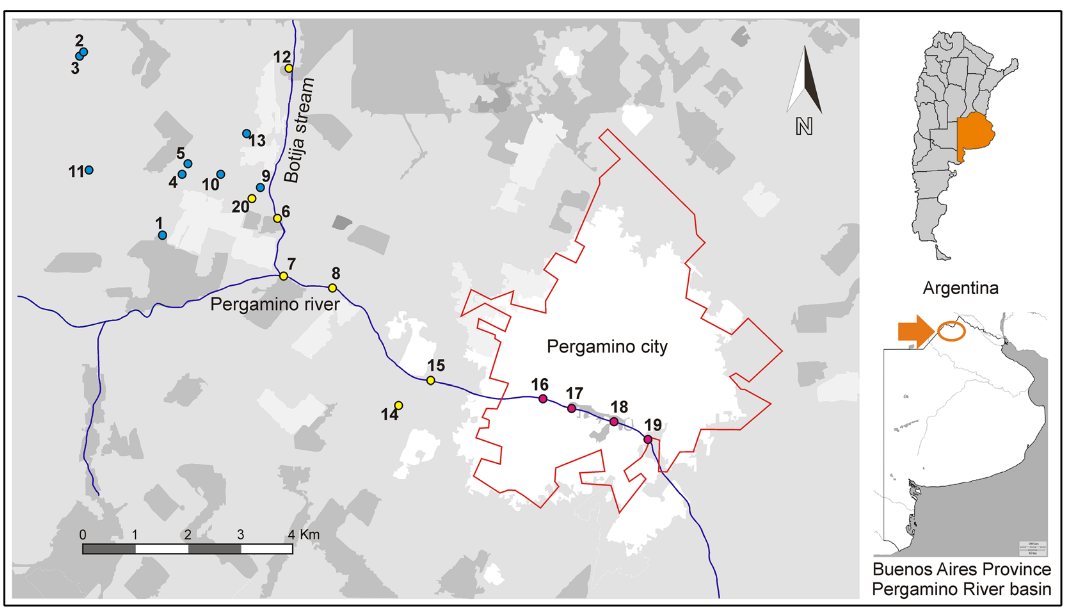

Fig. 1 Map of the Pergamino River basin, Buenos Aires, Argentina

directly from the piezometer using a water sampling bailers. The water-table level was between 2 and 8 m and the thickness of the Pampean aquifer was variable (Sainato et al. 2000).

Each water sample was split into three aliquots: the first one was collected in a 0.5-L plastic bottle to measure anions, cations, carbonates, bicarbonates, and sulfates. The second one was placed into a 250-mL plastic bottle with 0.5 mL 10% hydrochloric acid solution for *N*-phosphonomethyl glycine (glyphosate) and aminomethylphosphonic acid (AMPA) determinations. The third one was collected in a 50-mL falcon conical tube for the *C. elegans* bioassay. Samples were taken, stored, transported, and preserved according to Standard Methods (SM 1060-C) (APHA 2012).

Water samples were taken from July 2014 to February 2015 to assess temporal variability. These months of the year were chosen coincidently with chemical fallow preparation and soybean crop production. Sampling operations were not carried out in January 2014 due to operational difficulties in accessing the area.

2.2 Physicochemical Parameters

Temperature (T, °C), pH, oxidation-reduction potential (ORP), and electrical conductivity (EC) were determined in situ using a multiparameter probe (Horiba U-10 Model). Potassium (K^+), magnesium (Mg^{2+}), sodium (Na^+), calcium (Ca^+), copper (Cu^{2+}), zinc (Zn^{2+}), total chrome (Cr), cadmium (Cd^{2+}), and iron (Fe^+) were determined by atomic absorption spectrophotometry following Standard Methods (Perkin-Elmer 2000). Other physicochemical parameters were measured according to standardized methods (APHA 2012; ASTM 2014). Sulfate (SO_4^{2-}) was measured by the turbidimetric method (SM 4500- SO_4^{2-}.E); carbonates (CO_3^{2-}) and bicarbonates (HCO_3^-) were measured by acid-base titration (SM 2320-B) (Jackson 1976); chlorides (Cl^-) were determined by the argentometric method (SM 4500-Cl–B); and nitrates (NO_3^-) were determined directly using HACH® test strips. Information on aquifer level was obtained using a chalked steel tape or provided by local technicians.

2.3 Water Quality Index

The Water Quality Index of the Canadian Council of Ministers of the Environment is based on a combination of three factors (CCME 2001): scope (F1) refers to the percentage of parameters where water quality guidelines are not met; frequency (F2) refers to the percentage of samples where water quality guidelines are not met; and amplitude (F3) refers to the amount by which the water quality guidelines are not met.

All these three factors are combined to produce a single value so that water quality is expressed as a number ranging from 0 (highly polluted water) to 100 (unpolluted water). Then, these values are divided into five descriptive categories: excellent (95–100), good (80–94), fair (65–79), marginal (45–64), and poor (0–44) (Statistics Canada 2014).

The WQI for the monitoring sites at the Pergamino River basin was calculated considering complete datasets from the analyzed period. On the basis of local normative regulation, the parameters selected to build the WQI were T, pH, EC, Cl^-, Cu^{2+}, NO_3^-, SO_4^{2-}, Zn^{2+}, Cr, Cd^{2+}, and Fe^{2+}. The maximum allowable limits were determined according to different regulations. The allowable limits for Cu^{2+}, Cr, and Cd^{2+}, established by Decree N° 831/93 for the protection of freshwater aquatic life of the Federal Hazardous Wastes Act N° 24051 of Argentina (SAyDS 1993), were used. The allowable limits for Zn^{2+}, pH, Cl^-, and SO_4^{2-} were set by Resolution N° 42/06 (ADA 2006) and for Mn^{2+} and Fe^{2+} by Resolution N° 336/03 (ADA 2003) for acceptable discharge parameters of the Provincial Water Code Act N° 12257 of the Water Authority of the Ministry of Infrastructure and Public Services of the Province of Buenos Aires. Water temperature limits were established by Resolution N° 3/09 for protection of aquatic life of the Matanza-Riachuelo basin Authority Act N° 26168 of the Ministry of the Environment and Sustainable Development of Argentina (ACUMAR 2009). EC limits for irrigation water use were set at less than 1500 μS cm^{-1} (not permissible according to the classification for groundwater for irrigation purposes) (Alsheikh 2015; UCCC 1974). The allowable limits for NO_3^- concentrations was established at a maximum values of 45 mg L^{-1} according to the Joint Resolution by the Secretariat for Health Policies, Regulation and Relations (Res. N° 68/2007) and the Secretariat for Agriculture, Livestock Farming, Fisheries and Food (Res. N° 196/2007) of Argentine Food Code Act N° 18284 of the Ministry of Health of Argentina (CAA 2007). For more details about the maximum allowable limits set by different regulation, see supplementary data (Table A2).

2.4 Glyphosate and Aminomethyl Phosphonic Acid Measurements

To quantify glyphosate and AMPA, 3 mL of each water sample were transferred to a 15-mL polyethylene flask. A combined protocol with several modification was used to quantify both substances (Goscinny et al. 2012; Nedelkoska and Low 2004; Schuster and Gratzfeld-Hüsgen 1992). Separation was carried out by a high-performance liquid chromatography technique (HPLC) using an Agilent 1100 with a C18 reverse column and a fluorescence detector (excitation 266 nm, emission 305 nm). Two different gradient elution programs were developed for the separation of F1 and F2 using acetonitrile and a 0.002 M KH_2PO_4 with 7% acetonitrile (pH 7). A flow rate of 0.5 mL min^{-1} was used throughout. Quantitation of pesticides in water samples was calculated by comparing the peak areas for each compound with those obtained from the injection of standard solutions after derivatization.

The percentage for glyphosate and AMPA were calculated considering the total number of samples and for each water groups.

2.5 The Nematode *Caenorhabditis elegans* Toxicity Test

2.5.1 Nematode Cultures

Caenorhabditis elegans var. Bristol (strain N2) was used throughout the experiment. The strain was obtained from the *Caenorhabditis* Genetics Center (CGC) and maintained as stock cultures on nematode growth medium agar plates (NGM) (per liter: 17 g bacto agar, 2.5 g bactopeptone, and 3 g NaCl; with the addition after autoclaving of 1 mL 1 M $CaCl_2$, 1 mL 1 M $MgSO_4$, 25 mL 1 M KH_2PO_4, and 1 mL of a solution containing 5 g L^{-1} cholesterol, prepared in ethanol) seeded with *Escherichia coli* OP50-1at 20 °C as described by Brenner (1974). Gravid *C. elegans* hermaphrodites were washed off the plates with M9 buffer (6 g L^{-1} Na_2HPO_4, 3 g L−1 KH_2PO_4, 5 g L^{-1} NaCl, 3 g L^{-1} $MgSO_4{\cdot}7H_2O$) and synchronized by exposure to a bleaching mixture (0.45 N NaOH, 2% HOCl) following standard procedures (Stiernagle 2005).

2.5.2 C. elegans Growth Bioassay

The nematode bioassay was carried out, with a few modifications, according to Standard Methods (ISO 2010). Endpoints for toxicity testing were body size measurements (Höss et al. 2012). Exposures were performed in 24-well sterile tissue culture plates. In each well, 0.5 mL of the collected water sample was incubated with 15 µL M9 buffer containing ten L1 stage worms supplemented with *E. coli* OP-50.1. The final *E. coli* concentration was OD_{600nm} = 1. One growth control was performed for each monthly sampling using M9 buffer instead of water samples. After 96 h of incubation at 20 °C, the bioassay was stopped by heat killing the worms at 50 °C. The samples were stained with 0.5 mL of an aqueous solution of Rose Bengal (0.5 g L^{-1}) for easier visualization. Four replicates were set up for the control and for each collected water samples.

2.5.3 C. elegans Body Length Measurement

Rose Bengal-stained nematodes samples were photographed using an optical microscope Nikon Eclipse 50i at 40× magnification (100× for L1 stage worms). Then body length, along the body axis, was measured using the ImageJ software (Schneider et al. 2012). The mean initial body length of L1 stage worms was 221 ± 20 µm (n = 30). The body length in M9 controls at the end of the bioassay ranged between 1200 to 1300 µm. Nematode growth was calculated as the difference between the mean body length after sample exposure and the mean initial body length. Results are expressed as nematode relative growth over the control.

2.5.4 Toxicity Ranking Based on C. elegans Relative Growth

To characterize the magnitude of the toxic effect, a toxicity category ranking was built based on relative growth rate of the nematode (Höss et al. 2009). The toxic effects categories for *C. elegans* relative growth were established as follows: non-toxic effect for values above 0.90, slightly toxic effects for values between 0.75 and 0.89, moderately toxic effects for values between 0.74 and 0.50, and highly toxic effects for values below 0.49. The relative percentage of each category was calculated for each monitoring site.

2.6 Statistical Analysis

Data were analyzed using the R program (R Core Team 2016). To compare samples discriminated by location and season, as well as by water origin groups, the non-parametric Mann-Whitney test was used (Conover 1980). This rank-based test makes no assumptions about the distribution of variables and maintains a reasonable asymptotic relative efficiency.

For the most important chemical parameters, a one-way univariate analysis (ANOVA) and the Kruskal-Wallis (non-parametric ANOVA) test were conducted considering a significance level of 5%. Boxplots were developed to display the spread of data groups, the median position, and the atypical values.

In the case of SO_4^{2-}, the non-parametric Mann-Whitney test was done. For Zn^{2+}, glyphosate, and AMPA, median test was performed. This test is a non-parametric analysis, a special case of Pearson's chi-squared test, used for testing whether two groups differ in their median value. The median test focused on whether the two groups come from populations with the same median in order to compare them. Correlation between *C. elegans* growth and the physicochemical parameters was analyzed using the Pearson and Spearman test with a significance level of 5%.

3 Results and Discussion

3.1 Physicochemical Parameters Analysis

The minimum, maximum, mean, and median values for the seven monitoring datasets of physicochemical parameters are shown in Table 1. The difference between mean and median has long been used to determine the most frequent value from the average dispersion among series (Antonopoulos et al. 2001; Mozejko 2012). Physicochemical water parameters exhibited a wide range of values among sites according to origin: surface, underground, and urban waters.

There was a drastic variation in the EC parameter among the water groups. Table 1 and Fig. 2a show that there were significant differences among the three water groups (χ^2; P value < 0.05). EC for urban water presented a higher salinity condition, probably due to domestic and urban discharges that contribute to water deterioration. The guideline for irrigation water quality from the University of California states that EC values higher than 1500 μS cm^{-1} are indicative of moderate salinization risk and are classified as not permissible for water irrigation purposes (UCCC 1974). In view of this criterion, RSW and USW are far from reaching the best quality for agricultural use. The Na^+ concentration revealed a profile similar to EC with the lowest values in RGW and the highest in USW. Comparison of the three water groups revealed significant differences (χ^2; P value < 0.05), specifically between RGW and the other groups (Table 1 and Fig. 2b). Cl^- values were the highest in USW followed by RSW, with the lowest values corresponding to RGW. As shown in the boxplot analysis in Fig. 2c, highly significant differences were found between RGW and USW (χ^2; P value < 0.05). Moreover, in RSW sites N° 7, 8, and 15, as well as in all USW, Cl^- values exceeded local regulatory limits for aquatic life protection (< 625 mg L^{-1}) (ADA 2006).

Also, the SO_4^{2-} anion showed the lowest values in RGW and RSW, with no significant differences between these two groups (Table 1 and Fig. 2d). However, these groups exhibited highly significant differences in mean values compared to USW (χ^2; P value < 0.001). Several reports demonstrated that the Cl^- and SO_4^{2-} ions remain on the soil surface as salts grains due to evaporation of groundwater, then are lifted by the wind to be returned to the ground, and are finally washed away by rain (Ferraris and Couretot 2007). Urban discharges could also contribute to increase the ionic concentration that affects water quality.

In the case of pH, high values were measured at monitoring sites N° 20 and 14 located on a stream flowing through an out-of-use dumping point. Both exceeded the guideline for aquatic life protection (pH > 9) (ADA 2006). Sample analyses revealed that NO_3^- at sites N° 2 and 11 did not comply with drinking water regulation (< 45 mg L^{-1}; CAA 2007) (Table 1). Site N° 11 exhibited the highest concentration along the year with average values around 65 mg L^{-1}, which involve risks for human water consumption. Even if sites N° 1 and 9 also showed high values of NO_3^-, site N° 1 water is used for irrigation purposes and site N° 9 is just used for groundwater monitoring, so they did not pose a risk to human health.

The concentration of NO_3^- at sites N° 1, 2, and 3 increased in September (data not shown) possibly due to the crop fertilizer compounds used in agricultural activities. Figure 2e shows a significant difference in NO_3^- concentration between ground and surface water samples (χ^2; P value < 0.001). As regards metal

Table 1 Physicochemical parameters and relative growth of water samples

Site		°C	pH	EC µS cm^{-1}	ORP	HCO_3^- me L^{-1}	NO_3^- mg L^{-1}	SO_4^{2-} mg L^{-1}	Cl^- mg L^{-1}	K^+ mg L^{-1}	Ca^{2+} mg L^{-1}	Na^+ mg L^{-1}	Mg^{2+} mg L^{-1}	Gly µg L^{-1}	AMPA µg L^{-1}
1	Mean	22.1	7.8	1046	119	9.7	45	38.2	36	81.4	9.1	171.8	8.1	1.45	1.89
	Median	20.3	7.8	1040	126	9.8	47	54.5	36	74.5	8.8	175.3	7.2	0.89	1.53
	SD	6.0	0.1	83	33	0.3	4	24.4	0	49.3	11.1	128.1	5.5	1.70	1.17
	Min	18.3	7.6	967	71	9.5	39	11.0	36	36.0	2.0	83.3	5.7	0.20	0.94
	Max	33.4	7.9	1194	154	10.0	48	65.2	36	170.5	29.5	415.5	20.0	3.82	3.19
2	Mean	21.0	7.4	1711	134	13.5	28	86.5	103	79.4	11.5	251.7	8.0	1.12	2.50
	Median	19.4	7.4	1735	132	13.5	26	83.5	89	79.9	10.4	260.0	8.7	0.41	2.50
	SD	3.6	0.1	132	26	0.9	9	16.3	44	71.4	13.3	103.9	2.1	1.62	0.87
	Min	18.3	7.3	1505	114	12.5	21	68.0	71	30.4	4.7	123.7	4.0	0.12	1.89
	Max	28.2	7.7	1883	179	15.0	47	111.4	178	240.5	35.0	391.5	11.0	3.52	3.11
3	Mean	19.9	7.5	1553	121	12.4	17	66.9	85	62.9	8.0	261.6	6.3	0.86	0.74
	Median	19.5	7.6	1513	117	12.5	16	69.0	89	57.1	5.8	242.0	6.3	0.72	0.74
	SD	2.7	0.1	160	53	1.0	3	10.7	23	58.8	9.5	109.5	1.5	0.74	0.67
	Min	16.0	7.4	1364	69	11.5	14	55.2	53	21.3	3.1	124.0	4.6	0.20	0.27
	Max	23.7	7.7	1777	213	14.0	21	79.8	107	196.0	28.5	421.0	9.0	1.90	1.21
4	Mean	20.7	8.0	1221	120	10.0	28	68.7	57	27.1	3.7	210.2	1.9	1.04	6.00
	Median	21.3	8.0	1189	121	10.0	32	70.0	53	33.5	2.7	221.0	3.2	0.79	6.00
	SD	3.3	0.3	127	30	1.8	5	8.8	15	39.6	4.2	58.0	1.5	0.69	6.00
	Min	14.5	7.5	1073	89	8.0	22	55.5	36	4.1	1.7	116.2	0.1	0.27	6.00
	Max	24.3	8.4	1452	160	13.0	34	77.3	71	113.5	13.0	276.5	4.5	1.97	6.00
5	Mean	20.7	8.0	1490	111	10.7	27	68.4	120	71.7	5.3	290.1	4.3	0.86	2.04
	Median	20.2	8.0	1480	108	10.8	26	80.0	115	89.8	5.0	295.0	4.6	0.27	2.04
	SD	2.0	0.2	133	47	0.6	4	20.7	62	72.1	6.7	133.0	0.7	1.09	2.24
	Min	19.0	7.7	1356	74	10.0	24	40.4	53	23.9	2.0	124.7	3.5	0.20	0.46
	Max	23.7	8.3	1680	180	11.5	35	84.8	195	203.5	19.5	521.5	5.0	2.12	3.63
6	Mean	21.4	8.3	2286	123	13.1	12	64.0	298	68.9	14.7	368.1	9.2	1.02	0.80
	Median	21.1	8.3	2320	121	13.5	12	90.8	302	67.8	16.5	380.0	10.3	1.09	0.30
	SD	4.4	0.2	214	25	1.5	4	55.5	85	73.4	10.8	159.5	2.2	0.55	1.06
	Min	14.7	7.9	1942	98	11.0	8	8.9	213	31.0	4.9	148.0	6.1	0.40	0.07
	Max	28.8	8.7	2570	153	15.0	19	143.2	391	245.0	39.0	601.0	12.1	1.75	2.02
7	Mean	18.7	8.4	4444	110	12.7	9	103.4	951	127.3	48.3	698.1	28.9	0.82	2.05
	Median	19.8	8.5	4560	132	13.0	8	72.3	1083	151.1	40.2	935.3	31.3	0.64	2.31
	SD	5.6	0.2	778	31	0.9	8	126.9	547	79.3	44.9	379.2	8.8	0.71	0.52
	Min	10.5	8.0	3363	77	11.5	5	67.3	0	42.8	26.8	163.0	15.1	0.27	1.45
	Max	27.4	8.7	5620	140	13.5	25	324.4	1385	249.0	143.0	1177.5	39.2	1.75	2.40
8	Mean	19.4	8.3	5004	113	12.7	6	128.3	941	119.2	54.8	750.7	39.0	0.86	3.98
	Median	19.5	8.4	5080	133	13.0	5	75.0	1189	111.1	45.1	927.0	40.1	0.57	4.22
	SD	4.7	0.3	920	28	1.0	1	144.9	756	87.5	64.2	367.4	20.7	0.64	0.45
	Min	11.3	7.9	3999	79	11.0	5	66.1	0	34.7	27.3	161.5	16.3	0.42	3.47
	Max	25.4	8.7	6640	139	13.5	7	381.7	1846	283.5	206	1252.0	84.5	1.60	4.26
9	Mean	19.6	7.6	1385	126	13.6	21	59.1	36	66.3	10.5	260.9	6.4	1.36	3.24
	Median	18.4	7.6	1434	126	13.5	22	77.1	36	75.9	11.5	318.0	6.5	1.53	3.46
	SD	3.3	0.1	224	20	0.5	14	23.7	0	108.0	14.9	102.4	1.8	0.86	2.40
	Min	16.0	7.5	1008	102	13.0	10	35.9	36	7.9	3.8	111.1	3.9	0.42	0.74
	Max	25.0	7.7	1647	155	14.5	50	83.0	36	323.5	46.5	422.5	10.0	2.12	5.52

Table 1 (continued)

Site		°C	pH	EC µS cm^{-1}	ORP	HCO_3^- me L^{-1}	NO_3^- mg L^{-1}	SO_4^{2-} mg L^{-1}	Cl^- mg L^{-1}	K^+ mg L^{-1}	Ca^{2+} mg L^{-1}	Na^+ mg L^{-1}	Mg^{2+} mg L^{-1}	Gly µg L^{-1}	AMPA µg L^{-1}
10	Mean	19.7	7.5	895	152	9.6	22	46.6	25	97.2	30.7	136.2	11.2	0.41	2.27
	Median	19.7	7.5	1007	151	9.5	21	51.3	18	99.6	22.2	185.0	14.3	0.27	2.24
	SD	2.1	0.1	219	25	0.7	2	18.7	10	69.6	58.4	86.6	7.2	0.34	1.69
	Min	17.6	7.3	518	127	8.5	20	25.1	18	38.0	11.3	27.0	2.4	0.20	0.60
	Max	23.7	7.7	1141	183	10.5	25	70.8	36	228.5	172.5	278.5	25.5	1.01	3.98
11	Mean	20.7	8.0	1322	119	9.4	49	75.8	92	43.2	3.3	256.9	3.3	1.07	1.87
	Median	19.7	8.0	1273	121	10.0	61	75.4	89	42.4	2.1	276.0	3.3	0.98	1.87
	SD	4.7	0.3	122	28	1.2	22	3.4	23	83.2	6.0	124.0	1.1	0.95	2.26
	Min	15.0	7.3	1169	88	8.0	13	72.1	71	5.5	1.6	112.3	2.2	0.20	0.27
	Max	29.5	8.3	1472	164	1.0	75	79.6	124	252.5	18.0	431.0	5.5	2.12	3.47
12	Mean	20.4	8.2	2508	79	13.3	12	115.0	364	46.9	13.6	390.8	7.6	0.25	2.00
	Median	21.0	8.2	2498	117	13.6	11	118.9	355	52.0	13.3	387.5	12.0	0.20	1.53
	SD	5.8	0.1	395	55	1.6	5	36.7	117	18.7	15.9	104.4	4.5	0.16	1.33
	Min	12.8	8.0	1960	11	11.5	8	75.5	249	26.4	6.2	278.0	2.6	0.12	0.98
	Max	28.5	8.3	2920	154	14.8	21	164.4	497	77.4	45.0	566.0	12.4	0.49	3.51
13	Mean	20.4	7.8	1315	114	10.7	32	76.2	36	29.1	3.0	216.9	3.2	0.84	1.73
	Median	20.0	8.0	1336	127	11.0	33	76.5	36	28.5	2.8	228.0	3.1	0.20	1.73
	SD	2.8	0.3	131	37	1.0	5	2.5	22	63.7	2.3	90.2	0.5	1.34	0.00
	Min	16.5	7.3	1120	55	9.5	24	72.0	0	2.2	1.4	115.8	2.5	0.12	1.73
	Max	24.6	8.1	1462	170	12.0	38	78.6	71	192.5	7.4	378.5	4.0	2.86	1.73
14	Mean	19.8	8.4	1491	109	12.6	8	68.0	56	64.5	19.5	261.0	6.5	0.72	1.10
	Median	22.4	8.3	1544	107	12.8	8	69.3	71	63.5	17.2	288.5	6.1	0.72	1.00
	SD	4.7	0.4	132	25	0.9	8	7.4	28	70.8	42.3	110.5	3.8	0.53	0.73
	Min	10.9	7.9	1227	75	11.5	5	56.1	0	26.2	5.9	137.5	3.0	0.22	0.42
	Max	23.9	9.1	1643	137	14.0	25	74.7	71	233.0	125.5	465.5	15.0	1.53	1.88
15	Mean	21.3	8.4	5088	118	12.4	7	306.9	1377	125.1	55.1	757.6	33.3	0.50	5.46
	Median	20.9	8.4	5210	110	12.3	7	290.8	1278	117.0	51.1	1014.5	35.5	0.49	5.46
	SD	4.5	0.3	988	19	1.2	3	42.2	270	112.2	45.1	452.9	15.1	0.13	5.74
	Min	15.5	7.9	3999	101	11.3	5	271.5	1136	34.9	27.4	160.0	14.3	0.38	1.40
	Max	29.2	8.7	6860	144	14.0	15	374.1	1811	361.0	148.5	1548.5	64.0	0.72	9.52
16	Mean	21.3	8.4	4672	110	12.0	6	276.5	1235	129.2	52.9	824.6	30.7	0.50	3.93
	Median	21.3	8.5	4680	107	12.5	5	286.3	1243	104.9	44.0	1064.0	33.4	0.42	3.67
	SD	4.6	0.3	573	19	2.3	1	47.0	147	117.7	46.4	421.0	8.4	0.44	2.89
	Min	14.2	7.9	3999	92	8.5	5	209.7	1101	56.7	30.8	163.2	19.5	0.20	0.66
	Max	28.7	8.8	5420	141	15.0	7	340.0	1456	406.5	157.0	1353.0	41.5	1.45	7.70
17	Mean	21.3	8.4	4939	111	12.5	7	312.8	1338	129.7	50.9	821.0	32.1	1.59	11.35
	Median	21.4	8.4	5030	109	12.5	7	308.2	1243	110.0	49.7	1161.0	32.7	0.64	2.60
	SD	5.0	0.3	867	20	0.6	2	47.2	243	108.8	41.0	421.1	8.8	2.33	18.38
	Min	15.0	7.9	3999	94	12.0	5	266.6	1101	31.3	27.6	161.6	16.4	0.24	1.30
	Max	30.5	8.8	6550	142	13.5	9	372.7	1740	321.5	146.0	1369.5	43.5	6.77	38.89
18	Mean	20.4	8.4	4632	86	12.6	7	282.7	846	146.5	58.8	738.8	34.4	1.07	43.19
	Median	21.7	8.5	4705	109	12.4	7	286.1	1136	171.0	44.6	925.2	33.6	0.72	5.02
	SD	6.3	0.3	666	37	0.6	1	35.5	672	101.3	54.4	367.8	14.1	0.80	79.59
	Min	12.1	7.7	3999	31	12.3	6	239.5	0	39.5	33.1	162.7	19.8	0.27	0.34
	Max	30.4	8.7	5370	124	13.5	9	325.9	1491	326.0	169.0	1221.5	62.0	2.21	162.40

Table 1 (continued)

Site		°C	pH	EC µS cm^{-1}	ORP	HCO_3^- me L^{-1}	NO_3^- mg L^{-1}	SO_4^{2-} mg L^{-1}	Cl^- mg L^{-1}	K^+ mg L^{-1}	Ca^{2+} mg L^{-1}	Na^+ mg L^{-1}	Mg^{2+} mg L^{-1}	Gly µg L^{-1}	AMPA µg L^{-1}
19	Mean	21.6	8.4	4566	108	12.9	7	299.2	1251	159.3	54.0	801.2	32.7	1.31	16.12
	Median	20.8	8.5	4460	109	12.9	6	296.6	1278	198.4	43.3	1002.2	33.9	0.19	0.11
	SD	4.7	0.2	687	27	0.7	2	23.4	110	93.5	32.0	437.2	6.6	0.74	5.78
	Min	15.7	8.0	3999	80	12.0	5	279.5	1101	52.2	33.7	163.5	24.4	0.57	1.10
	Max	28.9	8.7	5400	145	13.8	9	326.6	1349	325.0	109.0	1445.5	41.0	0.53	8.97
20	Mean	21.3	9.3	947	92	6.2	7	48.0	18	49.2	3.8	225.0	3.7	0.96	1.49
	Median	21.4	9.4	927	100	6.1	5	46.5	18	47.5	2.8	240.8	3.3	0.79	1.49
	SD	5.1	0.4	116	25	1.1	10	4.4	16	73.3	14.1	119.6	2.7	0.47	1.12
	Min	15.0	8.5	830	68	5.0	5	44.8	0	20.0	1.4	112.7	2.0	0.41	0.70
	Max	30.5	9.7	1150	122	7.8	25	54.7	36	215.5	37.0	405.0	9.5	1.53	2.28

For each site, statistical parameters were calculated from $n = 7$

EC electric conductibility, *ORP* oxidation-reduction potential, *Gly* glyphosate, *AMPA* aminomethylphosphonic acid

concentration, Cd^{2+} was only detected in October and November in all water samples with the exception of those from sites N° 11, 12, 19, and 20. Cu^{2+} was measured in August but not detected in most of the water samples with the exception of those from sites N° 11 (0.174 mg L^{-1}) and 13 (0.26 mg L^{-1}). Both Cd^{2+} and Cu^{2+} values exceeded the current regulatory limit of 0.0002 and 0.002 mg L^{-1}, respectively (SAyDS 1993). Fe^{2+} was detected only at monitoring site N° 5 in August with a value of 2.3 mg L^{-1}, just above the regulatory

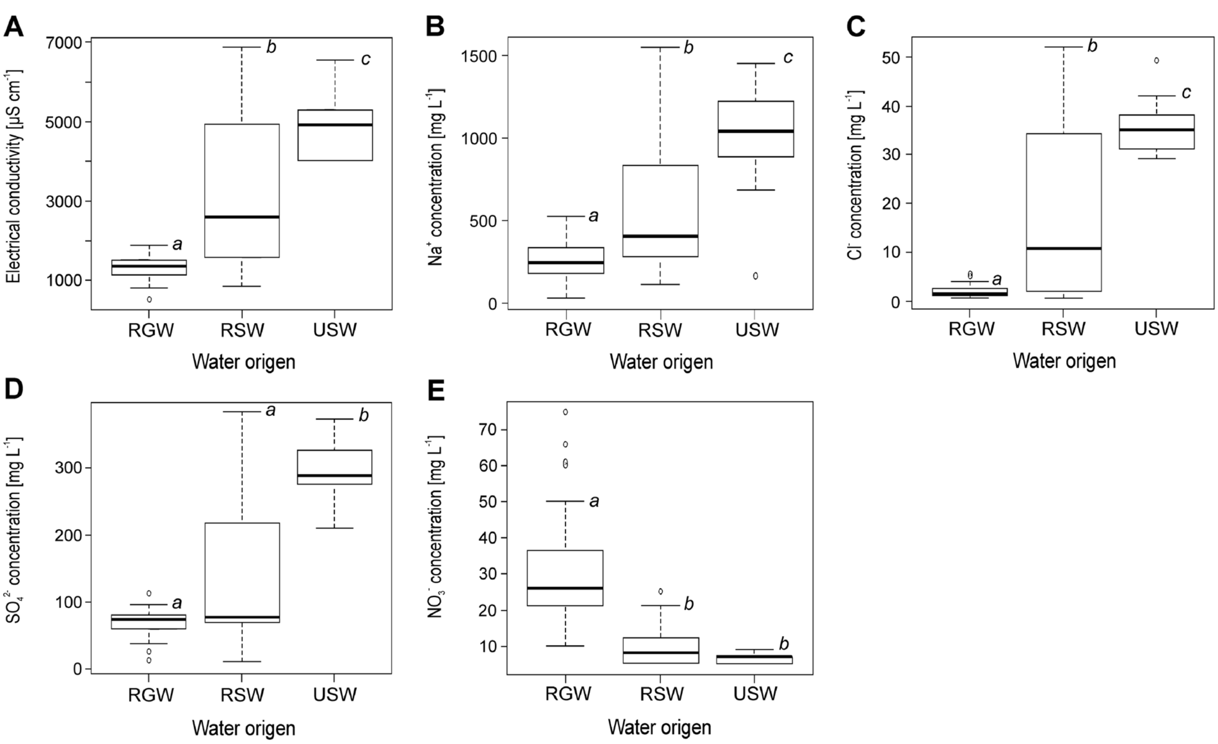

Fig. 2 Box and whiskers plots of water groups vs. electrical conductivity (**a**), vs. [Na^+] (**b**), vs. [Cl^-] (**c**), vs. [SO_4^{2-}] (**d**), and vs. [NO_3^-] (**e**). Box plots with same letter are not different at P value < 0.05 based on t test

limit of 2 mg L^{-1} (ADA 2003) (Table A3). Mn^{2+} was detected in all water samples in concentrations under the regulatory limit of 0.5 mg L^{-1} (ADA 2003). The presence of Zn^{2+} was detected in all water samples, and all UGW samples exceeded the regulatory limit (< 0.0075 mg L^{-1}; ADA 2006), in almost every tested month. Unexpectedly, sites N° 2 and 3, used as drinking water supply sources, exceeded the regulatory limits in all seven tested months reaching a maximum value of 0.721 mg L^{-1}. Regarding surface water samples, the Zn^{2+} concentration exceeded regulatory limits at all sites at least once, with the exception of monitoring site N° 14. All together, these results demonstrated a great variability in the physicochemical parameters, suggesting that an integrated approach is necessary to comprehend water quality and to facilitate the decision-making process.

3.2 Water Quality Index

In order to integrate all physicochemical parameters and had an overview of the water quality conforming to the current legislation, an annual WQI was calculated. According to the Canadian Council of Ministers of the Environment for the Water Quality Index calculation (CCME 2001), all values belonged to the marginal, fair, or good water quality category (Fig. A2). Most RGW sites in this study belonged to the marginal category, with a range of 49.7 to 60.5, except for site N° 10 (65.6), which was in the fair category. The depth of drilling varied from 37 to 86 m (Table A1) but no correlation was found between depth and WQI values. The low WQI values could be explained by the fact that heavy metals were found in the water samples. In particular, Cd^{2+}, Zn^{2+}, and occasionally Cu^{2+} were the main pollutants in groundwater. As groundwater flow is very low, contamination by heavy metals could be due to residues from past agrochemical applications as well as to natural soil composition (Auge 2004; Galindo et al. 2007). These marginal WQI values show the compromised condition of water quality in the Pergamino aquifer especially at sites N° 2, 3, and 11 which are used as drinking water supply sources. As for RSW at sites N° 12 and 20, both used for recreational purposes, WQI values were 81.1 and 84.3, respectively, which corresponds to the good category. The RSW at the other sites falls into the marginal category with WQI values ranging from 53.9 to 62.6. This low WQI is due to a combination of multiple factors such as Fe^{2+}, EC, NO_3^{2-}, heavy metals, and ions, with values above the regulatory limits (Tables 1 and A2).

The lowest WQI values were calculated for USW at sites N° 16, 17, 18, and 19 (60.4, 59.9, 63.2, and 62, respectively), all in the marginal category. These results are consistent with the physicochemical parameters analyses, where high values were observed for EC, Cl^{-}, Cr, Cd^{2+}, and Fe^{2+} at almost all the USW sites. Although most of WQI values for surface water along the river fell into the marginal category, different parameter profiles were obtained depending on the pollution from rural or urban activities.

Even if the WQI analysis revealed low water quality, other pollutants or interactions among them could exert unknown effects on aquatic environment. For this reason, integrated tools are required for a complete water quality assessment.

3.3 Determination of Glyphosate and AMPA

The occurrence of glyphosate and AMPA has been detected in water bodies and soils worldwide, despite the known relatively short half-life of glyphosate (Arunakumara et al. 2013; Coupe et al. 2012; Liu et al. 1991; Lupi et al. 2015; Mamy et al. 2010). As the predominant crop in the study area is glyphosate-resistant transgenic soybean, glyphosate and AMPA (its degradation metabolite) were determined in order to evaluate agrochemical contamination in the rural area and in the basin down the river. Glyphosate and AMPA were detected at least once in all monitoring sites (Table 1) indicating a persistent discharge of these chemicals into the Pergamino River basin according to the high agricultural activity. Figure 3 shows percentages of samples positive for glyphosate, AMPA, or the presence of both chemicals together in each water group. The presence of glyphosate in the three water origin groups exhibit similar proportions, having the highest percentage in the RGW group. This result could indicate either an easy percolation process or a long and persistent use of glyphosate in the area. AMPA-positive samples varied among water groups finding the highest percentage in RGW group once again. This could be explained by a natural attenuation capacity of the microbial community that degrade glyphosate into AMPA as it was mentioned in several reports from other regions (Arunakumara et al. 2013; Vera et al. 2010). Moreover, RGW presented the lowest percentage of both chemical together indicating an efficient transformation process

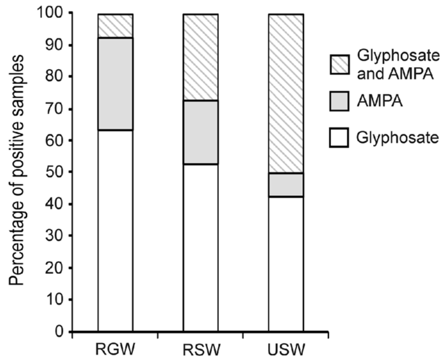

Fig. 3 Percentage of water samples for glyphosate, AMPA, or both chemicals in each water group

of glyphosate into its degradation metabolite. While in RSW and USW, the percentage of both compounds together was four and eight times higher, respectively.

Besides, having both chemical simultaneously could be explained by glyphosate coming from nearby rural areas, since rainfall plays an important role in the pesticides mobilization via runoff process after application, as well as a higher half-life of AMPA (Peruzzo et al. 2008).

Regarding the concentration values for glyphosate and AMPA for each water group, the Mann-Whitney median test shows higher values for glyphosate in surface water samples compare to groundwater (*P* value = 0.013). This result is consistent with previous reports which stated that despite the relatively short half-life of glyphosate, it was presented in soils regardless the application event (Aparicio et al. 2013; Lupi et al. 2015). Glyphosate concentration values ranged from 0.18 to 6.77 μg L^{-1}; this unique high value was found at monitoring site N° 17 in December 2014 and could be attributed to a singular agrochemical discharge from the surrounding crop fields. This phenomenon was reported previously by Coupe et al. (2012) who observed higher concentrations of glyphosate in stream water associated with a new application event. It is worth mentioning that none of the analyzed samples exceeded the CCME Water Quality Guidelines for Aquatic Life Protection for glyphosate. This guideline establishes that the maximum allowable limit in short- and long-term glyphosate exposure is 2.7 and 0.8 mg L^{-1}, respectively (CCME 2001). However, the fact that glyphosate and AMPA were found in water samples implicates that agriculture practices are not executed in a sustainable manner and could represent a risk for aquatic species. In this sense, multiple research have pointed out the presence of the adjuvants and surfactants, which include ethoxylated tallowamines, alkylpolyglycosides, or petroleum distillates used in commercial formulations. Those substances not only increase the environmental threats but also alter both the environmental fate and residue levels of glyphosate and AMPA (Myers et al. 2016). Regarding the monitoring sites used as drinking water source, none of the samples exceeded the guidelines for Drinking Water of the Federal Hazardous Wastes Act (SAyDS 1993) which establishes a limit of 0.28 mg L^{-1} for glyphosate.

The Mann-Whitney median test for AMPA concentration shows no significant differences between water sample groups (*P* value = 0.078). Contrary to glyphosate, there are no regulatory limits for AMPA despite its potential usefulness to identify polluted sites. As it was mentioned before, the presence of AMPA in stream water is not necessarily indicative of glyphosate degradation in situ but also of a delivery process (such as runoff or wind erosion) from surrounding crop fields, as it is a well-known fact that AMPA is more persistent in soils than glyphosate (Busse et al. 2001; Mamy et al. 2010; Ratcliff et al. 2006). A more persistent presence of AMPA was found in winter (*P* value < 0.01) compared to spring and summer, contrary to glyphosate concentration that shows no differences among season (data not shown).

Altogether, these results show that agrochemical pollution should be measured and analyzed not only in the water resource in the crop field but also in urban areas close to them. Especially because urban pollution could inhibit in a significant manner the agrochemical biodegradation in stream environment and consequently increase water contamination. Even more, despite the fact that the concentration level of glyphosate and AMPA detected in the river were low, a deteriorated water quality and the presence of heavy metals could have a synergy effects on glyphosate dynamic that may injure aquatic organisms. This idea was supported by Tsui that described synergic effects between glyphosate-based herbicides and Hg and Se (Tsui and Chu 2008).

3.4 *C. elegans* Growth Response Measurements

To characterize the ecological risk at the Pergamino River basin, a relative growth bioassay of nematode *C. elegans* was performed. The minimum, maximum, mean, and median valued obtained for the 7-month dataset are shown in Table A4. In order to find which parameters could be responsible for the decrease in *C. elegans* growth in the bioassay, further statistical analyses were performed. With the Spearman and Pearson tests, a significant positive correlation was found only between nematode's relative growth and CO_3^{2-} (Spearman coefficient 0.249; P value < 0.005), Ca^{2+} (Pearson coefficient 0.204; P value < 0.005), Mn^{2+} (Spearman coefficient 0.253; P value < 0.005), and Fe^{2+} (Spearman coefficient 0.44; P value < 0.005). These ions may be responsible for promoting nematode growth through different physiological mechanisms. For instance, high intracellular concentrations of Mn^{2+} protected against oxidative damage extending the nematode life cycle (Lin et al. 2006). Regarding Fe^{2+} and Ca^{2+}, they has been reported that it is involved in many biological processes essential for sustaining life, such as the insulin-like pathway, oxygen transport, neurodevelopment, as well as its ability to serve as a cofactor for enzymes involved in a variety of biological processes including DNA synthesis and energy production (Anderson and Leibold 2014; Gourley et al. 2003; Tanimoto et al. 2017).

On the contrary, in the case of Cd^{2+}, a negative correlation was found (Spearman coefficient − 0.215; P value < 0.005). Moreover, Table A3 shows that in some samples, Cd^{2+} concentrations are above the LOEC value (0.14 mg L^{-1}) for *C. elegans* growth (Traunspurger et al. 1997). These results are consistent with several reports that show that Cd^{2+} decrease growth, life span, and reproduction and also affect behavior in *C. elegans* (Boyd et al. 2010; Chen et al. 2013; Höss et al. 2011; Hsu et al. 2012).

Mean values of relative nematode growth for each monitoring site were used to perform a boxplot analysis in order to discriminate between water origin and season (Fig. 4a, b). Outliers are shown as isolated dots. A Kruskal-Wallis test (P value = 0.44) revealed no significant differences in nematode relative growth among the different groups of monitoring sites (RGW, RSW, and USW). However, significant differences were obtained when comparing among season with greater values in summer (P value = 0.0325).

The relative percentages of the four toxic effect categories for *C. elegans* growth are plotted in Fig. 5. Data show that monitoring sites exhibit different toxic biological effects along the year and fall into at least one of the three categories (slight, moderate, or highly toxic). Remarkably, most of the samples were in the highly toxic effect category (percentages ranging from 14.3 to 50) with the exception of sites N° 1, 2, 10, 11, 12, and 14. All USW samples had elevated percentages in the highly toxic effect category, in particular site N° 18 with the 50% in this category that constitutes the highest value in the bioassay. This analysis confirmed that *C. elegans* proved to be helpful to identify threatening conditions for aquatic life and for human health, especially when WQI exhibits an almost fair condition. In the non-toxic effect category, sites exhibited values ranging from 14.3 to 83.3%. Particularly, sites N° 1, 10, 20 (83.3%), and 14 (80%) had the highest non-toxic effect values. The percentages of water samples from the Botija stream and the Pergamino River in the non-toxic effect category was around 50%, showing that river water quality does not fluctuate much in this category.

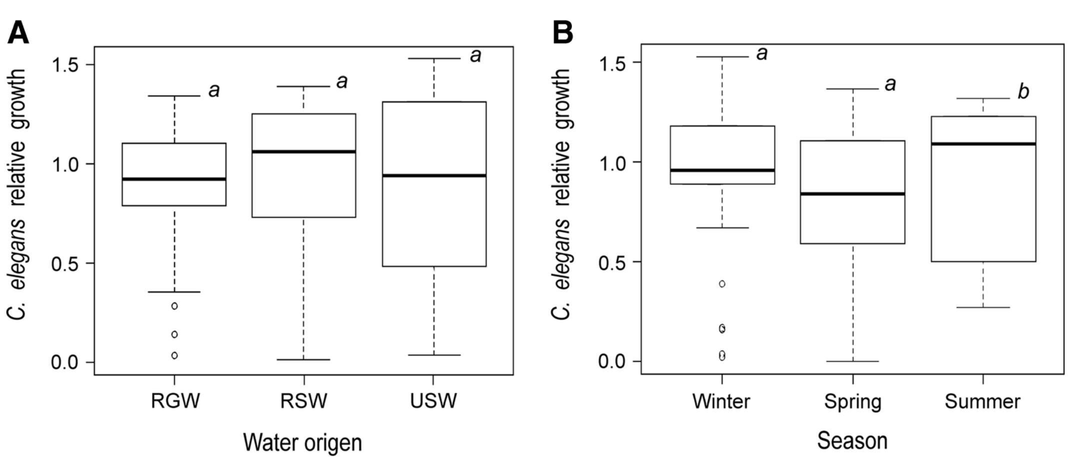

Fig. 4 Boxplot of *C. elegans* relative growth bioassay vs. the three water origin groups (RGW, RSW, USW) (**a**) and vs. seasons (winter, spring, and summer) (**b**). Box plots with same letter are not different at P value < 0.05 based on t-test

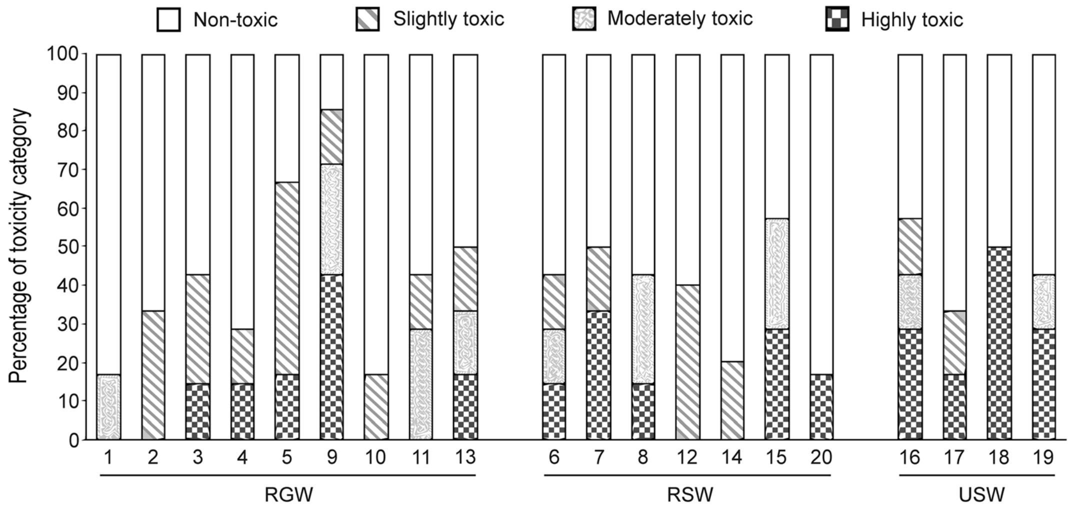

Fig. 5 Toxicity ranking based on *C. elegans* relative growth of water samples

The RGW group shows different scenarios regarding the *C. elegans* toxicity bioassay with multiple combinations of the four toxic effect categories. Also, the RGW group comprises the least contaminated condition at site N° 1 (16.7% slightly toxic and 83.3% non-toxic effect) and the worst one at site N° 9 (42.9% highly toxic, 28.6%, moderately toxic, 14.3% slightly toxic, and 14.3% non-toxic effect). Such variability in RGW could be due to multiple factors that influence the complexity of the aquifer system. When comparing toxicity with the results of the WQI analysis, no correlation was found, indicating physicochemical parameters measured were not associated with toxicity (data not shown). This underlines the fact that not only unknown compounds in the samples but also the complex interaction within the samples could be responsible for the toxic effect. All together, these results illustrate the complexity of executing a complete and an integrated water quality assessment, where water uses and ecosystem services are provided for economic and social development.

4 Conclusions

The uneven distribution, availability, and demand of water resources are determined by the environment and people necessities and greatly influenced by socioeconomic activities such as agriculture. The constant change in the landscape due to anthropic activities has an impact in quality and ecotoxicological status of water resources. The Pergamino River, used as a case of the study because of its long-term intensive agricultural and fast-growing urban development, showed different pollution effects as long as it flowed across the soybean fields and the city. WQI showed that most of the monitoring sites fell into the marginal water quality category, indicating that the whole area presented fragile and compromise environmental conditions, in particular for irrigation purposes. Additionally, glyphosate and AMPA were detected in ground and surface water along the year; the first one with higher values in the warm season which coincides with the soybean crop production. Toxicity ranking has proved to be a useful tool in interpreting the biological response of *C. elegans*. Toxicological effects were detected in all the three water groups and *C. elegans* response was particularly valuable to detect toxicity even when water parameters met regulatory requirements and water quality seemed to be adequate. These results contribute to the idea that pollution status of the water does not come from specific compounds, such as glyphosate and AMPA, but from synergetic effects of multiple substances.

At present, we are working on identifying other emerging pollutants and implementing *C. elegans* ecotoxicological assays to complement the current water impact assessment analysis in other basins.

In conclusion, reinforcing the concept that the impact of anthropic activities on water quality needs to be assessed from a holistic point of view, our integrated approach helps to understand how multiple stressors contribute to the deterioration of the environmental water quality. Therefore, we strongly recommend this kind of studies to obtain an overall view of the aquatic ecosystem that will ensure sustainable urbanization and agricultural practices and contribute to enhance water resources management plans and policies.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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