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Original Article

Evaluation of Water Quality in Streams Under Strong Influence of Sugarcane Cultivation Using the *Allium cepa* Test

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Abstract

The Ivinhema River Basin is affected by the expansion of sugarcane cultivation areas, which impacts the water quality of its streams. The aim of this study was to evaluate the cytotoxic, genotoxic, and mutagenic potential of water samples from three streams of the Ivinhema River Basin using the *Allium cepa* test. Sampling was conducted in March 2017. Surface water samples collected from the Piravevê, Vitória, and Rosário streams induced cytotoxic and genotoxic effects in *A. cepa*. Reductions in both germination and cell division were observed, which may have been related to the flow of toxic residues from the sugarcane monoculture into the stream's waters. Therefore, there is an urgent need to develop restoration projects aimed at conserving the water quality and biodiversity in these aquatic environments.

Keywords: Ivinhema River Basin, Paraná River Basin, cytotoxicity, genotoxicity, mutagenicity, meristematic cells.

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INTRODUCTION

Agriculture is considered one of the main anthropogenic activities that negatively impacts water quality worldwide (Martíni *et al*., 2021). The increase in agricultural activities in Brazil has caused concern because of its negative environmental impacts, including deforestation and the destruction of natural habitats (Gonino *et al*., 2019; Benvindo-Souza *et al.,* 2022). Brazil is one of the largest producers of sugarcane and ethanol (FAO, 2020; Tomei *et al*., 2020). Despite providing raw materials for the production of biofuels, which are an important renewable source of energy, sugarcane cultivation is considered one of the main sources of pollution in Brazil (Gonino *et al*., 2019; Kumar *et al.*, 2022). The application of pesticides such as atrazine, fipronil, thiamethoxam, sulfentrazone, simazine, ametrine, clomazone, diuron, hexazinone, metribuzin, and pendimethalin is common in agricultural areas occupied by sugarcane monocultures (Correia *et al.,* 2013; Araldi *et al.,* 2015; Fontanetti & Bueno, 2017; Trovato *et al.,* 2020). The high consumption of pesticides in sugarcane crops can affect several non-target organisms, including native plants and animals, in addition to negatively affecting the structure and dynamics of natural ecosystems (Ogura *et al.,* 2022). In the Midwestern region of Brazil, the State of Mato Grosso do Sul is considered an epicenter of sugarcane production, a crop for which high amounts of pesticides and fertilizers are used (Tomei *et al.,* 2020; Ogura *et al.,* 2022). In 2016, sugarcane production accounted for approximately 2% of the total production in the State of Mato Grosso do Sul (Tomei *et al*., 2020).

The Paraná River Basin, considered the second largest and most important hydrographic basin in South America, has recently undergone a significant expansion of sugarcane cultivation, causing fragmentation and loss of habitats, in addition to the degradation of water quality (Gonino *et al.,* 2019). In particular, the Ivinhema River Basin is one of the regions with the greatest expansion in sugarcane cultivation, in addition to the installation of new industrial plants for the production of sugar, ethanol, and bioenergy (Ferreira & Silva, 2016).

The contamination of several water bodies in the Upper Paraná River Basin by metals and pesticides resulting from the runoff of agricultural activities and industrial and urban effluents has damaged aquatic life (Peluso *et al*., 2021). Investigation of water toxicity in the aquatic environments of the Paraná River Basin is essential to assess its environmental quality, as its water resources are used for various purposes, especially irrigation (Mato Grosso do Sul, 2015). When agricultural crops are irrigated with water contaminated by pesticides, including those used in sugarcane production, phytotoxic effects such as reduced germination and growth can occur in numerous plant species of economic interest (Ogura *et al*., 2022). In addition, aquatic ecosystems near sugarcane cultivation areas are becoming increasingly fragile and degraded because of the runoff of various types of toxic contaminants, which can lead to the loss of local biodiversity (Jayawardena *et al*., 2021).

The *A. cepa* test is widely used and is recommended by several environmental regulatory agencies for the assessment of phytotoxicity and genotoxicity in aquatic environments monitoring studies (Leme & Marin-Moraes, 2009; Garcia-Mediana *et al*., 2020; Kumar *et al*., 2022). This test allows the evaluation of phytotoxic and genotoxic endpoints, such as alterations in seed germination, root development, and cell cycle interference, in addition to alterations in the genome of plant cells (Gameiro *et al*., 2020). Furthermore, the *A. cepa* test has a high sensitivity for a wide range of pure chemicals or mixtures of chemicals (Leme & Marin-Moraes, 2009; Kumar *et al.,* 2022). Leme & Marin-Morales (2009) and Gallego *et al*. (2021) demonstrated that a test using *A. cepa* yielded satisfactory results in the evaluation of various pollutants.

Thus, the objective of this study was to evaluate the cytotoxic, genotoxic, and mutagenic potential of water samples from three streams under the strong influence of sugarcane cultures belonging to the Ivinhema River Basin, which is located in the Upper Paraná River Basin, using the *A. cepa* test. The hypothesis of this study is that the increase in areas occupied by sugarcane monoculture caused an increase in cytotoxic, genotoxic, and mutagenic potential in *A. cepa* exposed to water samples from streams located in the surroundings of these agricultural areas.

MATERIALS AND METHODS

Sampling sites

The Ivinhema River Basin, the second largest hydrographic basin in the State of Mato Grosso do Sul, covers an area of approximately 46,688 km^2 (Fortes *et al*., 2005; Mato Grosso do Sul, 2015). This has ecological significance because the Ivinhema River floodplains are located in priority areas for environmental conservation and are part of the ecological corridor that connects the Pantanal and Serra da Bodoquena with the Paraná River (Pott *et al.,* 2014). This basin is located in the Várzeas do Rio Ivinhema State Park (PEVRI), an important integral conservation unit (Dos Santos *et al.,* 2020). However, although the Ivinhema River Basin provides important ecosystem services, it is located in a region with intense agricultural activities, especially cattle ranching and sugarcane monoculture, which are the most predominant crops in the basin (Mato Grosso do Sul, 2015; Dos Santos *et al.,* 2020). Water samples were collected during March 2017 from the Piravevê, Vitória, and Rosário streams, all belonging to the lower portion of the Ivinhema River Basin, which is located in the Upper Paraná River Basin, in the Brazilian State of Mato Grosso do Sul (Mato Grosso do Sul, 2015) (Figure 1). It is important to highlight that all streams had reduced native riparian vegetation. The banks of the Rosário and Vitória streams are predominantly agricultural areas with an emphasis on sugarcane monoculture cultivation. The banks of the Piravevê stream are predominantly occupied by pasture areas. The Rosário stream is approximately 1.5 m wide and the Vitória stream is approximately 5.5 m wide, characterizing both as second order streams, whereas the width of the sampled section in Piravevê ranges 7–15 m, characterizing it as a third order stream.

Physicochemical parameters

The physicochemical parameters dissolved oxygen (mg L^{-1}), hydrogen ionic potential (pH), electrical conductivity (µS cm^{-1}), and temperature (ºC), were determined using a Horiba multiparameter probe previously calibrated directly in the waters from sampling sites at Piravevê, Vitória, and Rosário streams.

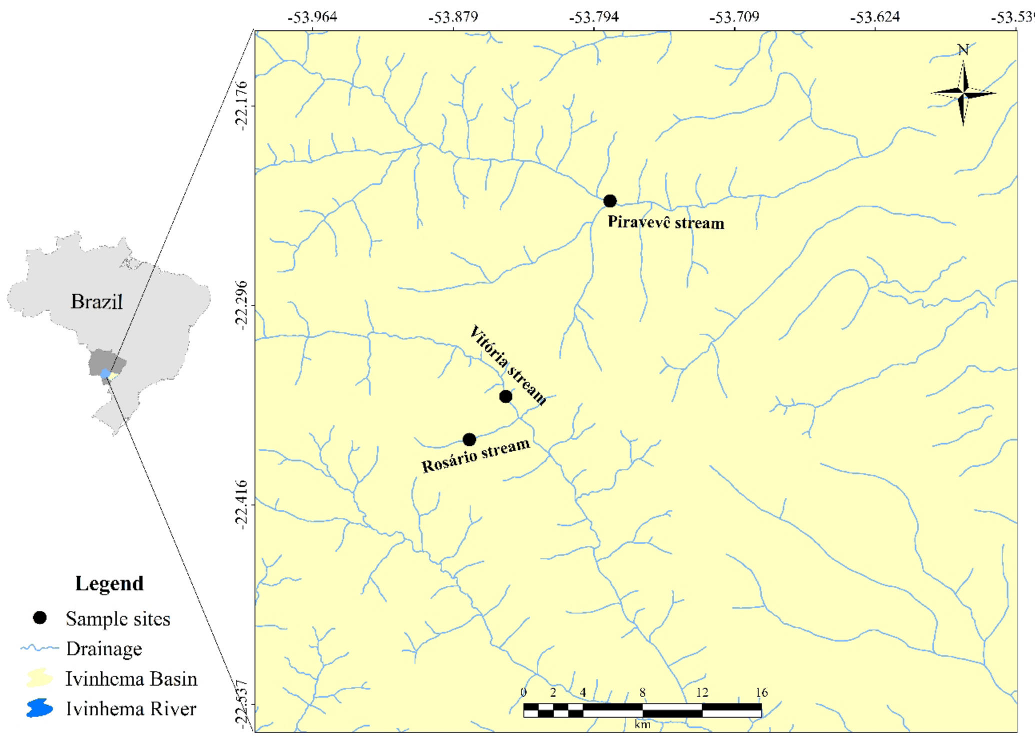

Figure 1. Location of the sampling sites in Piravevê, Vitória, and Rosário streams, Ivinhema River Basin, Mato Grosso do Sul, Brazil.

Allium cepa test

Aliquots (50 mL) of water were collected in previously decontaminated and sterilized polyethylene bottles to a depth of approximately 5 cm below the surface, transported, stored at room temperature, and then taken to the laboratory for assays.

Seeds of the *A. cepa* were sown in approximately 3 mL of each water sample. The herbicide trifluralin (0.84 mg L^{-1}) was used as positive control (PC), and distilled water was used as negative control (NC). All tests were performed with 30 seeds per plate in triplicate. These seeds were left to germinate in temperature of 23 ± 3 °C for a period of 96 h, according to the procedure adapted from Leme & Marin-Morales (2009). After germination, the seeds were counted manually, and the roots were measured with digital calipers (DigMess). The roots were collected and fixed in Carnoy's solution (3:1 absolute ethanol:glacial acetic acid, v/v). Then, the roots were hydrolyzed using HCl (1 mol L^{-1}), washed with distilled water, and stained with Schiff's reagent overnight. After staining, the root meristems were covered with a coverslip and slightly crushed in a drop of 45% acetic carmine. Ten slides of the root meristem were prepared for each treatment, and 500 cells per slide were analyzed using an optical microscope (Nikon Eclipse, E200) at a magnification of 400×, totaling 5000 cells per treatment. Cytotoxicity was determined based on the percentage germination (GR), relative mean root growth (RRG), mitotic index (MI), and cell death rate (CDR). Genotoxicity was investigated by evaluating chromosomal alterations in *A. cepa* root meristematic cells and determined using the chromosomal alteration index (CAI). Specifically, mutagenicity was evaluated by counting the micronuclei in the meristematic cells to determine the mutagenicity index (MTI). The indices were calculated using the formulae described by Francisco *et al*. (2018).

Statistical analysis

Normality was verified using the Shapiro-Wilk test. Kruskal-Wallis nonparametric test was applied with Dunn's post hoc test ($p \leq 0.05$) was used to evaluate the cytotoxicity, genotoxicity, and mutagenicity in *A. cepa* cells. A heat map was generated using the "pheatmap" package, being a grouping color-coded by specific streams, in relation to the cytotoxicity, genotoxicity, and mutagenicity endpoints obtained in the *A. cepa* tests. The greater the color intensity of the squares, the greater the cytotoxicity and genotoxicity. All analyses were performed using the R platform (R Development Core Team, 2021).

RESULTS AND DISCUSSION

Physicochemical parameters

Among the physicochemical parameters evaluated in the three streams, only the pH showed values that did not comply with the Brazilian legislation (Brasil, 2005), for Class II freshwater bodies, at sampling sites Vitória (5.08 ± 0.72) and Rosário (5.00 ± 0.47). The acidic pH observed in both streams may be related to intense agricultural activities in their surroundings. In sugarcane monocultures, different types of fertilizers are widely used for soil preparation in addition to fertigation with vinasse. Some of these products can be transported to streams, causing severe risks and damage to aquatic life (Christofoletti *et al.*, 2013). Notably, the indiscriminate use of vinasse in the fertigation process has been identified as the cause of acidity in aquatic environments, which has had a serious impact on the maintenance of local biodiversity (Ogura *et al.*, 2022). In the three streams, the dissolved oxygen values ranged 6.09–8.00 mg L^{-1}, the electrical conductivity of the water ranged 18.50–44.00 µS cm^{-1}, and the temperature ranged 26.14–27.11 °C.

Allium cepa test

The water samples collected from the three streams significantly inhibited the germination of *A. cepa* seeds compared to the NC ($p < 0.05$) (Table 1 and Figure 2). The lowest germination percentage (GR) was induced by the water sample collected in the Piravevê and Rosário streams (Table 1 and Figure 2). The mean relative root growth (RRG) parameters did not differ significantly for any of the samples or the NC group ($p > 0.05$) (Table 1 and Figure 2).

Considering the MI, the water samples from the three streams caused a decrease in cell division in *A. cepa* root meristem cells, with significant differences ($p < 0.05$) in NC. No difference in the CDR was found between streams, and low frequencies of cell death were observed in all water samples. Regarding the CAI, the water samples from all streams differed from the NC ($p < 0.05$). The CAI did not differ between the Piravevê and Vitória streams ($p > 0.05$). No significant differences ($p > 0.05$) were observed in the MTI (Table 1 and Figure 2).

All the water samples significantly inhibited the germination of *A. cepa* seeds. Mitosis was negatively affected in samples collected at all sampling sites. The water sample from the Rosário stream induced a significant increase ($p < 0.05$) in CAI compared with the NC. These results demonstrate that the waters of these three streams were cytotoxic and genotoxic to *A. cepa*. However, none of the water samples exhibited mutagenic effects (Table 1 and Figure 2). The inhibition of germination, decrease in MI, and increase in CAI values indicate the presence of cytotoxic and genotoxic chemicals in the water from the three sampling sites. The MI

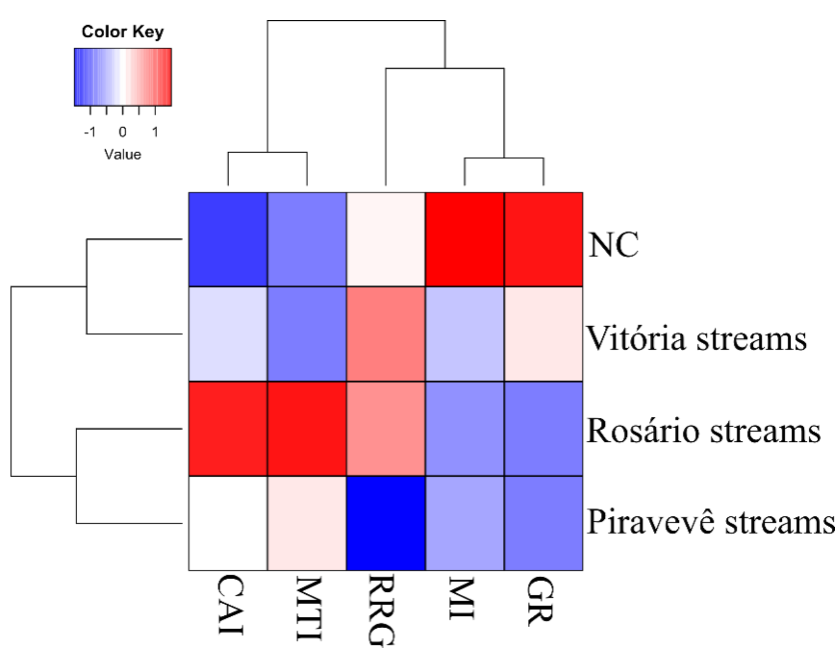

Figure 2. Hierarchical clustering of mean cytotoxicity and genotoxicity values obtained in tests with *A. cepa* induced by water samples collected from Rosário, Piravevê, and Vitória streams, belonging to the Ivinhema River Basin, Mato Grosso do Sul, Brazil.

Table 1. Cytotoxicity and genotoxicity in *A. cepa* (median|interquartile shift) induced by water samples collected in Rosário, Piravevê, and Vitória streams, belonging to the Ivinhema River Basin, Mato Grosso do Sul, Brazil.

Effects	Parameter	NC	Rosário	Piravevê	Vitória	PC
Citotoxicity	GR (%)	100.00|0.00a	57.14|2.68b	57.14|9.82b	75.00|7.14c	71.43|4.46
	RRG (cm)	5.28|1.36a	6.07|2.34a	3.19|1.05a	6.17|1.59a	2.83|0.86
	MI (%)	80.09|5.66a	11.17|10.45b	15.08|4.44b	20.84|3.03b	17.90|6.76
	CDR (%)	0.00|0.02a	0.00|0.00a	0.00|0.00a	0.00|0.00a	0.00|0.03
Genotoxicity	CAI (%)	0.29|0.29a	5.42|5.05bc	2.73|0.82b	2.23|1.25b	5.83|2.02
Mutagenicity	MTI (%)	0.00|0.00a	0.34|0.07a	0.14|0.07a	0.00|0.00a	0.17|0.00

% GR: germination; RRG: relative mean root growth; MI: mitotic index; CDR: cell death rate; CAI: Chromosomal alteration index; MTI: mutagenicity index; NC: negative control; PC: positive control. Different letters represent statistically significant difference.

is an indicator of the cytotoxic potential of several substances that can inhibit or increase cell proliferation (Leme & Marin-Morales, 2009; Francisco *et al*., 2018). In contrast, chromosomal alterations measured using CAI indicate the presence of clastogenic and aneugenic agents responsible for genotoxicity (Athanásio *et al.*, 2014). Athanásio *et al.* (2014) reported a decrease in MI and an increase in CAI in *A. cepa* cells after exposure to water samples from urban streams that received mixed industrial and urban wastewater discharge and runoff from contaminated agricultural areas. Ogura *et al.* (2022) highlighted the importance of phytotoxic effects on several plant species resulting from pesticide residues used in agricultural crops. Garcia *et al.* (2017) observed that the agricultural residues of sugarcane, mainly vinasse, cause genotoxicity in *A. cepa* by inducing the formation of nuclear buds, anaphasic bridges, micronuclei, chromosome loss, and chromosome breakage.

In addition, chemical contaminants present in aquatic environments that inhibit plant growth can cause impairment in relation to the use of these waters for irrigation of agricultural crops (Yadav *et al*., 2019; Ebo Yahans Amuah *et al.,* 2022). Ebo Yahans Amuah *et al*. (2022) reported that the use of contaminated water for the irrigation of agricultural crops is a major problem worldwide, as it affects both productivity and public health. Alberti *et al*. (2022) reported that approximately 70% of the world's freshwater is used for agriculture and that its contamination is a significant global problem. It is important to highlight that approximately 97% of the water extraction in the Ivinhema River Basin, where the streams of the study are located, is used for irrigation activities, mainly for the cultivation of sugarcane (Mato Grosso do Sul, 2015). Thus, the cytotoxic and genotoxic alterations induced by chemical contaminants observed in the test with *A. cepa* may be associated with the flow of toxic waste resulting from the expansion of agricultural activities in its surroundings. These chemicals can enter and contaminate water through direct application, runoff, and atmospheric deposition.

CONCLUSION

Water samples from the three streams induced cytotoxicity and genotoxicity in the meristematic cells of *A. cepa*, as indicated by a decrease in cell division and an increase in chromosomal alterations. This damage may be associated with the ingress of chemical residues into streams, resulting from the expansion of agricultural areas intended mainly for sugarcane monoculture. The surroundings of the sampling sites were not influenced by urban areas, supporting the initial hypothesis of the study. Water pollution with toxic and genotoxic chemicals can make its use for the irrigation of agricultural areas unfeasible, in addition to compromising the safety of the native biota. Therefore, we emphasize the need to develop environmental monitoring programs for water bodies to conserve these important water resources.

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CREDIT AUTHOR STATEMENT

EMSS: Data collect, investigation and writing-original draft. **LFV**: Conceptualization the study, data collect, analysis the results, preparation of the location map and writing-reviewing and editing. **BAC**: Conceptualization the study, data collect, methodology, investigation, produced the results, writing reviewing, and editing. **LFVF**: Methodology, produced the results and writing-original draft. **YRS**: Data collect and writing-reviewing and editing. **FK, AB** and **APB**: Writing-reviewing and editing. All Authors revised the paper.

CONFLICTS OF INTEREST

Authors declare that there are no conflicts of interest.

REFERENCES

Alberti, M. A., Blanco, I., Vox, G., Scarascia-Mugnozza, G., Schettini, E., & Pimentel da Silva, L. (2022). The challenge of urban food production and sustainable water use: Current situation and future perspectives of the urban agriculture in Brazil and Italy. *Sustainable Cities and Society*, 83, 103961. https://doi.org/10.1016/j.scs.2022.103961

Araldi, R., Velini, E. D., Gomes, G. L. G. C., Tropaldi, L., Silva, I. P. F., & Carbonari, C. A. (2015) Performance of herbicides in sugarcane straw. *Ciência Rural*, 45(12), 2106-2112. http://dx.doi.org/10.1590/0103-8478cr20141244

Athanásio, C. G., Prá, D., & Rieger, A. (2014). Water quality of urban streams: the *Allium cepa* seeds/seedlings test as a tool for surface water monitoring. *The Scientific World Journal*, 2014, 391367. https://doi.org/10.1155/2014/391367

Benvindo-Souza, M., Hosokawa, A. V., dos Santos, C. G. A., de Assis, R. A. Pedroso, T. M. A., Borges, R. E., Pacheco, S. M., de Souza Santos, L. R., & de Melo e Silva, D. (2022). Evaluation of genotoxicity in bat species found on agricultural landscapes of the Cerrado savanna, central Brazil. *Environmental Pollution*, 293, 118579. https://doi.org/10.1016/j.envpol.2021.118579

Brasil. (2005). Conselho Nacional do Meio Ambiente (CONAMA) - Resolução no 357 de 17 março de 2005. http://www2.mma.gov.br/port/conama/legiabre.cfm?codlegi=459 Accessed date November 10, 2022.

Christofoletti, C. A., Escher, J. P., Correia, J. E., Marinho, J. F. U., & Fontanetti, C. S. (2013). Sugarcane vinasse: environmental implications of its use. *Waste Management,* 33(12), 2752-2761. http://dx.doi.org/10.1016/j.wasman.2013.09.005

Correia, N. M., Camilo, E. H., & Santos, E. A. (2013). Sulfentrazone efficiency on *Ipomoea hederifolia* and *Ipomoea quamoclit* as influenced by rain and sugarcane straw. *Planta Daninha*, 31(1), 165-174. http://dx.doi.org/10.1590/S0100-83582013000100018

Dos Santos, S. L., Viana, L. F., Merey, F. M., Crispim, B. A., Solorzano, J. C., Barufatti, A., Cardoso, C. A. L., & Lima-Junior, S. E. (2020). Evaluation of the water quality in a conservation unit in Central-West Brazil: Metals concentrations and genotoxicity *in situ*. *Chemosphere*, 251, 126365. https://doi.org/10.1016/j.chemosphere.2020.126365

Ebo Yahans Amuah, E., Amanin-Ennin, P., & Antwi, K. (2022). Irrigation water quality in Ghana and associated implications on vegetables and public health. A systematic review. *Journal of Hydrology*, 604, 127211. https://doi.org/10.1016/j.jhydrol.2021.127211

FAO. Food and Agriculture Organization of the United Nations. (2020) FAOSTAT, Agri-environmental Indicators/Pesticides.

Ferreira, P. S., & Silva, C. A. (2016) A expansão da cana-de-açúcar na bacia hidrográfica do Rio Brilhante, Mato Grosso do Sul: o uso da técnica de NDVI como instrumento para evidenciar dinâmicas territoriais. *Geografares*, 22, 6681. https://doi.org/10.7147/GEO23.12340

Fontanetti, C. S., & Bueno, O. C. (2017). Cana-de-açúcar e seus impactos: uma visão acadêmica. Bauru: Canal 6. Bauru, SP:275 p. ; 23 cm. ISBN 978-85-7917-421-6. https://www.canal6.com.br/livros_loja/Ebook_Cana.pdf?_ga=2.169904224.1767696250.1675787056-854537533.1675787056&_gl=1*jh4nb1*_ga*ODU0NTM3NTMzLjE2NzU3ODcwNTY.*_ga_LTS0RDE5Z8*MTY3NTc4NzA1Ni4xLjEuMTY3NTc4NzA1Ny4wLjAuMA. Accessed date November 10, 2022.

Fortes, E., Stevaux, J. C., & Volkmer, S. (2005). Neotectonics and channel evolution of the Lower Ivinhema River: A right-bank tributary of the upper Paraná River, Brazil. *Geomorphology*, 70(3–4), 325–338. https://doi.org/10.1016/j.geomorph.2005.02.011

Francisco, L. F. V., Crispim, B. A., Viana, L. F., Nascimento, H. D. S., Raposo Junior, J. L., & Grisolia, A. B. (2018). Cytotoxicity, genotoxicity and mutagenicity of aluminum, manganese and lead in meristematic cells of root *Allium cepa*. *Orbital: the electronic journal of chemistry*, 10(1), 60–65. http://dx.doi.org/10.17807/orbital.v10i1.1037

Gallego, J. L., & Olivero-Verbel, J. (2021). Cytogenetic toxicity from pesticide and trace element mixtures in soils used for conventional and organic crops of *Allium cepa* L. *Environmental Pollution*, 276, 116558. https://doi.org/10.1016/j.envpol.2021.116558

Garcia, C. F. H., Souza, R. B., de Souza, C. P., Christofoletti, C. A., & Fontanetti, C. S. (2017). Toxicity of two effluents from agricultural activity: Comparing the genotoxicity of sugar cane and orange vinasse. *Ecotoxicology and Environmental Safety,* 142, 216-221. https://doi.org/10.1016/j.ecoenv.2017.03.053

García-Medina, S., Galar-Martínez, M., Gómez-Oliván, L. M., Torres-Bezaury, R. M. D. C. Islas-Flores, H., & Gasca-Pérez, E. (2020). The relationship between cyto-genotoxic damage and oxidative stress produced by emerging pollutants on a bioindicator organism (*Allium cepa*): The carbamazepine case. *Chemosphere*, 253, 126675. https://doi.org/10.1016/j.chemosphere.2020.126675

Gameiro, P. H., Assis, K. H., Hasenack, H., Arenzon, A., Dias Silva, K. U., Torres de Lemos, C., & Ferrão Vargas, V. M. (2020). Evaluation of effect of hazardous contaminants in areas for the abstraction of drinking water. *Environmental Research*, 188, 109862. https://doi.org/10.1016/j.envres.2020.109862

Gonino, G. M. R., Figueiredo, B. R. S., Manetta, G. I., Zaia Alves, G. H., & Benedito, E. (2019). Fire increases the productivity of sugarcane, but it also generates ashes that negatively affect native fish species in aquatic systems. *Science of The Total Environment,* 664, 215–221. https://doi.org/10.1016/j.scitotenv.2019.02.022

Jayawardena, U. A., Wickramasinghe, D. D., & Udagama, P. V. (2021). Cytogenotoxicity evaluation of a heavy metal mixture, detected in a polluted urban wetland: Micronucleus and comet induction in the Indian green frog (*Euphlyctis hexadactylus*) erythrocytes and the *Allium cepa* bioassay. *Chemosphere,* 277, 130278. https://doi.org/10.1016/j.chemosphere.2021.130278

Kumar, V., Ameen, F., Islam, M. A., Agrawal, S., Motghare, A., Dey, A., Shah, M. P., Américo-Pinheiro, J. H. P., Singh, S., & Ramamurthy, P. C. (2022). Evaluation of cytotoxicity and genotoxicity effects of refractory pollutants of untreated and biomethanated distillery effluent using *Allium cepa*. *Environmental Pollution,* 300, 118975. https://doi.org/10.1016/j.envpol.2022.118975

Leme, D. M., & Marin-Morales, M. A. (2009). *Allium cepa* test in environmental monitoring: a review on its application. *Mutation Research/Reviews in Mutation Research*, 682(1), 71-81. https://doi.org/10.1016/j.mrrev.2009.06.002

Martíni, A. F., Favaretto, N., de Bona, F. D., Durães, M. F., Souza, L. C. P. & Goularte, G. D. (2021). Impacts of soil use and management on water quality in agricultural watersheds in Southern Brazil. *Land Degradation & Development*, 32(2), 975-992. https://doi.org/10.1002/ldr.3777

Mato Grosso do Sul. (2015). Instituto do Meio Ambiente de Mato Grosso do Sul/ IMASUL, SEMAC. Plano de recursos hídricos da bacia hidrográfica do Rio Ivinhema. Campo Grande: IMASUL, 2015. Accessed date November 10 2022.

Ogura, A. P., Moreira, R. A., da Silva, L. C. M., Negro, G. S., Freitas, J. S., da Silva Pinto, T. J., Lopes, L. F. P., Yoshii, M. P. C., Goulart, B. V., Montagner, C. C., & Espíndola, E. L. G. (2022). Irrigation with Water Contaminated by Sugarcane Pesticides and Vinasse Can Inhibit Seed Germination and Crops Initial Growth. *Archives of Environmental Contamination and Toxicology*, 82(3), 330-340. https://doi.org/10.1007/s00244-022-00914-x

Peluso, J., Pérez Coll, C. S., Cristos, D., Rojas, D. E., & Aronzon, C. M. (2021). Comprehensive assessment of water quality through different approaches: Physicochemical and ecotoxicological parameters. *Science of The Total Environment,* 800, 149510. https://doi.org/10.1016/j.scitotenv.2021.149510

Pott, A., Silva, J. S. V., & Gomes, E. L. (2014). Características da Bacia Hidrográfica do Rio Ivinhema. *Revista GeoPantanal*, 16,109-124.

R Core Team (2021) R: a language and environment for statistical computing. R foundation for statistical computing, Vienna. http://www.R-project.org. Accessed date November 10, 2022.

Tomei, J., Lyrio de Oliveira, L., de Oliveira Ribeiro, C., Lee Ho, L., & Montoya, L. G. (2020). Assessing the relationship between sugarcane expansion and human development at the municipal level: A case study of Mato Grosso do Sul, Brazil. *Biomass and Bioenergy*, 141, 105700. https://doi.org/10.1016/j.biombioe.2020.105700

Trovato, V. W., Portilho, I. I. R., Barizon, R. R. M., & Scorza Júnior, R. P. (2020). Herbicide runoff from a soil with different levels of sugarcane straw coverage in Brazil. *Ecotoxicology and Environmenatl Contamination*, 15(1), 25-35. http://dx.doi.org/10.5132/eec.2020.01.04

Yadav, A., Raj, A., Purchase, D., Ferreira, L. F. R., Saratale, G. D., & Bharagava, R. N. (2019). Phytotoxicity, cytotoxicity and genotoxicity evaluation of organic and inorganic pollutants rich tannery wastewater from a Common Effluent Treatment Plant (CETP) in Unnao district, India using *Vigna radiata* and *Allium cepa. Chemosphere*, 224, 324-332. https://doi.org/10.1016/j.chemosphere.2019.02.124