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AVALIAÇÃO ECOTOXICOLÓGICA DO RIO URUGUAY E EFLUENTES PRÉ E PÓS APLICAÇÃO DE PESTICIDAS UTILIZANDO *Caenorhabditis elegans* COMO BIOMONITOR

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RESUMO

O rio Uruguai é o rio mais importante do oeste do Rio Grande do Sul, separando o Brasil da Argentina e do Uruguai. O rio apoia atividades de pesca e fornece água potável para a população, no entanto, a poluição é uma questão preocupante devido a atividades antropogênicas como a agricultura. A região é conhecida pelas abundantes culturas de arroz, sendo uma das principais cidades produtoras de arroz, mas também pela aplicação de grandes volumes de pesticidas legais e ilegais, por ser região de fronteira. Em longo prazo, esta prática poderá causar danos irreversíveis devido ao acúmulo de resíduos, inclusive no meio aquático. Assim, nosso estudo teve como objetivo analisar as possíveis alterações de três pontos hídricos do município pré e pós a época de plantio e aplicação de pesticidas, sendo eles o Rio Uruguai, o arroio do Salso localizado na BR 472 e uma barragem localizada próxima a lavouras de arroz. Para biomonitorar o meio aquático, utilizamos o nematoide de vida livre *Caenorhabditis elegans*, que foram expostos por 24h às amostras em agitação e transferidos para meio solido para realizar os testes de sobrevivência, longevidade e reprodução. Nas amostras pré-pesticidas, observamos que mesmo que não tenha sido detectada a presença de pesticidas propriamente ditos, as análises indicaram a presença de metais nas águas coletas. Já nas amostras obtidas após a época de aplicação as análises demonstraram a presença de Tebuconazol, Imaetapir, Clomazone, curiosamente, os metais descritos nas amostras pré-pesticidas não foram relatados nas amostras pós. Observamos que tanto a sobrevivência quanto a viabilidade reprodutiva dos vermes expostos às amostras pré e pós-pesticidas foram alteradas em relação ao controle, indicando que tanto o Rio Uruguai quanto os outros cursos de água estão contaminados e não apenas pela presença de pesticidas, o tamanho da população também foi significativo reduzido em comparação ao grupo controle nas amostras obtidas no pós plantio. Esse trabalho ilustra a importância de estudos na área da ecotoxicologia, devido à contínua poluição do meio ambiente e o impacto que esse fato pode causar aos organismos que nele vivem.

Palavras-Chave: *Canorhabditis elegans*, Pesticidas, Metais pesados, Análises Limnologicas, Biomonitoramento.

ABSTRACT

The Uruguay River is the most important river in the west of Rio Grande do Sul, separating Brazil from Argentina and Uruguay. The river supports fishing activities and provides potable water for the population, however, pollution is a worrying issue due to anthropogenic activities such as agriculture. The region is known for abundant rice crops, being one of the main rice-producing cities, but also for the application of large volumes of legal and illegal pesticides, because it is a border region. In the long term, this practice may cause irreversible damage due to accumulation of waste, including in the aquatic environment. Thus, our study had as objective to analyze the possible changes of three water points of the municipality before and after the time of planting and application of pesticides, such as the Uruguay River, the Salso stream located in BR 472 and a dam located near crops of rice. In order to biomonitor the aquatic environment, we used the free-living nematode Caenorhabditis elegans, which were exposed for 24 h to the shaken samples and transferred to the solid medium to perform survival, longevity and reproduction tests. In the pre-pesticide samples, we observed that even if the presence of pesticides was not detected properly, the analyzes indicated the presence of metals in the collected waters. In the samples obtained after the application period the analyzes showed the presence of Tebuconazol, Imaetapir, Clomazone, interestingly, the metals described in the pre-pesticidal samples were not reported in the post-samples. We observed that both the survival and the reproductive viability of the worms exposed to the pre- and post-pesticide samples were altered in relation to the control, indicating that both the Uruguay River and other watercourses are contaminated and not only by the presence of pesticides, the size of the population was also significantly reduced in comparison to the control group in the samples obtained after planting. This work illustrates the importance of studies in the field of ecotoxicology, due to the continuous pollution of the environment and the impact that this can cause to the organisms that live in it.

Key words: *Caenorhabditis elegans*, Pesticides, Heavy Metals, Limnological Analysis, Biomonitoring.

SUMÁRIO

CONTEXTUALIZAÇÃO

Os defensivos agrícolas se tornaram bastante uteis no decorrer dos anos, especialmente em regiões favoráveis ao desenvolvimento de pragas, o que otimizou as produções em larga escala. Entretanto, com o uso incorreto destes químicos e a demanda mundial de alimentos aumentando consideravelmente ao longo das décadas, provocou um aumento desenfreado na utilização de pesticidas nas produções agrícolas.

Esse crescimento deu inicio ao problema de diversos resíduos tóxicos que passam a ser encontrado não só nos alimentos como no meio ambiente, causando toxicidade em diversas espécies acarretando danos deliberados. As fontes de águas costumam serem contaminadas pelas enxurradas e condições climáticas ou ainda a percolação desses resíduos no solo, atingindo os lençóis freáticos e águas subterrâneas. Sendo assim a biota aquática está constantemente exposta a um grande número de substâncias tóxicas, com uma ampla gama de agentes como metais pesados, agrotóxicos, compostos orgânicos, entre outros (ARIAS, 2007; SANCHES, 2003).

Não só esse problema da falta de consciência na dosagem de pesticidas como múltiplos impactos ambientais, por exemplo, a mineração, construção de barragens, o desvio de cursos naturais, lançamento de efluentes, exploração desenfreada de recursos pesqueiros tem provocado uma expressiva queda da qualidade da água e alteração dos ecossistemas aquáticos. (BERTI, 2009)

Graças ao avanço do campo da toxicologia aquática, temos agora uma maior compreensão dos efeitos dos poluentes sobre os organismos e é dada bastante ênfase à importância de avaliar respostas fisiológicas dos animais e o uso de espécies como meio de estudo. Deve-se notar que uma abordagem que faz uso de biomarcadores não substitui as estratégias de monitoramento químico-físico convencionais, mas os integra fornecendo grande contribuição na padronização de resultados e na avaliação de poluentes nos meios aquáticos, mesmo em baixas concentrações (VIARENGO, 2007).

Entre todas as regiões banhadas pelas águas do Rio Uruguai, a cidade de Uruguaiana, fronteira com Argentina, tem sua economia baseada na pecuária e a agricultura, destacando-se como forte produtor de arroz. O rio Uruguai pode ser considerado um dos rios mais importantes para o estado do Rio Grande do Sul e se apresenta como uma fonte natural de vida, não só no fornecimento de água para o abastecimento regional e agrícola da fronteira oeste e outros países latinos (SILVA, 2008), como também sendo abrigo de vasta diversidade ictiológica, considerada uma das principais riquezas desta região (PESSANO, 2008). Logo, é de extrema importância que as águas do Rio Uruguai sejam preservadas.

Ecotoxicological Assessment of Uruguay River effluents pre and post pesticides application using *Caenorhabditis elegans* as a biomonitor

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1. INTRODUCTION

The aquatic environments have the characteristic of being dynamic, exhibiting a great variability in the quality of water. And even if part of this variability is attributed to the intrinsic characteristics of the system and variations, such as climate change, drought, rainy seasons, among others, some variations can originate from pollution events. Anthropogenic activities (i.e. industrial wastewater, transport, agriculture) can easily release pollutants, which are the most important threats to the conservation of the world's water resources. (FERREIRA. et al., 2016)

The presence of agrochemicals in hydrological systems due to agricultural activities is the most common type of contamination. These are of particular concern because the commercial formulations contain many different components, such as potentially toxic metals and surfactants. These have potentially toxic properties, such as persistence in the environment, which may affect other living organisms and consequently lead to an elevated ecological risk (NUNES et al., 2010) (SILVA et al., 2011; GONZALEZ-MACIAS et al., 2006). Particularly, Brazil has been one of the largest consumers of agrochemicals since the 1970s, since it is the largest exporter in the world and according to surveys published by the IBGE in 2008, Brazil had agricultural production, with a growth of 9.1 % compared to the previous year (IBAMA, 2009).

Among the most important Brazilian agricultural commodities are soybeans, sugarcane, corn and particularly rice. For their mass production, the need of pesticides and genetically modified seeds is growing exponentially. As a consequence, the pesticide consumption factor of the state of Rio Grande do Sul (RS, southern Brazil) alone accounts for about 20 % of the pesticides consumed within the whole country (PRIMAL, et al., 2005). Particularly, the municipality of Uruguaiana, located in the extreme west of RS, is of the main producer of irrigated rice, being one of the most important in the Brazil (BUSATO, et al., 2002). The optimal climatic conditions, the vast lands and the presence of a major river as Uruguay River are the main reasons for this success. Although the regulatory agencies are always visiting the farms, the amount of pesticides applied and the traffic of illegal pesticides as paraquat, that enters Brazil thought the borders of Uruguay and Argentina, are still a current issue. There are many small dams and streams that have been created to allow the water flux to and from the rice crops. Therefore, the pesticides are dragged to the Uruguay River. The Uruguay River is very important for agricultural, fishing and also for recreational activities along its 1,838 Km, however the water quality has been neglected by regulatory agencies. Erosion and silting of the margins and near extinction of some vegetal species are consequences of the pollution.

It is known that continuous monitoring is one of the most reliable practices used to obtain information about the quality of natural water resources. Different analyzes have been used to detect more variations, in addition to the physical-chemical monitoring already standardized. However, most of these techniques cannot identify all the pollutants present in the aquatic environment. For this reason, ecotoxicological studies have been growing in recent years and are added to the monitoring plans (CLAVIJO, et al., 2016; RUAN, et al., 2009; SALEM, 2016).

Some criteria that must be met for an organism to be adopted in these tests, for instance they must be sensitive to the toxic agent and easy to handle and available throughout the year (CHU, 2002). Many authors have already demonstrated that the free-living nematode *Caenorhabditis elegans* is a viable model as a bioindicator in ecological risk assessments as it meets the requirements. In addition, *C. elegans* is a globally accepted model for environmental impact analysis (ASTM, 2014).

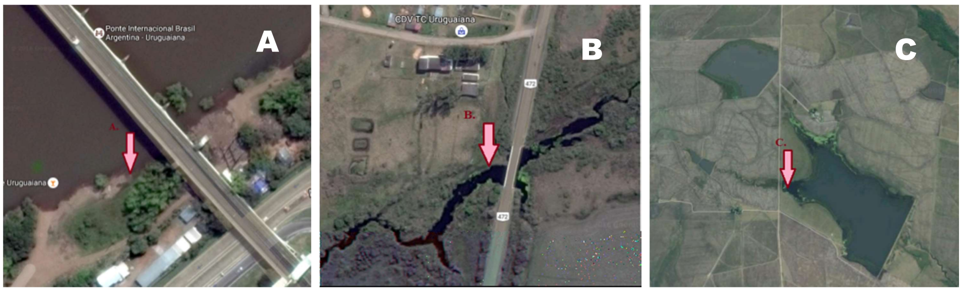
Remarkably, this model is very attractive to assess aquatic toxicity because it has short life cycle, small body size and is easy and low cost for maintenance. *C. elegans* has a high tolerance to pH, salinity and water hardness and offers a wide range of ecologically and toxicologically relevant parameters such as mortality, growth and reproduction (CHU, 2002; TEJEDA-BENITEZ, OLIVER-VERBEL, 2016).

Therefore, the objective of our study was to evaluate the quality of the water obtained from different locations of the municipality of Uruguaiana, Rio Grande do Sul state, Brazil before and after pesticides application in the rice crops by using the nematode *Caenorhabditis elegans* samples as biomonitor. In addition, we sought to correlate biologic endpoints and limnological water analysis.

2. MATERIALS AND METHODS

2.1. Samples Collection

Samplings were carried out in two periods, before the planting and pesticides application period and after pesticides application in the crops. The sampling sites were selected based on the different proximities to the rice crops: A) the margin of the Uruguay River basin, coordinates 29°44'50.3"S 57°05'19.6"W ; B) Salso Stream located in BR 472, known to be the drainage of the domestic sewage of the municipality, coordinates 29°47'54.2"S 57°05'31.6"W, and; C) Mezzomo Dam near plantation property rice knowledge, coordinates 29°59'14.5"S 57°07'19.3"W.



Collection of the pre-pesticides water samples were carried out on August 19th, 22nd and 29th / 2016 and post pesticides application on February 28th , March 2nd and March 3rd / 2017 at same period of the day, between 11:00 and 12:00 a.m. using sterilized flasks, from the top layer to the water flow. The pesticides application occurred between December and February. Following limnologic analysis, samples were stored at -20ºC.

2.2. Limnologic analysis

In order to determine the characteristics of the water samples, we have subjected them to physical and chemical analyses. Based on that, we evaluated the dissolved oxygen, temperature, pH and conductivity using a Portable Multiparameter, turbidity with a turbidimeter and ammonia and nitrite by colorimetric assays.

2.3. Pesticides Determination

The extraction was carried out using Strata X cartridges (500 mg / 3 ml), 6 mL of methanol, 6 mL of ultrapure water and 6 mL of acidified ultrapure water of pH3 were used for the conditioning. Two liters of sample were percolated maintaining the flow rate of 1 mL / min and subsequently 3 mL of acidified Ph:3 water was added to effect the cleaning of the cartridge. The analytes were eluted with 9 mL of methanol and under vacuum for another 10 minutes. The solvent was evaporated to dryness and the eluate was resuspended in 1.5 ml of methanol. Subsequently the samples were filtered through a 0.22 μm syringe filter and analyzed by HPLC-DAD.

The chromatographic system used was YL9100 (Young Lin, South Korea) equipped with a YL90 vacuum degasser YL9110, YL9150 autosampler, YL9131 column oven, YL9160 diode array detector. The control of the equipment and the data acquisition were made through the YL-Clarity software. The acetonitrile and methanol used in the chromatographic analyzes were HPLC grade (J.T. Baker, The Netherlands), ultrapure water was purchased from a Milli-Q system (Millipore, USA). Analyzes were performed using Synergi 4μ Fusion-RP 80Å (250 x 4.6 mm) and pre-column Fusion-RP (4 x 3.0 mm) chromatography column (Phenomenex, USA).

The method initially consists of acidified ultrapure water up to pH=3, methanol and acetonitrile (46/38/16, v / v) at the flow rate of 0.9 ml /min, after 10 minutes the mobile phase becomes 40% of water pH3 and 22% acetonitrile, maintaining the initial amount of methanol and with the flow rate of 1.0 ml/min, which is maintained up to 15 minutes. At 15 minutes the mobile phase becomes 36% water pH3 and 32% acetonitrile and methanol. After 30 min the mobile phase goes to 40% methanol, 36% acetonitrile and 24% water pH 3 to 35 min. At 35 minutes the mobile phase passes to 44% methanol, 36% acetonitrile and 20% water pH3 and flow of 1.2 mL/min. The method was finished at 40 minutes with mobile phase in 48% methanol and 16% water pH3. The wavelengths used were 220 nm for Tebuconazole and Clomazone and 248 nm for Imazethapyr, the injection volume was 20 μl of sample.

2.4. Metal Analysis

Metals as Aluminum, Manganese, Iron, Copper, Zinc, Arsenic, Cadmium, Lead and Magnesium were analyzed through inductively coupled plasma- mass spectrometry (ICP-MS) in order to investigate their presence in all samples.

2.5. Worms Maintenance and synchronization

Wild-type N2 worms were used in all the experiments and were obtained from the Caenorhabditis Genetics Center (CGC). Animals were kept in temperature-controlled environment at 21-22˚C.To obtain the animals at the same larval stage, the population was synchronized by exposing gravid adult worms to a lysing solution (0.45 N NaOH, 2% HOCl). After 14 hours the eggs hatch, releasing L1 larvae. (BRENNER, 1974).

2.6. Exposure

1,000 worms at L1 stage were placed in 100 ml Erlenmeyer containing 5 ml of the water samples and *Escherichia coli* OP50 as food source and left on constant agitation for 24 hours on an orbital shaker at 70 RPM in an incubator at 21 ° C. We have used K-medium as control, composed of sodium chloride, potassium chloride, distilled water, calcium chloride, magnesium sulphate and cholesterol, which was then autoclaved. Shortly after 24 hours, worms were pelleted and transferred to NGM (nematode growth medium) plates seeded with *E. coli* OP50.

2.7. Longevity Assay

When reaching L4 stage, 20 worms treated with each water sample were transferred to NGM plates, seeded with OP50, in duplicate. Survival was assessed daily until the worms were dead. Worms were transferred daily during the reproductive period in order to avoid progeny contamination. Each experiment was repeated three times.

2.8. Brood Size

One worm from each of the samples exposure was transferred daily to NGM plates with E. coli/OP50 and reproduction was evaluated by counting the size of the litter until the end of the reproduction period. Each experiment was repeated in triplicates and three independent experiments were performed.

2.9. Statistical analysis

All assays were performed at least three individual times and GraphPad Prism 6 software was used to generate charts and statistical analysis. One (Survival and Brood size) or two-way (Longevity Assay) ANOVA were used and $p < 0.05$ was considered statistically significant. Post hoc tests were performed in these cases through Tukey post hoc test. Values are normalized as percentage, assuming control as 100%.

3. RESULTS

In tables 1 and 2 are described the limnological data pre-pesticides application in the crops. It can be observed that dissolved O_2 was within the desired levels, However, the conductivity, which is an indirect indicator of pollution, presented high values, as well as the ammonia values present in the samples.

No residues of pesticides were found in these samples, however metals as Al and As were present, which is undesirable.

Table 1
Limnological analysis of samples prior to application of pesticides.

	Dissolved O_2 (mg/L)	Turbidity (NTU)	Ammonia (mg/L)	pH	Nitrite (mg/L)	Conductivity (µS/cm)
River 19	9.2	7.49	0.25	6.14	0	78.24
River 20	9.2	6.3	0.25	6.7	0	82.02
River 22	9.2	6.36	0.25	6.67	0	71.46
Stream 19	9.5	4.36	1	6.23	0.3	443.8
Stream 20	9.3	3.84	1	6.45	0.3	451.7
Stream 22	9.3	4.28	1	6.48	0.3	449
Dam 19	9.8	81.2	0.1	6.42	0	155.9
Dam 20	9.6	8.61	0.1	6.57	0	155.1
Dam 22	9.5	5.34	0.1	6.63	0	153.8

Table 2
Metal concentration in samples pre-pesticides (µg/L).

	Aluminum	Manganese	Iron	Copper	Zinc	Arsenic	Cadmium	Lead	Magnesium
River 19	54.84	2.49	50.52	1.14	0	0.38	0	0	28.6
River 20	12.48	31.41	67.78	0.75	0	0.43	0	0	65.34
River 22	0	26.04	21.64	0	0	1.37	0	0	56.8
Stream 19	58.17	3.37	58	1.41	0	0.4	0	0	32.14
Stream 20	0	10.43	27.26	0	0	0.44	0	0	96.34
Stream 22	0	60.7	35.55	0	0	1.41	0	0	86.77
Dam 19	136.5	1.86	104.1	0.98	0	0.21	0	0	41.77
Dam 20	6.33	18.57	67.34	1.03	0	0.44	0	0	89.9
Dam 22	0	69.56	51.65	0	0	1.44	0	0	103.02

Tables 3, 4 and 5 depict the limnological data from samples collected after the pesticides application. Overall, samples presented less dissolved O_2, higher turbidity and lower conductivity when compared to samples collected before pesticides application. In addition, pesticides residues of clomazone, imazetapyr and tebuconazole were found. On the other hand, a smaller amount of metals were present in these samples.

Table 3

Limnological analysis of samples after application of pesticides.

	Dissolved O_2 (mg/L)	Turbidity (NTU)	Ammonia (mg/L)	pH	Nitrite (mg/L)	Conductivity (µS/cm)
River 28	7.8	8.21	0	6.4	0	52.12
River 02	7.6	27	0.1	6.5	0	73.52
River 03	3.9	54.9	0.25	6.55	0	66.27
Stream 28	7.7	12.1	0.1	6.71	0.025	240.7
Stream 02	7.5	17.8	0.25	6.7	0.05	133
Stream 03	7.4	18.7	0.25	6.73	0	178.7
Dam 28	6.6	81.2	0	6.33	0	122
Dam 02	7.3	8.61	0.1	5.46	0	80.18
Dam 03	5.8	5.34	0.1	5.81	0	56.22

Table 4

Quantity of Pesticides detected in post-harvest analyzes.

	Clomazone (mg/L)	Imazetapir (mg/L)	Tebuconazol (mg/L)
River 28	0,003	0,00175	0,00338
River 02	0	0	0
River 03	0,01176	0,00158	0,00226
Stream 28	0,00318	0,00152	0,00343
Stream 02	0	0	0
Stream 03	0,00356	0,00146	0,00199
Dam 28	0,00868	0,00248	0,00366
Dam 02	0	0	0
Dam 03	0	0	0

Table 5

Metal concentration in samples post-pesticides (µg/L).

	Aluminum	Manganese	Iron	Copper	Zinc	Arsenic	Cadmium	Lead	Magnesium
River 28	17.7	0.17	29	0	0	0	0	0	0
River 02	185.3	7.1	245.3	0	0	0	0	0	0
River 03	160.6	0.78	143.5	0	0	0	0	0	0
Stream 28	0	0.11	3.5	0	0	1.4	0	0	0
Stream 02	171.7	0	307.9	0	0	0.4	0	0	0
Stream 03	0	18.6	0	0	0	1.1	0	0	0
Dam 28	13.2	0.05	8.2	0	0	0	0	0	0
Dam 02	0	0.11	0	0	0	0	0	0	0
Dam 03	1.4	0	2.5	0	0	0	0	0	0

In Fig. 1 A-C, we observed that *C. elegans* reproduction was reduced on the third sampling pre-pesticides application day from Mezomo Dam and Salso Stream. Following pesticides application, we have found that reproduction was compromised for all samples, but in different sampling days (in Mezomo Dam on D1 -Fig. 1D- and Salso Stream and Uruguay River on Day 20 -Fig. 1E). Notably, when we grouped all the pre (Fig. 2A) or post pesticides (Fig. 2B) samples, we observed that all samples affected worms reproductive capacity in relation to control.

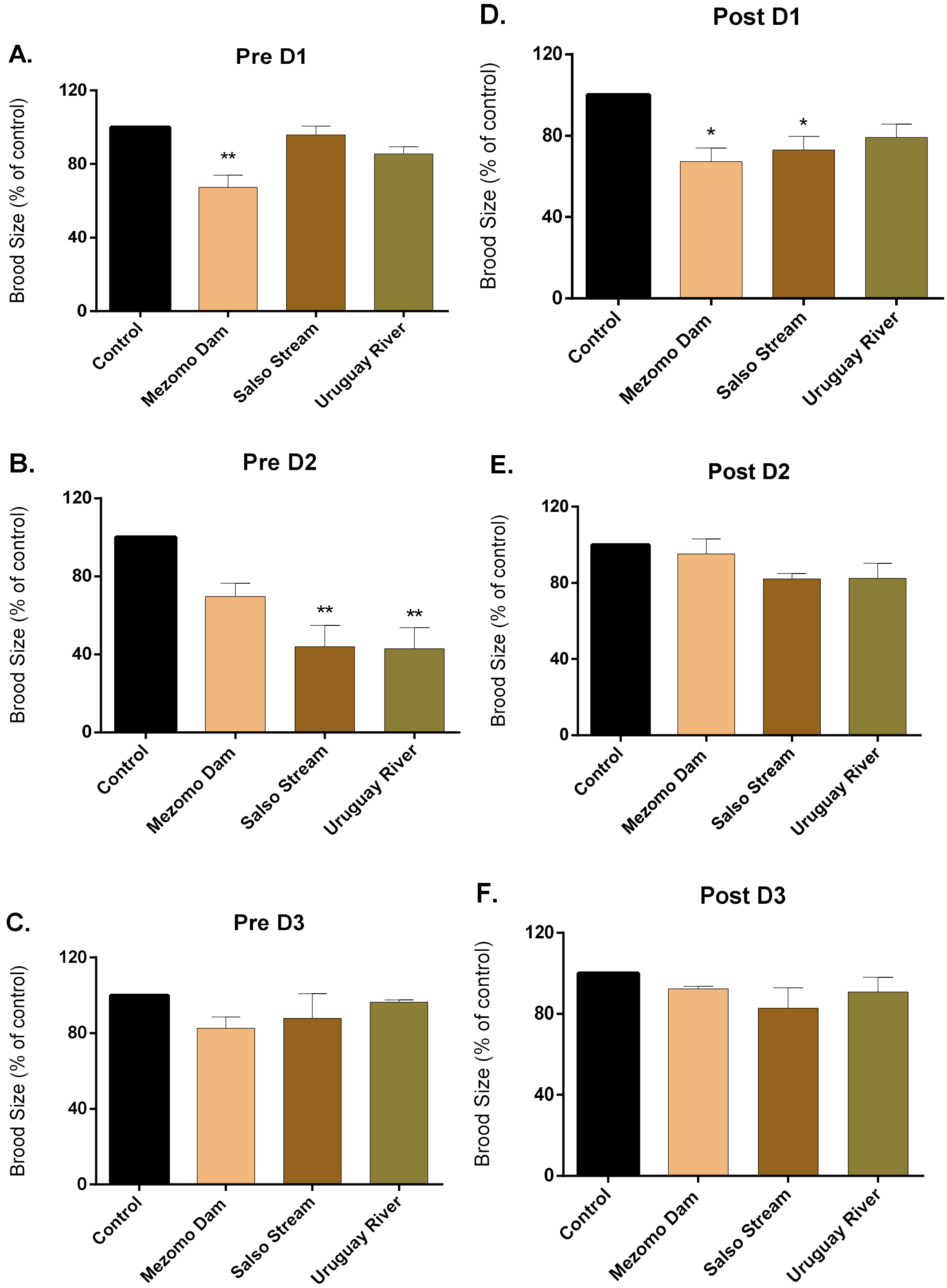


Fig.1. Reduced reproduction in *C. elegans*. A. worms exposed on first sample (D1) pre-pesticides; B. second sample (D2) pre-pesticides; C. third sample(D3) pre-pesticides; D. C. elegans exposed on D1 post-pesticides samples; E. D2 post-pesticides; F. *C. elegans* exposed on D3 post-pesticides. * indicates significant differences with control following One-way ANOVA ($p < 0.05$, Tukey post hoc test).

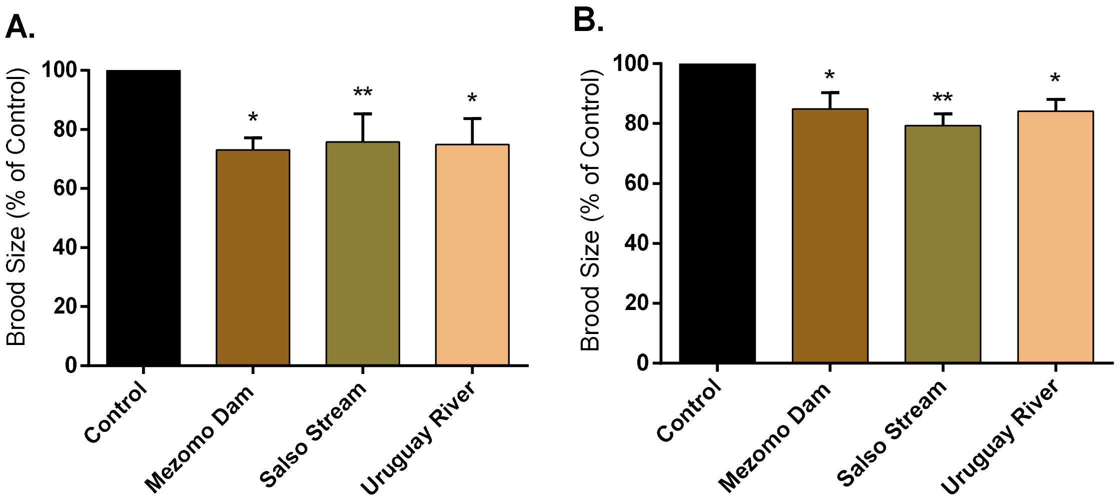


Fig. 2. Reduced reproduction in *C. elegans* when grouping samples from: A. pre-pesticides application and; B. post-pesticides application. * indicates significant differences with control following One-Way ANOVA ($p < 0.05$, Tukey post hoc test).

Next, we investigated the chronic effects of exposure to water samples in worms. At Pre D1 (Fig 3A), all water samples significantly reduced *C. elegans* life span. Pre D3 samples (Fig. 3C) also showed significant results, but only in samples collected in Mezomo Dam and Uruguay River. On the other hand, at D2 sampling (Fig. 3B) none of the samples caused a significant decrease in the life time in relation to the control group.

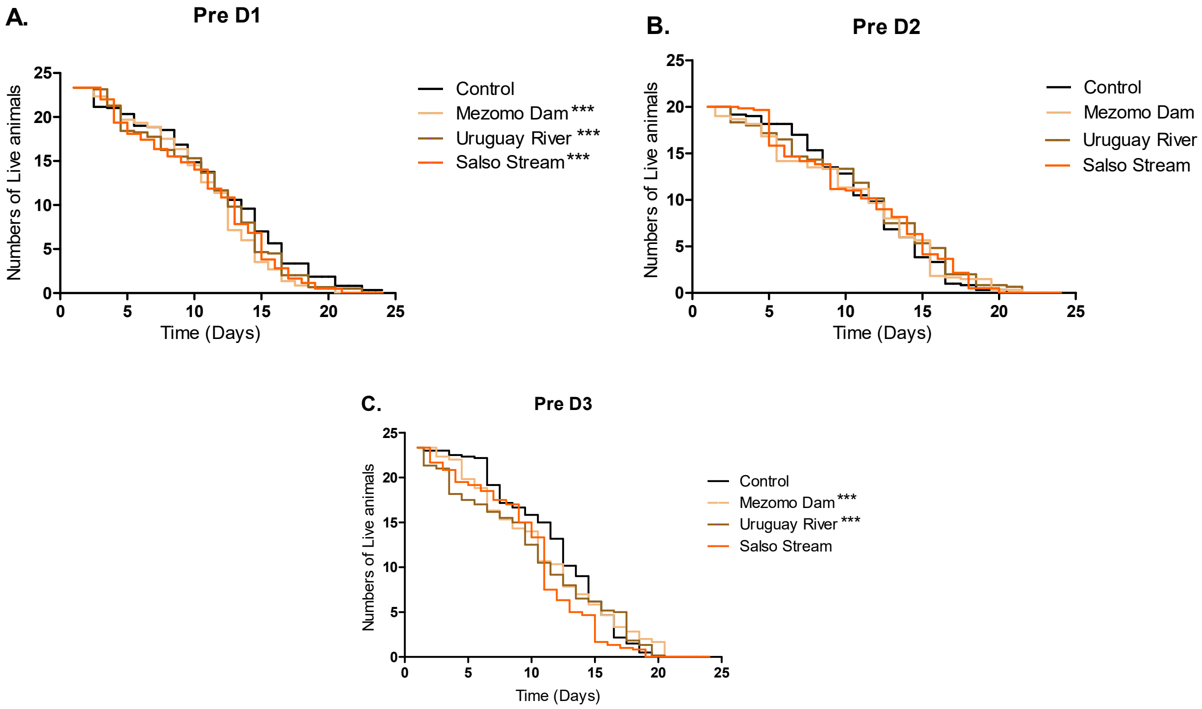


Fig. 3. Reduced survival in *C. elegans* exposed to pre-pesticides samples. A. D1; B. D2; C. D3. * indicates Significant differences from control following One-way ANOVA ($p < 0.05$, Tukey post-hoc test).

In Fig. 4, we can observe that all samples collected from the Mezomo Dam (Fig. 4A-C) after pesticide application caused a significant reduction in worms lifespan. For Salso stream, we have found that Post D2 (Fig. 4B) samples caused a significant decrease in worms lifespan.

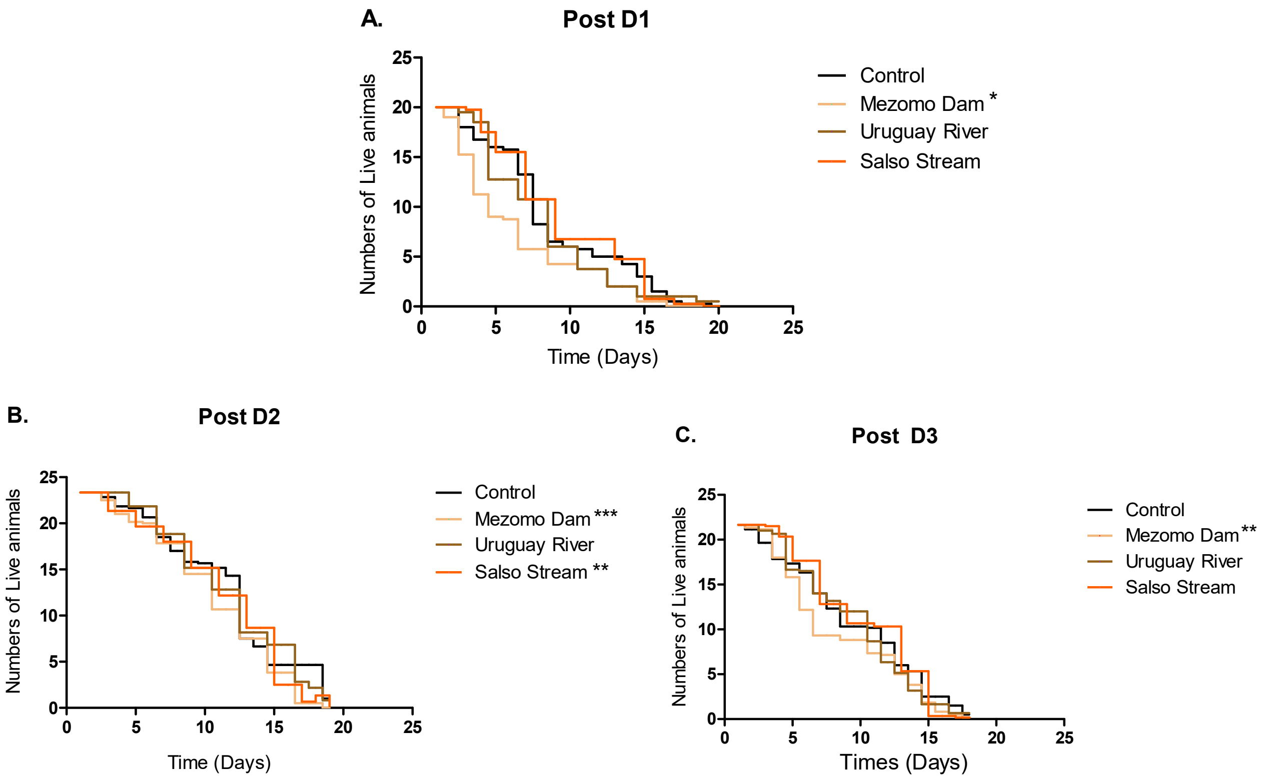


Fig. 4. Reduced survival in *C. elegans* exposed to post-pesticides samples. A. D1; B. D2; C. D3. * indicates Significant differences from control following One-way ANOVA ($p < 0.05$, Tukey post-hoc test).

In Fig. 5 is shown that worms exposed to the water samples collected after the application of pesticides in the crops represented a reduced survival rate in the first two days of collection (D1 and D2). However, the worms exposed to the samples of the third day did not present reduced survival (D3).

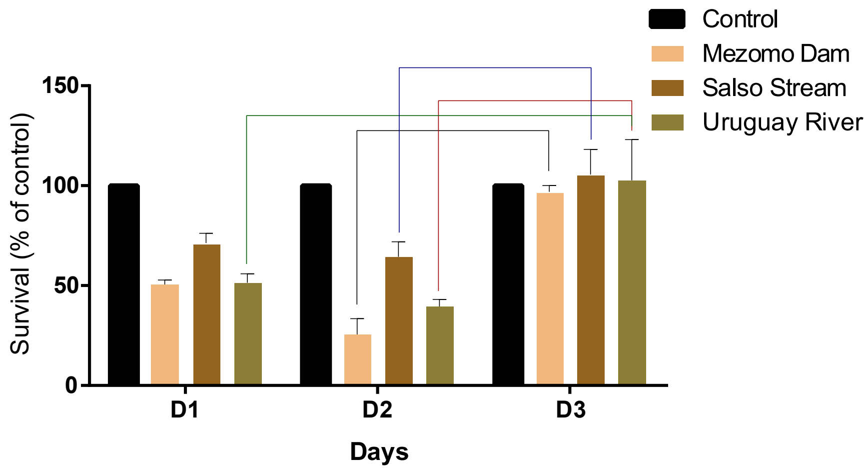


Fig. 5. Reduced Survival in *C. elegans* exposed in water samples collected after pesticides application. Lines indicate significant differences among groups by Two-way ANOVA ($p < 0.05$, Tukey post hoc test).

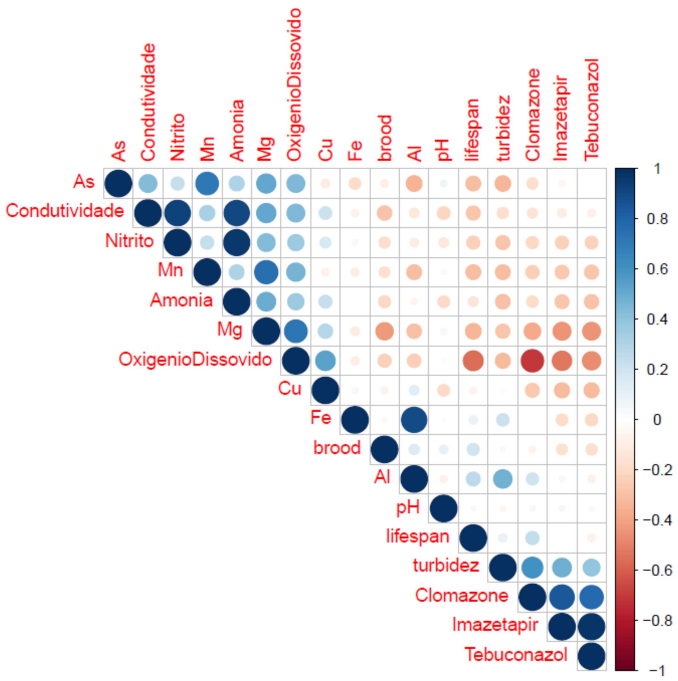
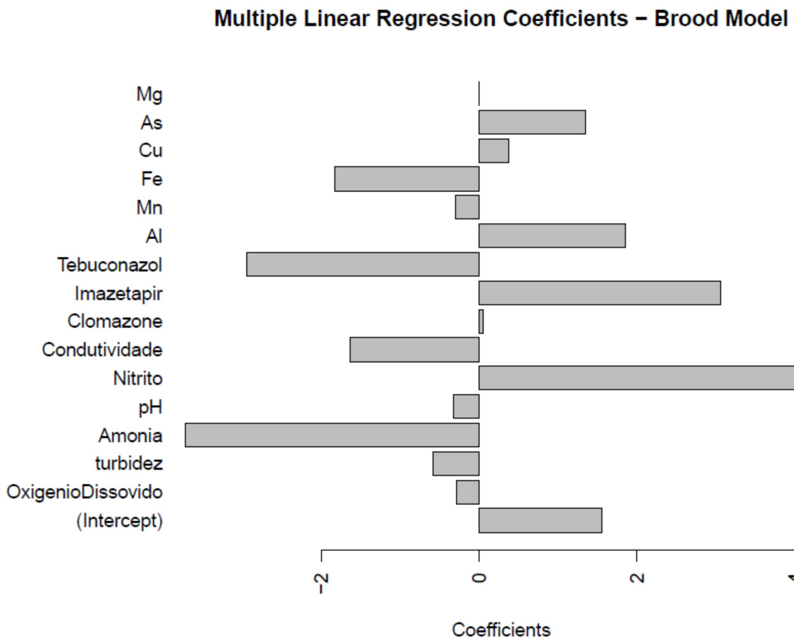


Fig. 6. Correlation analysis of all data.

We have also determined that many limnological data were also associated. For instance, we have found positive correlations for turbidity and pesticides levels and a negative correlation between dissolved oxygen and pesticides (Figure 6).



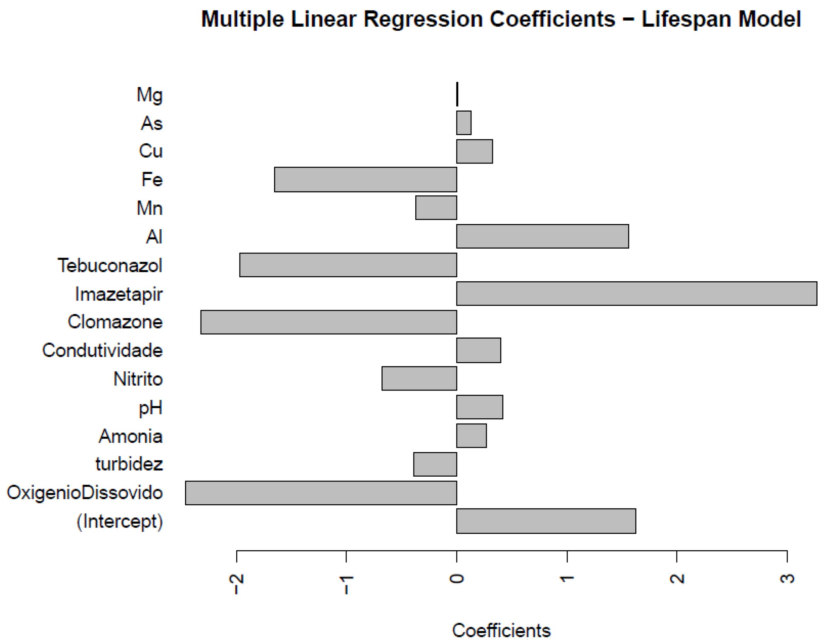


Fig. 7. Multiple linear regression coefficients. A. Reproduction; B. Survival

In order to determine which parameter would be causing alterations in the biological endpoints, we have performed a correlation analysis. We have observed that lifespan is negatively modulated by dissolved oxygen, conductivity, arsenium, manganese and magnesium levels in the samples; on the other hand, is positively correlated to clomazone levels (Figure 7A).

Brood size is negatively correlated to conductivity, magnesium, nitrite tebuconazole and Imazethapyr levels (Figure 7B). The normality tests demonstrated a great variation of the parameters in the samples, even those collected from the same sites in the same period. This may have impaired our analyzes.

4. DISCUSSION

Determination of water quality is critical for water management policies. In the present study, we have applied *C. elegans* as biomonitor to assess the quality or Uruguay River and two effluents that receive pesticides residues from the rice crops and outflow in Uruguay River. We have confirmed the presence of pesticides residues in the post-application samples, as well as observed limnological alterations in these samples that are not considered suitable for water quality indexes (CONAMA, 2005; GASPAROTTO, 2011; LÔNDERO and GARCIA, 2010.). In addition, we have found that independent on whether samples were collected before or after pesticides applications, worms depicted altered endpoints which indicate that the samples had poor quality.

One of the most important observations in this work is that *C. elegans* reproduction and lifespan were affected by changes in water quality, even when water samples the quality seemed to be adequate (pre-pesticides application samples). This aspect reinforces the concept that there must be other substances or chemicals in the water samples that were not measured in our study but even so exert toxicological effects. Notably, we have found higher amount of metals such as As and Al in pre-pesticides samples in relation to post-pesticides, however we could not relate these levels to the altered endpoints.

The presence of commonly used pesticides in rice crops (GLINSKI, et al 2018) as Clomazone, Imazethapyr and Tebuconazole was detected in the samples obtained after the pesticides application, even in the Uruguay River, which is the recipient of the affluent. Interestingly, when we compared the biological endpoints from worms treated with pre versus post-pesticides application, no significant changes were found. We expected a significantly higher toxicity in the post- samples considering the presence of pesticides. Notably, we could not measure some pesticides as we did not have standards or standardized methods for quantification.

Remarkably, worms exposed to all the post-pesticides samples collected from Mezzomo Dam showed a reduction in their longevity, even if no presence of metals was evidenced. As in longevity, survival (Fig. 5) was also compromised in worms exposed to the samples from all sites collected from D1 and D2. The hypothesis for such variations is that the water flow and the meteorological conditions on the days of sample collection may have diminished the possible presence of contaminants in the water column. This would explain variations in daily results, both pre and post pesticides application. However, even if the presence of metals in the post-planting samples has not been detected, the biological alterations in *C. elegans* were very significant which may be related to other contaminants or other factors present in these samples, as detected in the limnologic analyzes, and not only caused by the presence of metals or pesticides.

Another explanation for this phenomenon is that the solids present in pesticides and other contaminants may have decanted and are sedimented and accumulated, as reported by previous studies (TEJEDA-BENITEZ, 2016; CABALLERO –GALLARDO, 2015; KIM, 2018; TORO, 2016). This could indicate that these sites would have been already contaminated before the planting season in which the collection was carried out, therefore explaining the biological alterations observed in worms exposed to pre-pesticides samples.

Given all these variations, correlation analyzes were performed (Fig. 6) in an attempt to indicate a factor responsible for the biological alterations. We have observed that the lifespan is negatively modulated by the reduction of oxygen in the medium. It is known that *C. elegans* can enter into an alternative larval stage known as "dauer" in response to environments with low oxygen concentrations and can even die under highly anaerobic conditions due to energy deficiency (MILLER; ROTH, 2009; KITAZUME, et al., 2018; PADILLA, et al., 2002.).

Clomazone has been also correlated with reduced longevity reduction in our study. Clomazone is an herbicide widely used in various crops, including in rice. In 2009, clomazone was among the top ten most used herbicides in Brazil (IBAMA, 2010). Although very effective, it is known for environmental contamination due to its high solubility in water (1100 mg.L -1) (ZANELLA et al., 2002). Previous studies have already described that clomazone causes suppression of catalase antioxidant enzymes and increased lipid peroxidation in *Rhamdia quelen* (CRESTANI et al., 2007); to activate glutathione transferase enzyme activity and in *Prochilodus lineatus* (PEREIRA et al., 2013); and to cause high mortality, teratogenic effects and underdevelopment in *Danio rerio* embryos, proving toxicity (STEVANOVIC, et al., 2017). All these effects, in addition to liver damage, were also seen in different species of tadpoles (FREITAS, et al., 2017; OLIVEIRA, et al., 2016).

The correlation also showed effects of tebuconazole presence by reducing longevity and egg viability in *C. elegans*, which toxicity has already been reported leading to oxidative stress and endocrine disruption in rats (YANG, et al., 2018). Although imazethapyr has also been described as causing metabolic alterations in *Cyprinus carpio* (MORAES, et al., 2011), we have not found any correlations in our study in *C. elegans*.

In summary, our study demonstrated that all the samples did not present suitable characteristics not even before pesticides application and the biological data verified that worms, and putatively other animals, may suffer negative consequences from being in that environment. Notably, worms are very resistant animals, therefore minimal alterations in survival and reproduction are strong indicators of ecotoxicity. Therefore, the authorities need to be aware of these data and reevaluate regulatory policies in order to protect this very important river.

5. CONSIDERAÇÕES FINAIS

Considerando a grande importância de conhecer a qualidade da água para diversas áreas de interesse, nosso estudo com *C. elegans* demonstrou a importância do monitoramento em cursos de água, principalmente em regiões conhecidas pela produção agrícola e como modelos de bioindicador podem ser bastante uteis na avaliações de danos ao ambiente aquático e são valiosos adjuntos aos métodos de avaliação de qualidade.

Em nosso estudo, descobrimos que:

- ✓ As amostras de água do Rio Uruguai, Salso Strem e Mezzomo alteraram os parâmetros biológicos em C. elegans, independente de se as amostras foram coletadas antes ou depois da aplicação de pesticidas;
- ✓ Amostras de água apresentaram alterações nos dados limnológicos e a presença de metais não essenciais como As;
- ✓ A análise de correlação demonstrou que a condutividade, o oxigênio dissolvido e a presença de metais e pesticidas foram correlacionados às alterações biológicas observadas em *C. elegans.*

Destacamos a ameaça que o uso não consciente de agrotóxicos e outros contaminantes pode oferecer aos nossos recursos hídricos e à nossa fauna aquática devido à toxicidade gerada.

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