

Original Research

## Cropping and livestock impacts on surface waters in the Pampas region (Argentina)

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### Abstract

The rivers and streams of the cropping and livestock regions of Buenos Aires province (Argentina) contain variable concentrations of pollutants and high concentrations of nutrients that simultaneously affect aquatic ecosystem communities. In the present study, we evaluated the water quality of the Burgos stream and El Tala river (San Pedro, Buenos Aires province) by analyzing chemical contaminants (As, Cu, Pb, Zn, and glyphosate), nutrients (ammonium, nitrate, and phosphate), and algal toxicity bioassays. Surface water was collected at six sampling stations in three months (April and September 2019, and February 2020). By taking into account the percentages of algal growth inhibition (%I), the standard species *Raphidocelis subcapitata* and a species isolated from the study area, *Scendesmus acutus*, showed different sensitivities to the water samples, with a maximum %I of 70.64% and 34.62%, respectively. Moreover, algal growth stimulation was observed due to the high nutrient concentrations. A risk quotient (RQ) was applied to evaluate the environmental hazards of the chemical contaminants Cu, Pb, Zn, and glyphosate on the algal populations. According to the RQ values obtained for Pb (1.20 and 3.30) and Zn (1.17 and 2.12), the growth of algal populations could be at risk due to the maximum environmental concentrations measured of Pb (132 µg L<sup>-1</sup>) and Zn 106 µg L<sup>-1</sup>). The present study showed the first approach of an environmental risk analysis based on simultaneous determinations of chemical contaminants and laboratory bioassays with algae, using a strain of alga isolated from the aquatic ecosystems studied.

Keywords: algal bioassays, arsenic, glyphosate, heavy metals, risk quotient, toxicity

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## INTRODUCTION

The Pampas region, located in the southern cone of South America, represents a large plain of fertile land suitable for cropping and livestock. In Argentina, this region concentrates more than 50% of the country's population (21 million inhabitants) whose economy is mainly based on cropping or livestock production and industrialization. Currently, the most important crops are sorghum, wheat, corn, barley, sunflower, and soybean, which represent more than 80% of the total production of Argentina (Aparicio *et al.* 2013). In the last 40 years, profound changes have been recorded in the Pampas agroecosystems due to the growth of the cropping sector and the intensification of the livestock production system (Viglizzo *et al.*, 2010). Cropping activities have developed new technologies that depend on the use of fertilizers and pesticides affecting soils, and surface and groundwaters (Marino & Ronco 2005; Peruzzo *et al.* 2008; De Gerónimo *et al.*, 2011). Soybean cultivation has incorporated transgenic varieties resistant to glyphosate, which has increased the frequency and intensity of its application in recent years (Aparicio *et al.*, 2013).

Along with the cropping advances, there was also a marked intensification of livestock production based on reduced land areas, with a high density of animals subjected to intensive fattening or "feedlots" (Chagas *et al.*, 2014). These systems, used mainly for raising cattle, and other industrial activities, such as egg production, poultry, and pig farming, generate high amounts of excreta and organic matter, which reach surface waters as point effluents (Hatano *et al.*, 2005). In Argentina, there is a lack of specific legislation and scientific research on the risks associated with the management of manure from industrial and agricultural activities, as occurs in other countries. Along with the feces, certain trace contaminants, such as antibiotics, hormones, heavy metals and dioxins, may be discharged through agro-industrial effluents into surface waters and may even contaminate groundwater (Khan *et al.*, 2008). Among these chemicals, heavy metals have a recognized toxic effect on several living organisms (Wu *et al.*, 2016).

Therefore, several contaminants may be present simultaneously in the surface waters around crops and livestock areas, negatively affecting indigenous organisms. Glyphosate concentrations between 13 and 700  $\mu\text{g L}^{-1}$  were determined in several rivers and streams in cropping areas of Buenos Aires (Peruzzo *et al.* 2008; Bollani *et al.* 2019), as well as heavy metal concentrations that probably come from agrochemicals, phosphate fertilizers and organic fertilizers (Fergusson, 1990; Alloway, 1995; Pignata *et al.*, 2002). For example, concentrations of Cd (30  $\mu\text{g L}^{-1}$ ), Cu (252  $\mu\text{g L}^{-1}$ ), Pb (176  $\mu\text{g L}^{-1}$ ) and Zn (960  $\mu\text{g L}^{-1}$ ) were reported in a stream in Buenos Aires province (Bollani *et al.*, 2019). All these contaminants can be transported to surface waters with soil particles in runoff during rainy periods.

Chemical analyses of water samples are often conducted to detect the presence of agents potentially hazardous to

the aquatic environment and human health. From this information, effective quantitative methods were developed to evaluate the impact of pollution on human populations and aquatic ecosystems. The risk quotient (RQ) and aquatic quality guidelines are the most frequent methods for assessing the potential hazards of chemicals in aquatic ecosystems (Sanderson *et al.*, 2004; Nugegoda and Kibria, 2013). These methods are based on various ecotoxicological laboratory bioassays using different test organisms. In particular, photosynthetic algae, are selected models in ecotoxicological tests because they have rapid growth and can be cultivated in the laboratory using synthetic media that allow them to be kept in optimal physiological conditions. Regarding their ecological importance, algae are the main primary producers of the phytoplankton and the base of aquatic food chains. The standard species proposed as a reference organism by various environmental agencies is the green alga *Raphidocelis subcapitata* (USEPA, 2002; Environmental Canada, 2007; ISO, 2009). However, the effects of pollutants on a standard species are not necessarily the same as those that occur on strains of species isolated from water bodies in an area of interest. Native strains can provide relevant information to infer the effects of pollutants in their environment (Magdaleno *et al.*, 2014; Carusso *et al.*, 2018).

The objective of the present study was to evaluate the water quality of the Tala River and the Burgos stream, both located in cropping and livestock areas of Buenos Aires province, through the analysis of chemical contaminants (arsenic, heavy metals, and glyphosate) and algal toxicity bioassays. In addition, the risk quotient (RQ), a quantitative method, was applied to evaluate the environmental hazards of chemical contaminants in algal populations of the aquatic ecosystems.

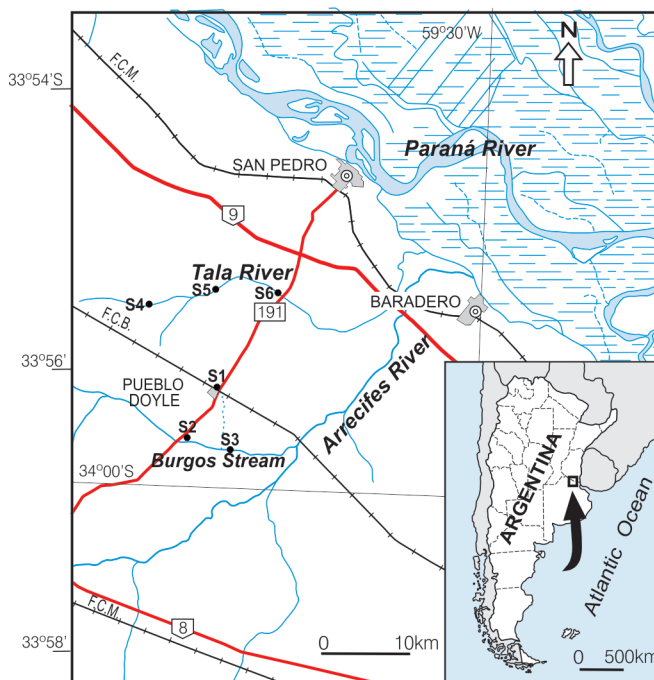
## MATERIALS AND METHODS

### Study area

The present study was carried out in the Tala river basin (800 km<sup>2</sup>) and the Burgos stream basin (430 km<sup>2</sup>) located in the Northeast of Buenos Aires province in the "Pampa Ondulada" region. The Tala river flows into the Paraná river, whereas the Burgos stream is a tributary of the Arrecifes river, which in turn is a tributary of the Paraná river (Fig. 1). This area is in a region with slightly undulating or flat slopes, where the rivers and streams have well defined channels. The climate is temperate, and the average annual rainfall is between 800 and 1200 mm, with maximums in December and minimums in July. The average annual temperatures are around 15 °C, with variations between 10 °C in winter and 20 °C in summer. The soils have a good field capacity but water erosion usually

occurs when the rains are intense and frequent during the humid months (Chagas *et al.*, 2010). The human population density is very low in both basins (47.452 inhabitants),

and the main crops are soybean, corn, and wheat. Cattle breeding takes place both extensively and intensively throughout this region.



**Figure 1:** Location of sampling sites in the Burgos stream basin and El Tala river basin (Buenos Aires, Argentina).

### Surface waters sampling

Water samples were collected at three sites in the Burgos stream basin (S1, S2, and S3), and three sites in the Tala river basin (S4, S5, and S6) (Fig. 1). Site S1 corresponds to the effluent outlet of a feedlot (with a capacity of 15,000 animals) into a natural channel. This channel crosses an area with soybean and corn crops and extensive cattle breeding. Sites S2 and S3 are located upstream and downstream of the discharge of this channel into the Burgos stream, respectively. Sites S4, S5, and S6 are located in the upper, middle, and lower Tala river basin (Fig. 1). Three months of sampling were selected according to the different periods of rains: April (fall) and September (spring) 2019, and February (summer) 2020. According to the precipitation values recorded by the National Institute of Agricultural Technology (INTA), the highest average annual rainfall occurs in the months of spring and summer (112.1-128.4 mm), while the lowest occurs in the months of autumn and winter (41.2-98.2 mm) (INTA, 2022). Surface water samples were collected in 250 mL polypropylene bottles and were stored in the dark at 4 °C for 24 h. For heavy metal quantifications, the water samples were immediately acidified, fixed to pH=2, with nitric acid 65% (Merck).

### Physical–chemical analysis of the water

The pH, dissolved oxygen, and temperature of the water samples were determined *in situ* with HANNA® multiple sensors. Inorganic nutrients: inorganic phosphate (PR<sub>S</sub>), nitrite (NO<sub>2</sub><sup>-</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), and ammonia (NH<sub>4</sub><sup>-</sup>) present in the water were evaluated according to Mackereth *et al.* (1989) and Strickland & Parsons (1972). Glyphosate and its degradation product, aminomethylphosphonic acid (AMPA), were also measured according to Goscinny *et al.* (2012) and Nedelkoska & Low (2004). Previously, water samples were filtered through 0.45 μm cellulose acetate membranes and then subjected to a derivatization process with 0.2 mL of fluorenylmethyloxycarbonyl (FMOC, 0.5 mM) and 0.3 mL of Buffer Borate up to a final volume of 4 mL. After 30 min reaction at room temperature, they were filtered through 0.45 μm nylon filters. Samples were then quantified by liquid chromatography using an Agilent 1100 Series kit. Two types of solvents were used: KH<sub>2</sub>PO<sub>4</sub> (0.002 M) in 7% acetonitrile (10 min), adjusted to pH=7, and acetonitrile (10 min). For glyphosate, the limit of detection (LOD) was 0.5 μg L<sup>-1</sup> and the limit of quantification (LOQ) was 2 μg L<sup>-1</sup>. For AMPA were 0.5 and 1 μg L<sup>-1</sup>, respectively.

Arsenic (As) concentrations were measured by hydride generation and the atomic absorption spectrometry method. The method, based on the absorption of As radiation at

a wavelength of 193.7 nm, consists of the following steps: generation and volatilization of the hydride generated from the reaction of As with NaBH<sub>4</sub> in acidic solution in a chamber, transport of the volatile hydride to a quartz cell, and dissociation and atomization (produced by exposing the cell to a flame at 900 °C). In this way, the determination of As was carried out by the generation of its hydrides by means of the flow injection system. The detection limit of this method was 0.27-0.54 µg L<sup>-1</sup> (Litter *et al.*, 2009). The LOD and LOQ were 0.27 and 0.54 µg L<sup>-1</sup>, respectively. Heavy metal concentrations of copper, lead, and zinc (Cu, Pb, and Zn) were quantified in both the total and dissolved fractions using a flame atomic absorption spectrophotometer, in an Analyst 200 (Perkin Elmer, Inc. Waltham, MA, U.S.A.), according to APHA *et al.* (2012). The dissolved fraction was obtained after passing the water samples through Whatman GF/C filters. The LOD (µg L<sup>-1</sup>), calculated as 3 x the standard deviation for 10 measurements of the blank, were Cu=5, Pb=10, Zn=3, and the LOQ (µg L<sup>-1</sup>) were Cu=7, Pb=20, Zn=6.

### Algal bioassays

The green alga *Raphidocelis subcapitata* (Koršhikov), (syn *Pseudokirchneriella subcapitata*), was obtained from the Culture Collection of Algae and Protozoa, U.K. (CCAP No. 278/4). The native Argentine strain of *Scenedesmus acutus* (Meyen), isolated from the Burgos stream (Afione Di Cristofano *et al.*, 2021), was also used to perform the bioassays. Both species (Chlorococcales, Chlorophyta) are maintained in the Culture Collection of the Phycology Laboratory, Department of Biodiversity and Experimental Biology of the Faculty of Exact and Natural Sciences, University of Buenos Aires. The axenic algae cultures were cultivated in 125 ml Erlenmeyer flasks containing 50 ml sterilized Bold's Basal Medium (BBM, Archibald & Bold, 1970) and agitated on a shaker at 80 rpm, under continuous cool-white fluorescent light (80 µmol photons m<sup>-2</sup> s<sup>-1</sup>). The flasks were maintained at 22 ± 2 °C for 7 days to obtain the inoculum at the exponential growth phase (approximately 5 x 10<sup>6</sup> cells mL<sup>-1</sup>).

The experimental procedure was performed according to the algal growth inhibition test using sterile 96-well microplates (Environment Canada, 2007). The bioassays were conducted in four replicate wells containing 200 µL water sample, previously filtered with a 0.45 µm membrane, and with an inoculum of initial cell density of 5 x 10<sup>4</sup> cells mL<sup>-1</sup>. Eight replicates of BBM culture medium were used as controls. The microplates were incubated under the same conditions as the inoculum cultures. Cell densities were estimated by absorbance at 620 nm after 96 h exposure.

### Data analysis

Inhibition of growth was expressed as a percentage of the control. One-way analysis of variance (ANOVA) followed by a Dunnett's post hoc test, was performed to evaluate the significant differences between the algal growth in the samples and the control. A *p*-value less than 0.05 was considered statistically significant.

The risk quotient (RQ) was estimated by taking into account of the environmental concentrations of heavy metals and glyphosate measured in the samples (MEC) and the effective concentrations 10, 20 and 50 (EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub>) for *R. subcapitata* and *S. acutus* obtained by Afione Di Cristofano *et al.* (2021), according to the formula: RQ=MEC/EC<sub>x</sub> (USEPA, 2022). The RQ values equal to or less than 1 mean that the algal populations are not affected by levels of contaminants in the environment, while the RQ values greater than 1 mean that they are affected by such contaminants.

## RESULTS AND DISCUSSION

The physical and chemical parameters obtained for water samples from the six sampling sites, collected in April and September 2019 (low rainfall period), and February 2020 (high rainfall period) are shown in Table 1. Water samples from S3-April 2019 and S1-February 2020 could not be collected. The temperature values were around those of a typical temperate water body with seasonal variations (Bollani *et al.*, 2019). The higher temperatures were recorded in summer (February) and early fall (April) with values between 23.8 and 33.3 °C, and the lower temperatures were recorded in the late winter (September) with values between 12 and 21 °C. The pH was below neutrality in April and February (between 6.3 and 6.7) and above neutrality in September (between 7.2 and 8.3). The surface waters showed oxygen levels ranging between 4.2 and 13.4 mg L<sup>-1</sup>, mainly due to photosynthetic activity. Moreover, the concentrations of nitrogen and phosphorus in the water samples were considered sufficient for algal growth. Nitrogen (incorporated as NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup>) is the most important nutrient for microalgae, whereas phosphorus (incorporated as PO<sub>4</sub><sup>3-</sup>) deficiency is one of the greatest limitations to algal growth (Grobbelaar, 2004). The levels of nitrogen as NH<sub>4</sub><sup>+</sup> (10.1-1157 µg L<sup>-1</sup>) or NO<sub>3</sub><sup>-</sup> (10-5181 µg L<sup>-1</sup>) in the water samples were similar or greater than the nitrogen concentration in the culture medium BBM (670 µg L<sup>-1</sup>). In the same way, the PRS concentrations in the water samples (116-2320 µg L<sup>-1</sup>) were similar or greater than the phosphorus (as PO<sub>4</sub><sup>3-</sup>) in the culture medium BBM (625 µg L<sup>-1</sup>).

Table 1 Physicochemical analysis of water samples collected in different sites of the study area in April and September 2019, and February 2020.

Parameter	April 2019						September 2019						February 2020					
	S1	S2	S3	S4	S5	S6	S1	S2	S3	S4	S5	S6	S1	S2	S3	S4	S5	S6
Temperature (°C)	33.3	27.4	-	23.8	24.6	27.9	21	13.6	16.5	12.5	12	15.4	-	28	28.7	25.9	26.2	29
pH	6.5	6.5	-	6.6	6.6	6.7	7.2	7.9	8.3	7.9	7.9	8.1	-	6.3	6.6	6.5	6.5	6.3
DO (mg L <sup>-1</sup> )	13.4	7.7	-	7.1	7.0	7.9	10.2	10.5	11.7	9.2	9.8	9.2	-	9.2	10	5	4.2	9.2
Electrical Conductivity (µS cm <sup>-1</sup> )	207	805	-	984	936	897	409	692	679	906	818	724	-	721	707	921	843	797
NH <sub>4</sub> <sup>+</sup> (µg L <sup>-1</sup> )	209	125	-	556	142	72	24	171	59	1157	329	108	-	35.1	27.6	10.1	86.1	43.1
NO <sub>3</sub> <sup>-</sup> (µg L <sup>-1</sup> )	394	1512	-	2064	1528	1355	10	539	529	683	896	655	-	2871	3315	4331	4047	5181
PRS (µg L <sup>-1</sup> )	2320	188	-	1182	966	819	1547	329	267	988	714	534	-	116	139	993	659	580
Glyphosate (µg L <sup>-1</sup> )	35.8	10.2	-	0.5	nd	6.5	2.26	2.14	nd	18.51	5.91	0.65	-	3.00	2.38	nd	nd	-
AMPA (µg L <sup>-1</sup> )	nd	nd	-	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	0.82	2.45	0.88	0.84	-
Arsenic (µg L <sup>-1</sup> )	7.2	45.0	-	33.3	35.3	29.5	11.0	52.8	48.0	40.0	32.8	16.8	-	40.4	45.7	47.6	39.7	50.9
Cu total (µg L <sup>-1</sup> )	17	8	-	13	15	19	18	7	7	10	7	6	-	9	11	15	13	14
Cu dissolved (µg L <sup>-1</sup> )	17	8	-	12	14	18	nd	nd	nd	nd	nd	nd	-	nd	nd	nd	nd	nd
Pb total (µg L <sup>-1</sup> )	80	81	-	133	89	120	74	64	63	58	68	62	-	21	24	37	26	28
Pb dissolved (µg L <sup>-1</sup> )	76	79	-	132	89	110	nd	nd	nd	nd	nd	nd	-	nd	nd	nd	nd	nd
Zn total (µg L <sup>-1</sup> )	81	59	-	108	55	53	56	26	26	49	24	26	-	16	12	36	19	16
Zn dissolved (mg L <sup>-1</sup> )	61	57	-	106	54	24	36	39	3	42	40	40	-	14	12	30	15	14

nd (not detectable) means <LOQ: glyphosate (2 µg L<sup>-1</sup>), AMPA (1 µg L<sup>-1</sup>), Arsenic (0.54 µg L<sup>-1</sup>), Cu (7 µg L<sup>-1</sup>), Pb (20 µg L<sup>-1</sup>), Zn (10 µg L<sup>-1</sup>).

The wide use of glyphosate is mainly due to the perception that it has low toxicity and little mobility in the environment (Battaglin *et al.*, 2014). However, according to Aparicio *et al.* (2013), once this herbicide is applied to the foliage, it can be washed off by rain, adsorbed by the soil particles and transported from terrestrial areas to surface waters. This transport is determined by the rainfall intensity, soil composition, slope characteristics, and vegetation (Borggaard & Gimsing, 2008). In the present study, concentrations of glyphosate were detected in all three sampling months with values ranging from 0.5 to 35.8 µg L<sup>-1</sup> (Table 1). These results are consistent with the herbicide application times on the soybean cultivation, both at sowing (September to December) and at harvest (April to June). The lowest concentrations of glyphosate detected, and also the degradation product AMPA (up to 3.00 µg L<sup>-1</sup>), occurred in February 2020 when the herbicide was not applied. The present study was carried on during a period (2019-2020) of relatively low rainfall (total precipitation = 834 mm) compared with the previous year 2018 (total precipitation = 1274 mm), according to the precipitation values recorded by the INTA (INTA 2022). The highest rainfall was recorded in February 2020 (58.4 mm) and the lowest in April and September 2019 (26.7 and 22.5 mm, respectively). However, the transport of herbicide occurred despite the lower rainfall events.

The arsenic concentrations were between 7.2 and 52.8 µg L<sup>-1</sup> (Table 1), which did not exceed the guideline level (As=150 µg L<sup>-1</sup>) established by USEPA for the protection of aquatic life (USEPA, 2009). However, in S2-September 2019 (52.8 µg L<sup>-1</sup>) and S6-February 2020 (50.9 µg L<sup>-1</sup>), the arsenic

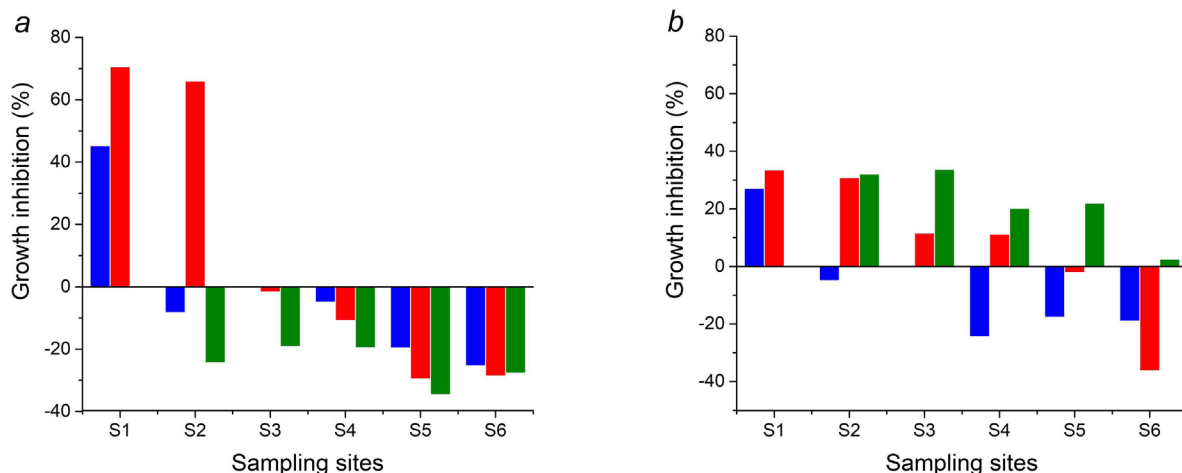
concentrations exceed the guideline level established by the Argentina Hazardous Wastes Law 24051/92 (50 µg L<sup>-1</sup>). Most of the research carried out in Argentina refers to the presence of arsenic in groundwater, where it occurs in almost two-thirds of the territory. The presence of arsenic in surface waters is less well known. However, recent studies revealed arsenic concentrations between 55 and 413 µg L<sup>-1</sup> in rivers, streams and lagoons located in the southwest of Buenos Aires province (Rosso *et al.*, 2011; Puntoriero *et al.*, 2014). In this way, the present study represents a new contribution to the knowledge of the presence of arsenic in surface waters in Argentina.

The maximum concentrations of Cu and Pb in the total fraction (19 and 133 µg L<sup>-1</sup>, respectively) exceeded the guideline levels established by USEPA (2009) for the protection of aquatic life (Cu=2 µg L<sup>-1</sup>, Pb=65 µg L<sup>-1</sup>). Likewise, the maximum concentrations of Cu, and Pb in the dissolved fraction (18 and 132 µg L<sup>-1</sup>) exceeded those levels (Table 1). On the other hand, Zn did not exceed the guideline level established by that agency (120 µg L<sup>-1</sup>) in either of the two fractions of water samples analyzed (total and dissolved fractions of 108 and 106 µg L<sup>-1</sup>, respectively). Taking into account the Argentina law, Cu and Pb concentrations exceeded the quality limits for the protection of aquatic life (2 and 1 µg L<sup>-1</sup>, respectively) in all water samples, both in dissolved and total fraction, while Zn exceeded that level (30 µg L<sup>-1</sup>) in several of the samples. Owing to their stability and persistence, the presence of these contaminants in water poses a serious threat to human health and ecosystems. According to the research carried out by Pignata *et al.* (2002) in Argentina, the high content of Zn in soils of cropping areas could be

related to the use of fungicides, whereas the high content of Cu could be related to the use of pesticides containing Cu. These products, together with wastewater used for irrigation and atmospheric deposition, contribute to metal pollution in crop soils and can be absorbed by the plants. Subsequently, metals deposited in soils could move to surface waters in runoff during rainfall events.

Algae bioassays provide simultaneous information about the substances that stimulate and inhibit the growth of algae populations (nutrient concentrations and toxic substances, respectively). In the present study, the water samples were analyzed without BBM medium dilution. There was obtained significant differences ( $p < 0.05$ ) between the growth in all the samples with respect to the controls, so the percentage inhibition of algal growth (%I) was plotted versus each sampling sites (Figure 2). Bioassays with the green alga *R. subcapitata* showed growth inhibition in three of the 16

samples analyzed, with a %I ranging between 45.26% and 70.64%. However, most samples showed growth stimulation, with negative inhibition values between -1.75% and -34.62% (Figure 2a). On the other hand, ten of the 16 samples analyzed inhibited the *S. acutus* growth, with %I ranging between 2.48% and 33.73%, and six samples showed growth stimulation, with negative %I values ranging between -2.09% and -36.26% (Figure 2b). Therefore, the native species showed greater sensitivity to detect inhibitory substances in more samples than the standard species. It is known that algal communities are composed of an array of species with different sensitivities. For this reason, the use of more than one representative species of a community in bioassays provides broader information about the risks associated with the presence of toxic substances in the aquatic ecosystem. In addition, the use of species isolated from natural environments can give more realistic information about the effects of xenobiotics on representative species of the region (Carusso *et al.*, 2018).



**Figure 2:** Percentage of algal growth inhibition (%I) in each sample site, in April 2019 (blue), September 2019 (red), and February 2020 (green) for: a) *Raphidocelis subcapitata*, and b) *Scenedesmus acutus*.

The highest values of %I occurred in S1 and S2 for both algal species. However, the concentrations of dissolved heavy metals and arsenic were not the highest in these two sites. On the other hand, in April 2020, all the sampling sites showed high concentrations of metals without differences between the total and dissolved fractions. In the case of algae, the dissolved fraction (not the total fraction) of heavy metals could be uptake into the cells. However, in natural water systems, dissolved metals can be partitioned between different physical states such as free or complexed, mainly associated with colloids. It is often assumed that the bioavailability of metals depends on the free labile ionic and labile form for microorganisms (Zhou *et al.*, 2008). The free metal concentrations of Cu, Pb and Zn in water samples could even be below the estimated  $EC_{50}$  for both species.

On the other hand, only the highest concentration of glyphosate occurred in S1-April 2019 (Table 1). It should be

noted that living organisms are exposed to complex mixtures of substances in aquatic ecosystems. Certain substances can exert synergistic or antagonistic effects at different environmental concentrations in those mixtures. In this way, the results obtained from laboratory bioassays provide realistic information about the inhibitory or stimulatory effects of the complex mixtures of nutrients and pollutants present in water samples. A previous study estimated the  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  of glyphosate, Cu, Pb and Zn for *R. subcapitata* and *S. acutus* (Afione Di Cristofano *et al.*, 2021). According to those results, only the concentrations of Zn and Pb were higher than the  $EC_{10}$  and  $EC_{20}$  for the two algal species in several samples (Table 2). The indigenous species *S. acutus* showed greater sensitivity than *R. subcapitata* when they were exposed to the complex mixture of nutrients and pollutants of water samples (Figure 2). These differences are due to specific physiological and morphological characteristics. Likewise, *S. acutus*

showed greater sensitivity than the standard species when it was exposed to low concentrations of Cu, Pb and Zn (Afione *et al.*, 2021). In this way, this strain could be incorporated as a new test organism for the analysis of water quality in natural areas from Buenos Aires, Argentina.

It is well known that heavy metals can affect algal growth in various ways by inhibiting different physiological processes. Photosynthesis in particular is the most sensitive process related to environmental stress. The metals Cu and Zn are essential metals for algae, as they participate in various physiological processes, such as photosynthesis and respiration (Starodub & Wong, 1987). However, when these elements are found in high concentrations, they can be toxic and inhibit population growth (Franklin *et al.*, 2002). On the other hand, Pb does not perform any cellular function, but it can displace other metal ions such as magnesium (Mg), calcium (Ca) and iron (Fe), which are involved in the photosynthetic apparatus and photosynthesis (Lamelas *et al.*, 2009). Likewise, there is evidence that glyphosate formulations, such as Roundup® and Atanor®, but not glyphosate itself, produce inhibitory effects on algae, such as reduction of superoxide dismutase and

catalase activity, in addition to growth inhibition (Romero *et al.*, 2011; Afione Di Cristofano *et al.*, 2021).

The environmental risk due to Cu, Pb, Zn and glyphosate concentrations in the Burgos stream and the Tala river was evaluated. The RQ values for these contaminants were estimated by taking into account of the EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values obtained by Afione Di Cristofano (2021) for the two algal species and the environmental concentrations of those toxic substances obtained in the water samples (MEC) (Table 2). The maximum concentrations of dissolved heavy metals and glyphosate were chosen in order to analyze the worst scenarios. Historically, EC<sub>10</sub> and EC<sub>20</sub> have been treated as no-effect-concentration (NOEC) analogs (Beasley *et al.*, 2015), and the risks associated with those values are related to chronic effects of the substances. In this way, the EC<sub>10</sub> or EC<sub>20</sub> could be considered environmentally relevant since they establish a protection threshold for the organisms. On the other hand, the EC<sub>50</sub> values are frequently related to the acute effects. In the present study, we evaluated both the NOEC (as EC<sub>10</sub> and EC<sub>20</sub>) and the EC<sub>50</sub> values in order to compare the two situations to which algae populations may be exposed.

Table 2: Risk quotients (RQs) based on MEC values and EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> (µg L<sup>-1</sup>), for copper, lead, zinc and glyphosate for *Raphidocelis subcapitata* and *Scenedesmus acutus*.

Toxic	EC <sub>10</sub> <sup>*</sup> (µg L <sup>-1</sup> )	EC <sub>20</sub> <sup>*</sup> (µg L <sup>-1</sup> )	EC <sub>50</sub> <sup>*</sup> (µg L <sup>-1</sup> )	MEC (µg L <sup>-1</sup> )	RQ <sup>1</sup>	RQ <sup>2</sup>	RQ <sup>3</sup>
<i>R. subcapitata</i>							
Cu	830	3090	7470	18	0.0271	0.0058	0.0024
Pb	nt	nt	nt	132	-	-	-
Zn	50	120	6510	106	2.1200	0.8833	0.0163
Glyphosate	nt	nt	nt	35.2	-	-	-
<i>S. acutus</i>							
Cu	170	290	10,920	18	0.1059	0.0621	0.0016
Pb	40	110	-	132	3.3000	1.2000	-
Zn	50	90	-	106	2.1200	1.1778	-
Glyphosate	nt	nt	nt	35.2	-	-	-

\* values obtained from Afione Di Cristofano *et al.* (2021)

$$RQ^1 = MEC/EC_{10}$$

$$RQ^2 = MEC/EC_{20}$$

$$RQ^3 = MEC/EC_{50}$$

nt = no toxic up to 20 mg L<sup>-1</sup> (data obtained by Afione Di Cristofano *et al.*, 2021)

By taking into account of all the situations analyzed, there would be no growth inhibition risk by environmental concentrations of Cu, Pb and glyphosate in the case of *R. subcapitata* (Table 2). All the RQs obtained for Cu were less than 1, while the RQs for Pb and glyphosate could not be estimated because they did not show any toxicity in the laboratory up to concentrations of 20 mg L<sup>-1</sup> (Afione Di Cristofano et al., 2021). However, the maximum concentration of Zn found in one of the samples could put algal growth at risk in the field (RQ for EC<sub>10</sub> was 2.12). On the other hand, there would be no growth inhibition risk by environmental concentrations of Cu or glyphosate in the case of *S. acutus*. Glyphosate was also non-toxic at the test concentrations up to 20 mg L<sup>-1</sup>. However, Pb and Zn concentrations in surface waters could be a risk for the growth of this species in a chronic way (RQ for EC<sub>10</sub> were 3.30 and 1.20, and RQ for EC<sub>20</sub> were 1.20 and 1.18, respectively) (Table 2). In this study, the risk associated with As concentrations in surface waters was not evaluated due to the lack of available toxicity data of algal bioassays.

The joint study of the chemical composition of water samples, together with laboratory bioassays and risk analysis, can be a useful tool for the evaluation of chemical contamination in aquatic environments. In the present study, a first approach to the analysis of the risks associated with some of the contaminants present in aquatic ecosystems of a cropping and livestock region was carried out. The use of a native algal strain allowed the analysis of the different sensitivity responses of living organisms to environmental chemical mixtures.

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