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Water quality assessment in the region of Vale dos Sinos using the alternative model *Caernorhabditis elegans*

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ABSTRACT

The determination of the condition of river water quality is critical to establishing sustainable water resource management policies. In this scenario, the objective of this study is to verify the use the nematode *Caenorhabidits elegans* as a bioindicator test to evaluate water quality to assess toxicological risks in aquatic environment. The samples of water were collected in three stretches of the Paranhana and Ilha rivers (P1, P2, and P3) in November (spring) of 2018, January (summer) of 2019, April (autumn) of 2019, and August (winter) of 2019. The physicochemical parameters were analyzed according to the standard methods (Standard Methods for the examination of Water and Wastewater 23rd edition). The nematode strains used are savage lineage N2 and were obtained through the *Caenorhabidits Genetics Center* (CGC) for the bioindicator test. The development evaluation was verified by the body surface area measurement of 20 nematodes quantified through the *ImageJ* software. Most physicochemical parameters were within limits recommended by CONAMA, but aluminum presented values ten times higher than the recommended limit. Significant differences were observed in the nematode development from all the samples compared to the control group (p<0,001). The results obtained in this study, using *C. elegans* as a bioindicator, showed possible toxicological effects due to pollutants present in aquatic environment that can affect live organisms. The nematode *C. elegans* was sensitive, showing that ecotoxicological assays are important for a realistic scenario of threats to water quality.

Keywords: Nematode, alternative model, water quality, bioindicador.

Introduction

The development of industrial innovation brought numerous advances and benefits for the population. However, there is an unequal growth of demand, degradation, and water pollution. Human activity consumes hydric resources and discharge chemicals and bacteriological pollutants in natural effluents; hence, anthropic activity is an important threat to the conservation water resource. Thus, in the last few years have been recommended the monitoring and assessment of the water, including physicochemical, ecologic, and toxicologic tests (Blume et al., 2010; Marengoni et al., 2013; Linden et al., 2015; Steffens et al., 2015; Clavijo et al., 2016; Brucker et al., 2021).

The watershed of Sinos River in Rio Grande do Sul involves 32 municipalities and supplies approximately 1,6 million inhabitants. Furthermore, this is the most studied watershed because of its demographic and industrial density, which increases the water contamination, mainly by the footwear-leather segment, predominant service in the south of the country (Lemos, 2014; IBGE, 2017). Numerous studies discussed the water quality assessment of the watershed of Sinos River, and in compliance with the physicochemical parameters evaluated in some studies, the sampling in specific locations of the Watershed of Sinos River, mainly in stretches nearby urbanization and industrialization, showed a decline in water quality (Blume et al., 2010; Cassanego et al., 2015; Steffens et al., 2015; Nascimento et al., 2016; Cassanego & Droste, 2017). A study by Steffens et al. (2015) evaluated the water quality by monitoring a particular species of fish, *Astyanax jacuhiensis*, and they observed morphologic alterations in the cellular nucleus of these animals that were exposed to water on the upper to the lower stretch of Sinos River, concluding that this location does not have good water quality (Steffens et al., 2015). Due to this it is fundamental to determine the condition of the water quality in rivers to establish sustainable politics of water resources management (Steffens et al., 2015; Cassanego & Droste, 2017).

Brazil's National Environment Council (CONAMA) is the agency responsible for the sustainable development of the ecologic function of prevention and precaution, which aims to control the release of pollutants in the environment at harmful or dangerous levels to human or other living beings, and its description is the assessment of the quality control through physicochemicals and bacteriological parameters. Even though regardless of all the technology used, it is still not possible to identify all the pollutants, and mainly its interactions, which are necessary items for the evaluation of the ecological water condition. For this reason, the use of biomarkers models in ecotoxicology has attracting attention in the scientific field (CONAMA, 2005; Clavijo et al., 2016; Peteffi et al., 2019; Roubicek, Rech & Umbuzeiro, 2020; Brucker et al., 2021,).

To an organism become a bioindicator, it must fulfill many criteria; some of them include: the organism must survive in healthy environments, however it must be sensitive to toxicants tests, easy to manipulate inside a laboratory, and easy obtention (Lins, 2010). The alternative *in vivo* model, *C. elegans*, used as a bioindicator organism in the evaluation of toxicologic risk in aquatic and ground environments, is shown by some authors (Leung et al., 2008; Xing. Guo & Wang, 2009; Clavijo et al., 2016; Peredney & Williams, 2000; Brucker et al., 2021). It is a free-living nematode and is worldwide accepted for toxicological risk assessment (Leung et al., 2008; ISO 10872, 2010; Hoss et al., 2012; ASTM, 2014). Many of the basics physiological process and stress response observed in superior organisms, including humans, are seen in the nematode. Furthermore, there are other benefits about using this alternative model in biological tests, for this reason, some items should be highlighted, like a low price, accessible acquisition, maintenance, and cultivation of the strains (Kaletta & Hengartner, 2006; Ávila et al., 2012; Charão et al., 2015). In addition, this organism has a short lifespan and is self-fertilizing reproduction, which assures an isogenic population. Because this model has decreased body size (length of 1mm in adulthood), it is easy to handle, and it can be manipulated in Petri dishes (Leung et al., 2008; Xing, Guo & Wang, 2009; Clavijo et al., 2016). Moreover, the genome of this organism is sequenced. Another advantage about using *C. elegans* is that the nematode is a multicellular organism that allows the assessment of the action of the compound systemically in an organism (Kaletta & Hengartner, 2006; Ávila et al., 2012; Charão et al., 2015; Stefanello et al., 2015).

Thus, this article aims to evaluate potential toxic effects using the alternative in vivo model *C. elegans* and the physicochemical parameters already proposed by CONAMA to verify the usefulness of this model as an extra tool in water quality monitoring.

Material and Methods

Collection of samples

The samples of water were collected at the locations in the intermediated stretch of the Sinos River. This is where the river receives water from the Ilha River, and Paranhana River in Rio Grande do Sul-Brazil.

Places where the sampling was carried out: Point 1 (P1): approximately 8 km upstream of the mouth of Ilha River (29°71′63,31″ S; 50°71′49,34″ W); Point 2 (P2): is located 30 m downstream of the mouth of Ilha River and from 13 km upstream of Paranhana River, approximately 8 km from P1 (29°69′18,48″ S; 50°74′67,42″ W); Point 3 (P3): approximately located 17 km from P2, 4 km from downstream of the mouth Paranhana River (29°68′62,29″ S; 50°85′09,88″ W). Figure 1 shows the sampling places 1, 2, and 3, named P1, P2, and P3, respectively.

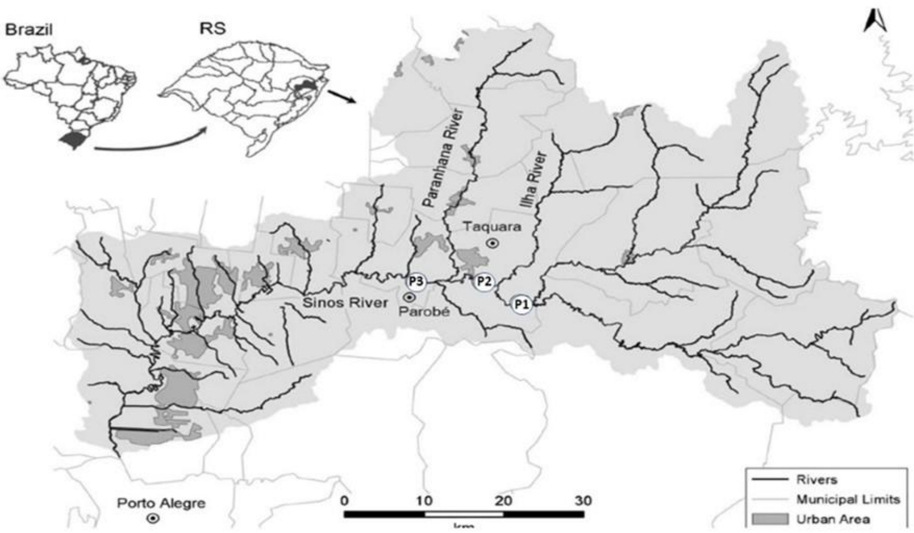

Figure 1. Water sampling places (P1, P2, and P3) in Sinos River, Rio Grande do Sul, Brasil. Font: Adapted from Dalzochio et al. (2018).

The location of P1 is an area with low environmental impact comparing to the others sampling sites. The P2 is located close to the Water Treatment Station of Taquara, where they collect, treat, and supply water to the population. Both sampling points 1 and 2 are in Taquara, and the common characteristic is the low population density, primarily because of the rural activity. The P3 is in Parobé and has a higher population density, receiving urban and industrial effluents, mainly from the footwear-leather segment.

Surface water samples were collected according to standard procedures established by the national guide for collection and preservation of samples (Brandão et al., 2011). In all samplings, surface water was collected near the riverside, in a 10 L recipient.

The samplings have been done in four distinct periods, including the four seasons of the year in Brazil, November (spring) of 2018, January (summer) of 2019, April (autumn) of 2019, and August (winter) of 2019, and they were made according to the standard method ISO 10872:2010 (ISO, 2010).

The precipitation graphic was built based on databases provided by the weather station of Campo Bom, considering total precipitation ten days before each collection, referring the three sampling locations together.

Physicochemical parameters

The parameters were analyzed according to the standard methods (Rice, Baird & Eaton, 2017). For metal analysis was used atomic absorption with graphite oven, aluminum (Al) by the method SM 3111 D (limit of quantification: 0,823 mg L^{-1}), lead (Pb), by the method SM 3111 B (limit of quantification (LOQ): 0,102 mg L^{-1}), cooper (Cu) by the method SM 3111 B (LOQ: 0,013 mg L^{-1}), total chromium (Cr) by the method SM 3111 D (LOQ: 0,182 mg L^{-1}), manganese (Mg) by the method SM 3111 B (LOQ: 0,059 mg L^{-1}), nickel (Ni) by the method SM 3111 B (LOQ: 0,031 mg L^{-1}), zinc (Zn) by the method SM 3111 B (LOQ: 0,010 mg L^{-1}), total phosphorus (P) by the method SM 4500 P D (LOQ: 0,023 mg L^{-1}). Nitrogen ammoniacal by the method SM 4500 NH_3 C (LOQ: 0,99 mg L^{-1} N), total solids by the method SM 2540 B (LOQ: 2,9 mg L^{-1}), biochemical oxygen demand (BOD) by the method SM 5210 D (LQ: 20 mgO_2 L^{-1}) and chemical oxygen demand (COD) by the method SM 5220 C (LQ: 45,07 mg L^{-1}).

Bioindicator test: Caenorhabditis elegans
Strains maintenance and synchronization

The nematode strains used are wild type N2 and were obtained from the *Caenorhabditis Genetics Center* (CGC) from the University of Minnesota, EUA. The strains have been maintained in the culture medium NGM (Nematode Growth Medium) that are used for strains conservation, beyond *Escherichia coli* OP50 was sown for the nutrition of the nematodes and incubated in 20ºC in a B.O.D incubator (Ávila et al., 2012; Charão et al., 2015; Brenner, 1974).

The nematodes were synchronized to obtain the same larval stage (L1 stage). The nematodes were briefly sub-cultivated in 8P dishes and supplied with *Escherichia coli* Na22 for nutrition (Brenner, 1974). The nematodes were taken from the 8P dishes after 3 days, and bleaching solution (NaOCl 1%; NaOH 0,25 M) was added for the disruption of the cuticle of the nematode and release of the eggs. After successive washes, the eggs were separated by gradient, using sucrose 30%. The dispersed eggs in the water were laid out in NGM dishes and, after 13-14 hours, we obtained the nematodes L1 stage (Ávila, et al., 2012; Charão et al., 2015).

Treatment and toxicity tests

The next experiments were performed three times (n = 3) in duplicate, according to Clavijo et al. (2016). Briefly, 1000 nematodes in L1 stage were treated with 2 mL of the water samples, supplied with of *E. coli* OP50 (final concentration OD 600nm=1) for feed, homogenized for 24 hours, and incubated in a B.O.D incubator at 20ºC. The control group was treated with basal solution (buffer solution) (Stiernagle, 2006). After 24 hours, the nematodes were laid out in NGM dishes, where remained for 48 hours incubated in 20ºC.

The nematodes were evaluated in two toxicity tests described in the *C. elegans* model: survival and development. The survival test evaluated the percentage of the number of living nematodes after the time in treatment relating to the control group (treated with basal solution). The development was evaluated by sampling. Development was determined by the nematodes in L4 stage (20 for treatment), they were placed in agarose blades, and the pictures were taken by the stereomicroscope with an attached camera. Subsequently, the superficial area of the nematodes body (μm²) was measured using *ImageJ* ® software.

Statistical analysis

Data were submitted to a statistical analysis using the program Prisma® 5,01. The results were compared by variation analysis (ANOVA), followed by the test *pos hoc* of Tukey, using the version 22.0 ® of the program SPSS. The values of $p < 0.05$ were considered statistically different.

Results and Discussion

Physicochemical parameters

The physicochemical parameters analysis (Table 1) had lower results than the limit of quantification for lead, total chromium, nickel, biochemical and chemical oxygen demand, and nitrogen ammoniacal in every place of sampling, not only in November (2018) but in January, April, and August (2019) too. The research of total phosphorus and total solids in November was positive in the three places of sampling, and the total phosphorus in the places P1 and P2 presented higher values than the recommended values promoted by CONAMA. Manganese was present in the places P2 and P3, and zinc in P1 and P3. In January, total phosphorus, manganese, cooper copper, and total solids were found in the places P1, P2, and P3, but zinc was positive in P3 only. In April, total phosphorus, total solids, and zinc indices were positive in the three sampling places, while manganese was only found in P3 and cooper in P1 and P2. In August, the levels of copper, total phosphorus, and total solids were positive again in P1, P2, and P3, whereas zinc and manganese were only found in place P1. Although the presence in the samples, most of these parameter's results are below the values recommended by CONAMA. However, aluminum was the only one that exceeded the recommended values established by CONAMA, being observed in some places, values ten times higher than the recommended limit.

Table 1. Physicochemical parameters result of the collected samples. Font: Oliveira et al. (2020).

Parameters	November 2018			January 2019			April 2019			August 2019			Measurement unit	Recommended values CONAMA
	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3		
Al	0,92	1,08	<LQ	0,84	<LQ	1,90	1,50	1,41	1,42	1,02	0,97	1,03	mg L^{-1}	0,1 mg L^{-1}
Pb	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	mg L^{-1}	0,01 mg L^{-1}
Cu	<LQ	<LQ	<LQ	0,005	0,003	0,002	0,002	0,001	<LQ	0,001	0,001	0,002	mg L^{-1}	0,009 mg L^{-1}
Total Cr	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	mg L^{-1}	0,05 mg L^{-1}
BOD_5	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	mg L^{-1} O2	3 mg L^{-1} O2
COD	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	mg L^{-1}	**
Total P	0,13	0,12	0,08	0,06	0,06	0,10	0,05	0,07	0,08	0,09	0,08	0,09	mg L^{-1}	0,1 mg L^{-1}
Mg	0,064	0,082	<LQ	0,079	0,099	0,11	<LQ	<LQ	0,06	0,07	<LQ	<LQ	mg L^{-1}	0,1 mg L^{-1}
Ni	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	mg L^{-1}	0,025 mg L^{-1}

NH_3	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	mg L^{-1} N	3,7 mg L^{-1} N
Total solids	60	74	60	79,5	72,5	113,5	107,0	101,5	94,5	147,5	123,5	123	mg L^{-1}	500 mg L^{-1}
Zn	0,014	<LQ	0,014	<LQ	<LQ	0,017	0,023	0,010	0,013	0,018	<LQ	<LQ	mg L^{-1}	0,18 mg L^{-1}

<LQ = Below the limit quantification; ** = Without recommended values by CONAMA.

It should be noted that Al is abundant in the environment. It is used in different areas such as the construction industry, food manufacturing, medicine, cosmetics, paints, and water treatment as coagulating agents like in the place P2 (Oliveira, 2016). The Al may be present because of the sewage release with no treatment, disposal of solid waste close to the river, and primarily because of the typical soil composition of the region (Dalzochio et al., 2018).

Caenorhabditis elegans model evaluation

According to the survival evaluation in *C. elegans* model (Figure 2), it was not observed significant differences between the samples collected and the control group (p = 0.810). A study by Anderson, Boyd & Williams (2001) showed that the survival parameters for toxicological evaluation in the *C. elegans* model are from 25 to 100 times less sensitive compared to other parameters, such as the development evaluation of the nematodes (Anderson, Boyd & Williams, 2001).

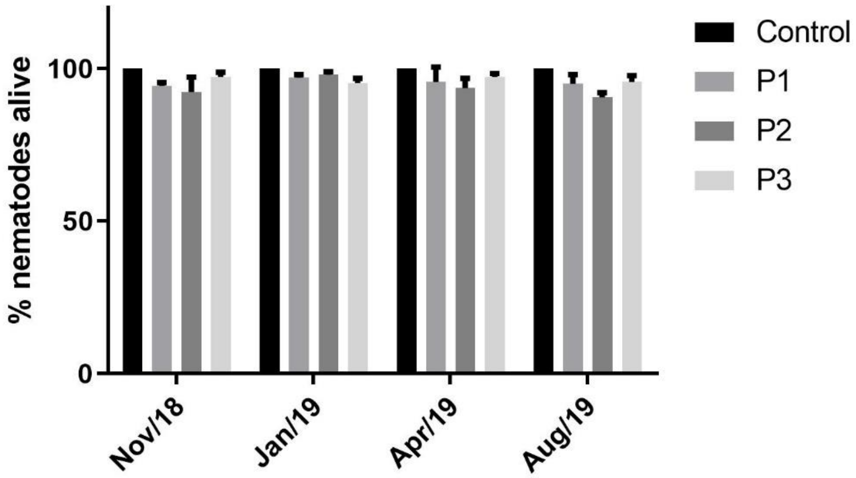

Figure 2. Nematode *Caenorhabditis elegans* survival after water treatment collected in November of 2018, January of 2019, April of 2019, and August of 2019. Results expressed as mean ± standard deviation. % concerning the control group. Font: Oliveira et al. (2020).

In this way, the development evaluation, verified by the body surface area measurement of the nematodes, showed a significant difference compared to the control group (p = 0,0001) in all collected periods and each collected place (Figure 3).

According to the obtained results, it was possible to verify values of the body surface area of 22 ± 5 µm² to the control group, while P1 presented 18 ± 4 µm², P2 18 ± 5 µm² and P3 19 ± 4 µm² for the samplings in November 2018. In the sampling of January of 2019, the body average of the control group was 20 ± 2 µm² whereas P1 presented values of 15 ± 3 µm², P2 14 ± 3 µm², and P3 15 ± 3 µm². In the sampling of April of 2019, the average values observed were 21 ± 4 µm² to control group, while P1 presented values of 15 ± 3 µm², P2 14 ± 3 µm², and P3 15 ± 3 µm². Finally, the sampling of August of 2019 denoted a body average in the control group of 22 ± 5 µm², in the place P1 the average was 17 ± 5 µm², P2 18 ± 4 µm², and P3 18 ± 3 µm².

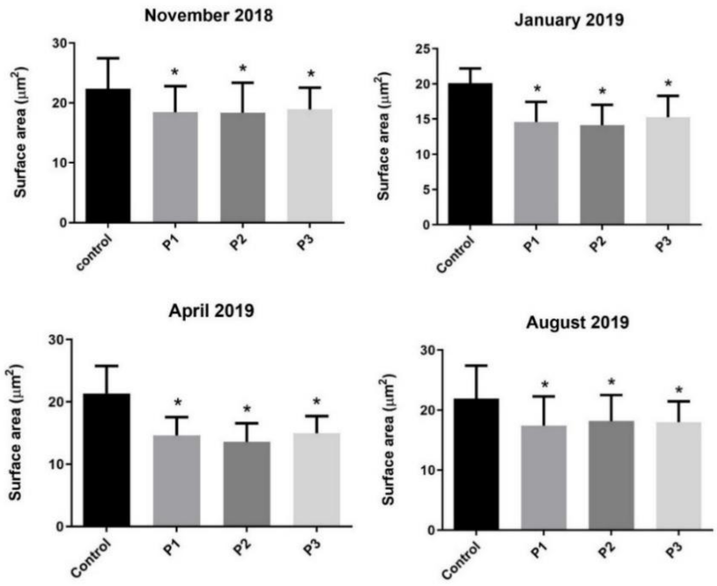

Figure 3. *Caenorhabditis elegans* body surface area after treatment with waters collected in November of 2018, January of 2019, April of 2019, and August of 2019. *p < 0,001 compared to control group. Font: Oliveira et al. (2020).

Although the development data in *C. elegans* demonstrated differences between the control group and all the sampling periods, indicating that the exposition to the water caused toxic effects in the nematode, it was not possible to establish a significant distinction between the three sampling places.

When compared the development of each place among the sampling periods (Figure 4), we observed that in November and August, the nematode development was less affected, with significant differences to January and April in P1, P2, and P3 (p<0,0001).

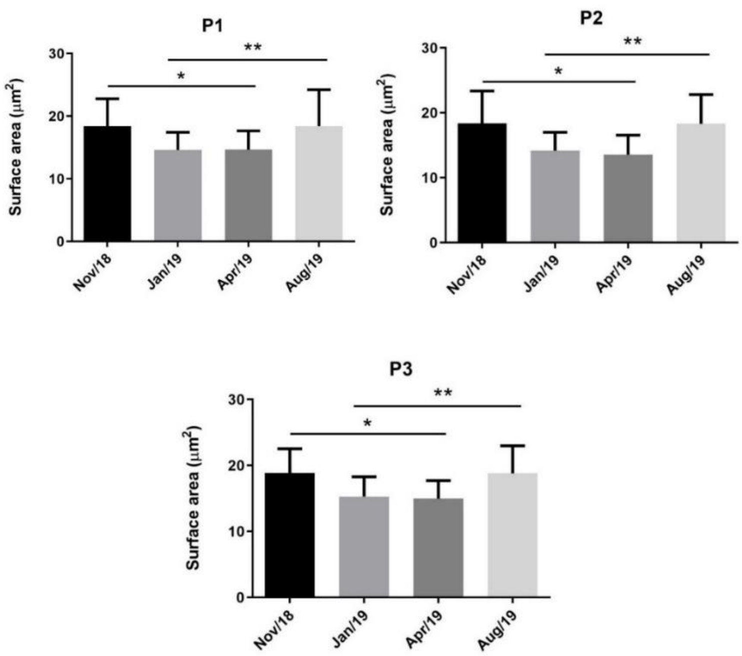

Figure 4. *Caenorhabditis elegans* body surface area compared to each point between the sampling places. *p < 0,0001 difference to the sampling of November of 2018. ** p < 0,0001 difference to the sampling of August. Font: Oliveira et al. (2020).

The intermediate section of the focal Sinos River research, it's a region that receives water contribution from the Ilha River and Paranhana River, that is why it has many distinct characteristics, such as low populational density and presence of agricultural activities (P1 and P2), upper population density and higher population density of the urban and industrials effluents (P3) (Dalzochio et al., 2018). The rain has important participation in the movement of the chemicals present in the ground, which can impact the pollutants concentration in the water (Dalzochio et al., 2018). The precipitation graphic in Figure 5 showed that the rainfall rates were lower in November of 2018 (Nov/18: 10 mm; Jan/19: 40 mm; Apr/19: 46,6 mm; Aug/19: 42 mm). In this way, it's possible to infer that the low toxicologic effects in November are because of the small rainfall rates observed in this period, reducing the movement of these stressful substances to the sampling places.

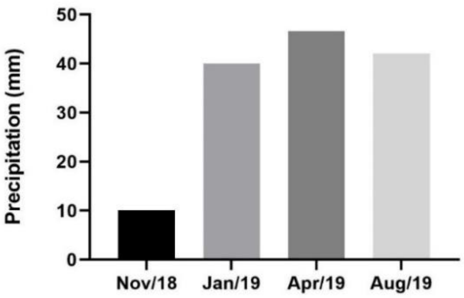

Figure 5. Total graphic of the total precipitation (mm) refers to the three sampling places accumulated 10 days before sampling in Sinos River in the fourth period, November of 2018, January of 2019, April of 2019, and August of 2019. Font: Oliveira et al. (2020).

The study region has as agricultural activity of rice, peanut, and corn plantation, which grows during September till November and the harvesting until the beginning of March, and during all this period there is an application of pesticides (IBGE, 2017). As observed, August presented fewer toxic effects, compared to January and April, coinciding with the period when there is no pesticides application in the region. However, we cannot assume for sure the effects of the pesticides on the *C. elegans* development because it was not possible to quantify these contaminants. Although most physicochemical parameters have submitted values according to the recommended by CONAMA, it was possible to determine the importance of using the nematode *C. elegans* as an integrative tool in the water quality assessment. The evaluation of a bioindicator is recognized as an environmental monitoring technique because it shows biological information (vegetable and animal) about the pollutants assessment on the environmental study (Lins, 2010).

Some species are used to evaluate and monitor water quality; within them, algae, microcrustaceans, and fishes stand out (Magalhães & Ferrão-Filho, 2008). A study by Dalzochio et al. (2017) evaluated the metals bioaccumulation in native fishes of places close to the study sites of this article and verified a compromised water quality through metals concentration evaluation in the fishes' muscles of the *B. iheringii* species.

Authors showed that the free-living nematode, *C. elegans*, can be employed as a bioindicator organism in the ground and underwater environments (Leung et al., 2008; Xing, Guo &Wang, 2009).

It has already been described that when there are stressful factors in the environment, a growth regulator triggers, and the nematode stops his development as a self-protection mechanism. Tepper et al. (2013) developed a study that describes the DAF-16 gene as a development regulator and nematode lifespan. After exposure to a stressful factor, DAF-16 is activated, and growth and development are suspended. In this way, for this model, this is a great mark parameter for toxicity evaluation. Even though Al is a portion of the rocky components in the region, beyond the recommended values, it is toxic and can lead to damages, not only for human beings but also to general biota, resulting in an unbalanced ecosystem (Tepper et al., 2013).

Clavijo et al. (2016) have done an experiment using the same experimental model to evaluate the water quality of the Rio Tunuyán watershed in the Mendonza province (Argentine), and it was verified through multivariate statistical analysis that even without major changes on physicochemical and bacteriological parameters evaluated, for water analysis the *C. elegans* was considered sensitive. This demonstrate that the nematode could identify the biological toxicity impact even when exposed to low pollutants doses or to a complex mixture of them that is not possible to reproduce inside a laboratory. They affirm that traditional studies to water quality assessment are not capable of preventing the potentially toxic effects in living organisms (Clavijo et al., 2016).

An experiment by Höss et al. (2001) evaluated the toxicity of cadmium in the *C. elegans* model with samples of sediments and water of the Starnberg Lake (German) using as parameters the nematode development. The authors verified that a positive correlation between the analyzed sediments and water and the model development concluded that it is a great bioindicator to environmental toxicological analysis (Höss et al., 2001).

There are others endpoint tests that can be used to evaluate the toxicological effects in *C. elegans*. However, according to Leung et al. (2008) development and behavior tests are more sensitive than the others like survival rate and brood size (Leung et al., 2008).

Recently, Brucker et al. (2021) evaluated different bioassays to assess the toxicity of drinking water collected in a rural area located in Brazil. The samplings were made in the period pre- and pos- application of pesticides. Considering the *C. elegans* model, it was observed changes in the development of the nematodes after treatment with water samples, indicating that C. elegans could be a complementary tool for monitoring the toxicity of drinking water (Brucker et al., 2021).

Similar study was conducted by Kuhn et al. (2021) in Uruguay River, located in Brazil. The authors used *C. elegans* as a bioassay to evaluate the quality and ecological safety of water before and after pesticides application in the region. Corroborating with our results, the authors did not find alteration in physicochemical parameters, however, it was observed toxic effects in C. elegans. These results reinforce the need for bioindicators in water quality assessment and ecotoxicological safety (Kuhn et al., 2021).

Studies report that the alternative model *C. elegans* can determine the biological toxicity impact even in a low pollutant dose or even with a complex mixture present in water that other methods cannot detect, being neglected (Clavijo et al., 2016; Brucker et al., 2021). Although most parts of the physicochemical parameters evaluated in this article are below recommended values, the water presented a toxic effect on the trial with the nematode. This demonstrated the importance of using bioindicators integrated to physicochemical analyses to evaluate the water quality. The response of bioassays can evidence possible adverse effects of pollutants presented in water in living organism. Moreover, the bioindicators are important tools to evaluate environmental hydric scenarios.

Conclusion

Even with the water quality verification determined by the monitoring companies are satisfactory, it was possible observe a toxic effect in *C. elegans*. This study reinforces the necessity of an integrated analysis, including a toxicology evaluation, intending to cover multiple stressful factors that contribute to the degradation and alteration of the ecological state of water. In this way, the bioassay with *C. elegans* turns out to be a great tool for the evaluation of water quality and environmental hydric scenarios.

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References

American Society for Testing and Materials (ASTM) E2172-01. 2014. Standard Guide for Conducting Laboratory Soil Toxicity Tests with the Nematode *Caenorhabditis elegans*. ASTM International, West Conshohocken, PA.

Anderson, G. L.; Boyd, W. A.; Williams, P. L. 2001. Assessment of sublethal endpoints for toxicity testing with the nematode *Caenorhabditis elegans*. Environmental Toxicology and Chemistry, 20, 833-838. https://doi.org/10.1002/etc.5620200419.

Ávila, D. S.; Somlyai, G.; Somlyai, I.; Aschner M. 2012. Anti-aging of deuterium depletion on Mn-induced toxicity in a *C. elegans* model. Toxicology Letters, 211, 319-324. https://doi.org/10.1016/j.toxlet.2012.04.014" \t "_blank.

Blume, K. K.; Macedo, J. C.; Meneguzzi, A.; Silva, L. B.; Quevedo, D. M.; Rodrigues, M. A. S. 2010. Water quality assessment of the Sinos River, Southern Brazil. Brazil Journal of Biology, 70, 1185-1193. https://doi.org/10.1590/s1519-69842010000600008.

Brandão, C. J.; Botelho, M. J. C.; Sato, M. I. Z.; Lamparelli, M. C. 2011. Guia Nacional de

Coleta e Preservação de Amostras: Água, Sedimento, Comunidades Aquáticas e Efluentes Líquidos. Companhia Ambiental do Estado de São Paulo e Agência Nacional de Águas, Brasília, Distrito Federal, BR.

Brenner, S. 1974. The genetics of *Caenorhabditis elegans*. Genetics, 77, 71-94. https://doi.org/10.1093/genetics/77.1.71.

Brucker, N.; Menezes, C.; Charão, M. F.; Silva, L. C.; Oliveira, T. S. S.; Menezes, J. M.; Muller, I.; Gioda, A.; Carvalho, B. R. F.; Calheiros, O. C. P.; Rizzetti, T. M.; Zanella, R.; Garcia, S. C. 2021. Bioassays to screen the toxicity in drinking water samples collected in Brazilian rural area. Toxicology Research, 10, 856-867. https://doi.org/10.1093/toxres/tfab072.

Cassanego, M. B. B.; Sasamori, M. H.; Petry, C. T.; Droste, A. 2015. Biomonitoring the genotoxic potential of the air on *Tradescantia pallida var. purpurea* under climatic conditions in the Sinos River basin, Rio Grande do Sul, Brazil. Brazilian Journal of Biology, 75, 79-87. https//doi.org/10.1590/1519-6984.05514.

Cassanego, M. B. B.; Droste, A. 2017. Avaliação do padrão espacial da qualidade da água de um rio no Sul do Brasil por meio da análise multivariada de indicadores biológico e químicos. Brazilian Journal of Biology, 77, 118-126. https://doi.org/10.1590/1519-6984.11215.

Charão, M. F.; Souto, C.; Brucker, N.; Barth, A.; Jornada, D. S.; Fagundez, D.; Ávila, D. S.; Eifler-Lima, V. L.; Guterres, S. S.; Pohlmann, A. R.; Garcia S. C. 2015. *Caenorhabditis elegans* as an alternative in vivo model to determine oral uptake, nanotoxicity, and efficacy of melatonin-loaded lipid-core nanocapsules on paraquat damage. International Journal of Nanomedicine, 50, 5093-5106. https://doi.org/10.2147/IJN.S84909.

Clavijo, A.; Kronberg, M. F.; Rossen, A.; Moya, A.; Calvo, D.; Salatino, S. E.; Pagano, E. A.; Morábito, J. A.; Minarriz, E. R. 2016. The nematode *Caenorhabditis elegans* as an integrated toxicological tool to assess water quality and pollution. Science of The Total Environmental, 569, 252-261. https://doi.org/10.1016/j.scitotenv.2016.06.057.

Conselho Nacional do Meio Ambiente (CONAMA). 2005. Resolução n. 357 de 03/2005. Available at: http://pnqa.ana.gov.br/Publicacao/RESOLUCAO_CONAMA_n_357.pdf. Access at: June 9, 2018.

Dalzochio, T.; Rodrigues, P. Z. G.; Simões, R. A. L.; Souza, S. M.; Petry, E. I.; Andriguetti, B. N.; Silva, H. J. G.; Silva, B. L.; Gehlen, G. 2018. In situ monitoring of the Sinos River, southern Brazil: water quality parameters, biomarkers, and metal bioaccumulation in fish. Environmental Science and Pollution Research International, 25, 9485-9500. https://doi.org/10.1007/s11356-018-1244-7.

Dalzochio, T.; Simões, R. L. A.; Souza, S. M.; Rodrigues, P. Z. G.; Petry, I. E.; Andriguetti, B. N.; Silva, H. J. G.; Gehlen, G. Silva, B. L. 2017. Water quality parameters, biomarkers and metal bioaccumulation in native fish captured in the Ilha river, southern Brazil. Chemosphere, 189, 609-618. https://doi.org/10.1016/j.chemosphere.2017.09.089.

Hoss, S.; Ahlf, W.; Bergtold, M.; Bluebaum-Gronau, E.; Brinke, M.; Donnevert, G.; Menzel, R.; Möhlenkmp, C.; Ratte, H. T.; Traunspurger, W.; von Danwitz, B.; Pluta, H. J. 2012. Interlaboratory comparison of a standardized toxicity test using the nematode *Caenorhabditis elegans* (ISO 10872). Society of Environmental Toxicology and Chemistry, 31, 1525-1535. https://doi.org/10.1002/etc.1843.

Höss, S.; Henschel, T.; Haitzer, M.; Traunspurger, W.; Steinberg, W. E. C. 2001. Toxicity of cadmium to *Caenorhabiditis elegans* (Nematoda) in whole sediment and porewater – The ambiguous role of organic matter. Environmental Toxicology and Chemistry, 20, 2794-2801. https://doi.org/10.1002/etc.5620201219.

Instituto Brasileiro de Geografia e Estatística (IBGE). 2017. Available at: https://www.ibge.gov.br/. Access June 9, 2018.

Instituto Nacional de Meteorologia (INMET). 2019. Available at: https://portal.inmet.gov.br/. Access at: 9 July, 2018.

International Organization for Standarization (ISO). 2010. ISO 10872: 2010. Water Quality - determination of the Toxic Effect of Sediment and Soil Samples on Growth, Fertility and Reproduction of *Caenorhabditis elegans* (Nematoda). Geneva, Switzerland.

Kaletta, T.; Hengartner, M. 2006. Finding function in a novel targets: *C. elegans* as a model organism. Nature Reviews Drug Discovery, 5, 387-398. https://doi.org/10.1038/nrd2031.

Kuhn, E. C.; Jacques, M. T.; Teixeira, D.; Meyer, S.; Gralha, T.; Roehrs, R.; Camargo, S.; Schwerdtle, T.; Bornhorst, J.; Ávila, D. S. 2021. Ecotoxicological assessment of Uruguay River and affluents pre- and post-pesticides' application using *Caenorhabditis elegans* for biomonitoring. Environmental Science and Pollution Research International, 28, 21730-21741. https://doi.org/10.1007/s11356-020-11986-4

Lemos, A. C. C. 2014. Análise geológico-geomorfológica da Bacia Hidrográfica do Rio Paranhana/RS para o reconhecimento de ambientes vulneráveis. Dissertação de Mestrado, Universidade Federal do Rio Grande do Sul. Porto Alegre, Rio Grande do Sul, Brasil. 102p.

Leung, M.; Williams, P. L.; Benedetto, A.; Au, C.; Helmcke, J. K.; Aschner, M.; Meyer, N. J. 2008. *Caenorhabditis elegans:* An Emerging Model in Biomedical and Environmental Toxicology. Toxicological Sciences, 106, 5-28. https://doi.org/10.1093/toxsci/kfn121.

Linden, R.; Antunes, V. M.; Heinzelmann, L. S.; Fleck, J. D.; Staggemeier, R.; Fabres, R. B.; Vecchia, A. D.; Nascimento, C. A.; Spilki, F. R. 2015. Caffeine as an indicator of human fecal contamination in the Sinos River: a preliminary study. Brazilian Journal of Biology, 75, 81-84. https://doi.org/10.1590/1519-6984.0513.

Lins, J. A.; Kirschnik, P. G.; Queiroz, V. S.; Cirio, S. M. 2010. Uso de peixes como biomarcadores para monitoramento ambiental aquático. Revista Acadêmica Ciência Animal, 8, 469-484. https//doi.org/10.7213/cienciaanimal.v8i4.11018.

Magalhães, D. P.; Ferrão-Filho, A. S. 2008. A ecotoxicologia como ferramenta no biomonitoramento de ecossistemas aquáticos. Oecologia Brasiliensis, 12, 355-381.
https://www.arca.fiocruz.br/handle/icict/27395.

Marengoni, N. G.; Klosowski, E. S.; Oliveira, K. P.; Chambo, A. P. S.; Gonçalves Júnior, A. C. 2013. Bioacumulação de metais pesados e nutrientes no mexilhão dourado do reservatório da usina hidrelétrica de Itaipu binacional. Química Nova, 36, 359-363. https://doi.org/10.1590/S0100-40422013000300002.

Nascimento, S.; Baierle, M.; Göethel, G.; Barth, A.; Brucker, N.; Charão, M.; Sauer, E.; Gauer, B.; Arbo, M. D.; Altknecht, M.; Dias, A. C. G.; Salles, J. F.; Sain'Pierre, T.; Gioda, A.; Moresco, R.; Garcia, S. C. 2016. Associations among environmental exposure to manganese, neuropsychological performance, oxidative damage and kidney biomarkers in children. Environmental Research, 147, 32-43. https://doi.org/10.1016/j.envres.2016.01.035.

Oliveira, V. E. 2016. Potencial neurotóxico do alumínio como modelo da doença de Alzheimer em *Caenorhabditis elegans*. Dissertação de Mestrado, Universidade Federal da Fronteira Sul, Erechim, Rio Grande do Sul, Brasil. 50p.

Peredney, C. L.; Williams, P. L. 2000. Utility of *Caenorhabditis elegans* for assessing heavy metal contamination in artificial soil. Archives of Environmental Contamination and Toxicology, 39, 113-118. https://doi.org/10.1007/s002440010086.

Peteffi, G. P.; Fleck, J. D.; Kael, I. M.; Rosa, D. C.; Antunes, M. V.; Linden, R. 2019. Ecotoxicological risk assessment due to the presence of bisphenol A and caffeine in surface waters in the Sinos River Basin - Rio Grande do Sul-Brazil. Brazilian Journal of Biology, 79, 712-721. https://doi.org/10.1590/1519-6984.189752.

Rice, E. W.; Baird, R. B.; Eaton, A. D. 2017. Standard methods for the examination of water and wastewater. American Public Health Association, 23rd edition. 1496p.

Roubicek, D. A.; Rech, C. M.; Umbuzeiro, G. A. 2020. Mutagenicity as a parameter in surface water monitoring programs-opportunity for water quality improvement. Environmental and molecular mutagenesis, 61, 200-211. https://doi.org/10.1002/em.22316.

Stefanello, S. T.; Gubert, P.; Puntel, B.; Mizdal, R. C.; Campos, A. M. M.; Salman, M. S.; Dornelles, L.; Ávila, D. S.; Aschner, M.; Soares, A. A. F. 2015. Protective effects of novel organic selenium compounds against oxidative stress in the nematode *Caenorhabditis elegans*. Toxicology Reports, 61, 961-967. https://doi.org/10.1016/j.toxrep.2015.06.010.

Steffens, C.; Klauck, R. C.; Benvenuti, T.; Silva, B. L.; Rodrigues, S. A. M. 2015. Water quality assessment of the Sinos River – RS, Brazil. Brazilian Journal of Biology, 75, 62-67. https://doi.org/10.1590/1519-6984.01613suppl.

Stiernagle, T. 2006. Maintenance of *C. elegans*. In: Chalfie, M. The *C. elegans* Research Community. [eds.]. WormBook. doi/10.1895/wormbook.1.7.1.

Tepper, R. G.; Ashraf, J.; Kaletsky, R.; Kleemann, G.; Murphy, T. C.; Bussemaker, J. H. 2013. PQM-1 complements DAF-16 as a key transcriptional regulator of DAF-2-mediated development and longevity. Cell, 154, 676-690. https://doi.org/10.1016/j.cell.2013.07.006.

Xing, X.; Guo, Y.; Wang, D. 2009. Using the larvae nematode *Caenorhabditis elegan*s to evaluate neurobehavioral toxicity to metallic salts. Ecotoxicology and. Environmental Safety, 72, 1819-1823. https://doi.org/10.1016/j.ecoenv.2009.06.006.