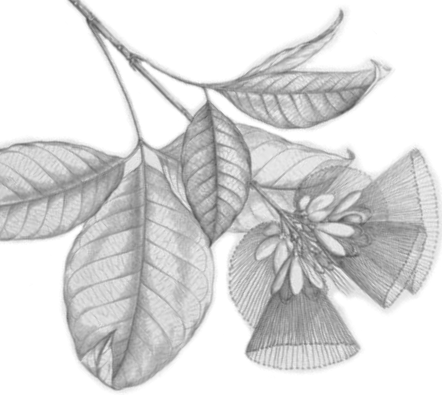

Urban Occupation Increases Water Toxicity of an Important River in Central Brazil

Elisa Flávia Luiz Cardoso Bailão [1]
Leciana de Menezes Sousa Zago [2]
Nathan Carvalho Silva [3]
Karine Borges Machado [4]
Patrícia Lima D'Abadia [5]
Pedro Henrique Francisco de Oliveira [6]
João Carlos Nabout [7]
Luciane Madureira de Almeida [8]

ABSTRACT

Meia Ponte River supplies water for two million people in Goiás State, Brazil. Despite its importance, the Meia Ponte River faces serious environmental problems such as the disposal of domestic and industrial effluents, what could impact the aquatic biota and the health of people who consumes its water. In this sense, here we aimed to evaluate the environmental quality and toxicity of surface water along the course of this river. Physicochemical analyses of water at Goiânia urban perimeter were higher than the limits of Brazil environmental regulations for fresh water. In relation to the diversity of species, phytoplankton classes associated to polluted environments were detected closer to urban perimeter. *Allium cepa* bioassay suggested that this river may contain substances with mitogenic activity.

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[1] Doutorado em Patologia Molecular pela Universidade de Brasília, UnB, Brasil; com período sanduíche em University at Buffalo. Docente na Universidade Estadual de Goiás, UEG, Brasil. https://orcid.org/0000-0001-6737-7548. elisaflavia@gmail.com

[2] Doutorado em andamento em Recursos Naturais do Cerrado pela Universidade Estadual de Goiás, UEG, Brasil. Mestrado em Recursos Naturais do Cerrado pela Universidade Estadual de Goiás, UEG, Brasil. https://orcid.org/0000-0001-8027-982X. lecianazago@hotmail.com

[3] Mestrado em andamento em Recursos Naturais do Cerrado pela Universidade Estadual de Goiás, UEG, Brasil. Docente na Faculdade Metropolitana de Anápolis, FAMA, Brasil. https://orcid.org/0000-0003-3061-3900. carvalho_nathan@hotmail.com

[4] Doutorado em Ecologia e Evolução pela Universidade Federal de Goiás, UFG, Brasil. Bolsista DTI-B na Universidade Federal de Goiás, UFG, Brasil. https://orcid.org/0000-0003-2744-7964. machadokb@gmail.com

[5] Doutorado em andamento em Recursos Naturais do Cerrado pela Universidade Estadual de Goiás, UEG, Brasil.
Mestrado em Recursos Naturais do Cerrado pela Universidade Estadual de Goiás, UEG, Brasil. https://orcid.org/0000-0003-4756-4869. patricialima.bio@gmail.com

[6] Mestrado em andamento em Ecologia e Evolução pela Universidade Federal de Goiás, UFG, Brasil. https://orcid.org/0000-0003-0681-3574. oliveira.pdroh@gmail.com

[7] Doutorado em Ciências Ambientais pela Universidade Federal de Goiás, UFG, Brasil. Docente na Universidade Estadual de Goiás, UEG, Brasil. https://orcid.org/0000-0001-9102-3627. joao.nabout@ueg.br

[8] Doutorado em Biociências pela Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP, Brasil. Docente na Universidade Estadual de Goiás, UEG, Brasil. https://orcid.org/0000-0003-1764-1480. almeidalm@hotmail.com

This result is in accordance with genotoxic analysis, because it was observed a significant increase in chromosomal aberrations. This data reveal the genotoxic potential of Meia Ponte River water. This genotoxicity represents a risk for aquatic biota and humans, once the genotoxic agents in water samples might cause the loss of DNA integrity, inducing damages and DNA breaks. In this context, the water utilization from Meia Ponte River without any treatment should be avoided and public policies need to be formulated and implemented to depollute this important river for Goiás State.

Keywords: Chromosomal Aberrations; Cytogenotoxicity; Meia Ponte River; Phytoplankton.

In recent decades the exponential growth of human populations and urban centers have impacted aquatic (Naiman and Turner 2000) and terrestrial (Wu et al. 2011) ecosystems. Anthropogenic activities such as emissions of domestic and industrial sewage, as well as chemical residues from agricultural activities, have caused profound alterations in the physicochemical characteristics of aquatic environments (Costa et al. 2015). Residues from industrial processes, domestic activities and agricultural practices, which are released into river basins, contain toxic substances such as metals, dyes, oils, emulsifiers, caustic soda, solvents, pesticides and organic and inorganic salts that can cause a serious problem for the health of the biota and humans that interact with these aquatic ecosystems (Mathur et al. 2007). As a result, reductions in water quality, losses of aquatic biodiversity, and changes in biological processes have been observed (Carvalho and Siqueira 2011; Costa et al. 2015).

Physicochemical factors such as pH, temperature, turbidity, electrical conductivity and dissolved oxygen are determined *in loco* to determine the water quality destined for human consumption. In Brazil, the Brazilian National Environment Council (Conselho Nacional do Meio Ambiente – CONAMA) on its Statutory Instrument 357/2005 (CONAMA 2005) establishes guidelines for framing water bodies, based on the quality levels that water should possess to meet community needs, health and human well-being, and the aquatic ecological balance. Characterization of the effluents using conventional physicochemical parameters alone may not be adequate for hazard assessments, considering the potential interactions that may occur among the complex chemical mixtures in the effluents (Pathiratne, Hemachandra, and De Silva 2015).

In that way, the phytoplankton community can help to qualify and quantify changes in water quality, applicable over large geographic areas and can also furnish data on background conditions and natural variability (Lee 1999). The micro algal diversity responds rapidly to environmental perturbations and is suitable bio-indicators of water condition (Onyema 2007).

In addition, bioassays can help in the environmental monitoring. Bioassays produce an integrated response to the complex mixtures of chemicals without prior knowledge on its chemical composition and its properties, which allow the exposition of the test organism directly to complex mixtures without previous treatment of the test sample (Rank and Nielsen 1993). Importance of incorporating mutagenicity/genotoxicity assays as additional parameters in risk assessments has been emphasized (Ohe, Watanabe, and Wakabayashi 2004). Nevertheless, adequate attention has not yet been given in many countries to test the mutagenicity/genotoxicity of the complex chemical mixtures found in domestic and industrial sewage.

Allium cepa bioassay has been frequently used in environmental monitoring and in the evaluation of cytotoxicity and mutagenicity of compounds present in environmental samples (Vujošević et al. 2008; Costa et al. 2015). It is very useful as a first analysis, because of its simplicity, low relative cost, versatility and minimum laboratory facilities required for its performance (Leme and Marin-Morales 2009; Ribeiro et al. 2016). Toxic effects are evaluated by analyses of the changes in the size and morphology of the onion roots, as well as the cell division rate and chromosomal changes. The results obtained with this test allow evaluating the risk of the compounds present in the environment for living organisms (Fiskesjö 1993). Accordingly, this test has been validated as efficient for genetic monitoring by the United Nations Environmental Program (UNEP), the World Health Organization (WHO) and the US Environmental Protection Agency (USEPA).

The Meia Ponte River is one of the most important rivers in the state of Goiás, Brazil. The Meia Ponte River Basin is located in the central-south region of the State of Goiás, in the central region of Brazil and is important for being responsible for the water supply of several cities (SANEAGO 2009). The Meia Ponte basin supplies water for 02 million people, fifty percent of the population of Goiás state, and a large part of the economy depends on it (World Heritage Encyclopedia 2002). Also, the river is used for various purposes, such as: water supply, irrigation of crops, supply of animal drinking water, and leisure. Despite its importance, the Meia Ponte River faces serious environmental problems due to unplanned urban development in the municipalities of the Meia Ponte Basin, the disposal of domestic and industrial effluents, and illegal solid waste disposal. This river receives municipal wastewater from the most populous cities of the state: Goiânia, Aparecida de Goiânia, and Anápolis. For example, the city of Goiânia discards over 90% of its untreated sewage into the river (World Heritage Encyclopedia 2002). In addition, the river receives industrial and agricultural effluents from anthropogenic activities (SANEAGO 2009; Carvalho and Siqueira 2011). In this context, this study aimed to evaluate the environmental quality and toxicity of surface water collected at five sampling points along the course of the Meia Ponte River. In this evaluation, in addition to environmental factors such as pH, temperature, conductivity and dissolved oxygen, the biological community was also analyzed. Cytogenotoxic potential of water samples were also measured using the *A. cepa* bioassay.

MATERIAL AND METHODS

STUDY AREA

Meia Ponte River is located in the Southern Central region of the state of Goiás, Brazil. The source of the river is located in Serra dos Brandões (16° 8' 1.82" S 49° 32' 55.24" W), in the municipality of Itauçu, about 60 km from Goiânia, the capital of the state of Goiás. Its extension is approximately 470 km and the mouth of the river is located in the municipality of Cachoeira Dourada de Goiás (18º 29' 41.7" S 49º 28' 25.8" W). The drainage area of the Meia Ponte basin is approximately 12,343 km^2, including 37 municipalities. Meia Ponte River is classified according to the Brazilian laws (CONAMA 2005), as class 02 river (intended for human consumption, agriculture, recreation and fishing activity).

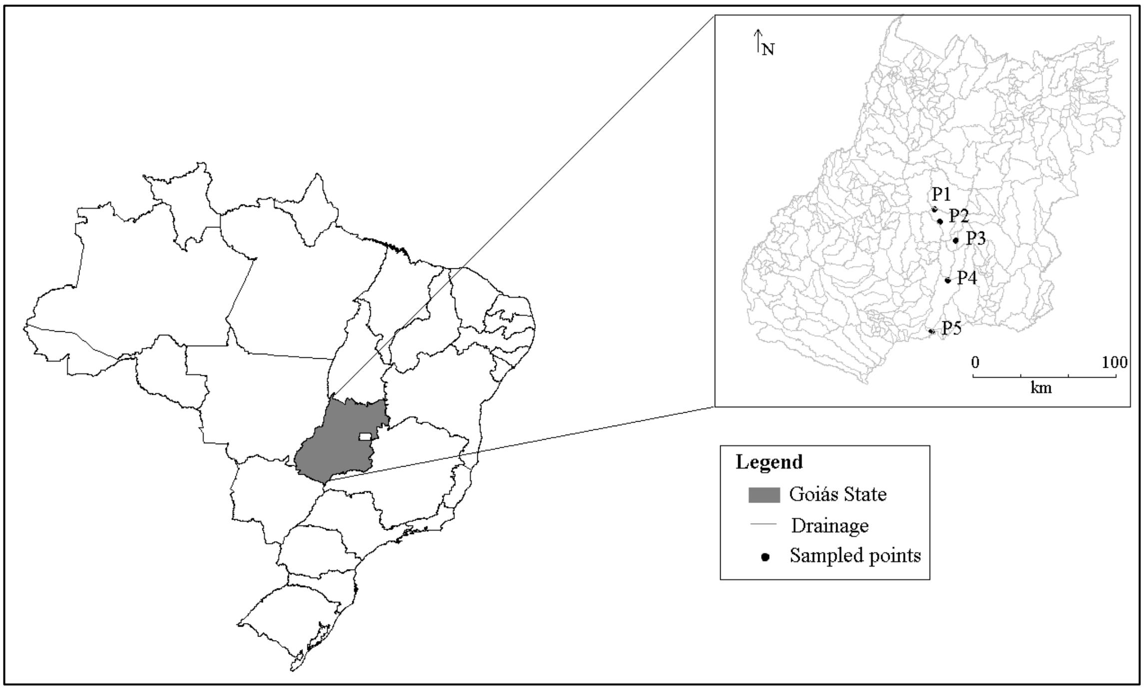
COLLECTION POINTS

Samples were collected at five locations along the Meia Ponte River in May 2016, a dry period in the area. Two liters of surface water were collected in polyethylene bottles at each sampling point. The containers were adequately labeled and kept under refrigeration (-4 °C) for further analysis. During sample collection, water temperature (ºC), electrical conductivity ($\mu s/cm^{-1}$), dissolved oxygen ($mg/l\,O_2$), turbidity (NTU) and pH were obtained using a multiparameter probe (Digimed). The sampling points were chosen based on their environmental characteristics (Figure 01). Point 1 (P1) is located in the municipality of Itauçu and is located at the source of the river (16° 8' 1.82" S 49° 32' 55.24" W), which is surrounded by native Cerrado vegetation. Point 2 (P2) is located in the rural area of the municipality of Inhumas, about 3.4 km beyond the urban area (16° 23' 17.17" S 49° 27' 16.18" W). In this region, residents of the municipality of Inhumas and neighboring cities use the river for fishing and leisure. The landscape around the collection point is predominantly composed of pasture and areas of corn and soybean cultivation. Point 3 (P3) is located within the urban perimeter of Goiânia (16° 44' 30.08" S 49° 8' 36.31" W). This area is characterized by the presence of garbage in abundance. Point 4 (P4) is located in the municipality of Pontalina, Goiás (17° 30' 38.99" S 49° 18' 18.99" W). The vegetation on the banks of the river is dense and the adjacent areas are, for the most part, agricultural areas. Point 5 (P5) is located at the mouth of the river, in the municipality of Cachoeira Dourada, Goiás (18° 28' 37.90" S 49° 36' 54.25" W). The area presents dense ciliary forest and is surrounded by areas of grain and sugar cane cultivation.

Phytoplankton Community

Phytoplankton was collected on the subsurface water (0.5 m depth) in 100 ml dark bottles and the samples were fixed in situ with modified acetic lugol. Phytoplankton was counted using the sedimentation technique (Utermöhl 1958), using an inverted microscope (Zeiss Axiovert 25) at a magnification of 400X. The identification was performed, wherever possible, to the species level.

Figure 01. Locations of the water samples collected from Meia Ponte River, located in Goiás state, Brazil.



Point 1 (P1): 16° 8' 1.82" S 49° 32' 55.24" W; (P2): 16° 23' 17.17" S 49° 27' 16.18" W; (P3): 16° 44' 30.08" S 49° 8' 36.31" W; (P4): 17° 30' 39.16" S 49° 18' 19.93" W; and (P5): 18° 28' 37.90" S 49° 36' 54.25" W.
Source: The Authors.

TOXICITY TEST USING *ALLIUM CEPA* ASSAYS

Bulbs of the common onion (*A. cepa*) were purchase at a local supermarket and ten bulbs were prepared for each sample. The bulbs were grown in distilled water at room temperature for 03 days, after which some newly-formed root tips were cut from each bulb. The root-tip length was evaluated and only tips of 02-03 cm were selected for the experiment, the others were cut and discarded. Later the bulbs were treated with surface water collected in the Meia Ponte River. Another set of onion bulbs was placed in distilled water to be a negative control. After 48 h in the test solutions, the length of each root was measured and the root tips from each bulb were harvested and fixed in Carnoy's solution (1 acetic acid: 3 ethanol) for 24 h. Then, root-tip was stored in 70% ethanol. After treatments, the root tips were rinsed a few times with distilled water. They were hydrolyzed with hydrochloric acid solution (5 M) at room temperature for 20 min. After hydrolysis, the roots were dissected in acetic acid (45%) and squashed with a cover slip. The cover slips were removed after freezing in liquid nitrogen and stained with Giemsa (5%) for 05 min. The slides were evaluated using an LEICA Optical Microscope with 40 to 100 times magnification. In total, 1,000 cells were analyzed per slide, with a total of 5,000 cells per treatment. The cytotoxic potential was calculated through observation of the mitotic index (MI). The MI was calculated for each treatment using the number of dividing cells/total number of cells (Seth et al. 2008). The genotoxic potential was estimated by the frequency of anomalies in the mitotic cycle (AMC) and the incidence of micronuclei (MN). The groups were divided into five treatments; each containing five replications, and the values were measured through analysis of variance (ANOVA) and compared through Tukey post-hoc tests. $p < 0.05$ was considered as indicative of significance.

RESULTS

PHYSICOCHEMICAL PARAMETERS

Table 01. Physicochemical characteristics of the surface water collected on different locations from Meia Ponte River.

Samples	Temperature (ºC)	Turbidity (NTU)	Electrical conductivity (µs/cm)	Dissolved oxygen (mg/l O_2)	pH
P1	21.2	49.0	31.0	9.7	6.7
P2	22.3	21.9	141.3	14.9	7.3
P3	22.9	35.7	286.6	2.2*	7.2
P4	23.3	18.5	107.9	7.9	6.8
P5	23.8	18.3	89.3	11.0	7.2
Levels permitted by CONAMA	**---**	**≤ 100**	**---**	**≥ 5.0**	**6.0 – 9.0**

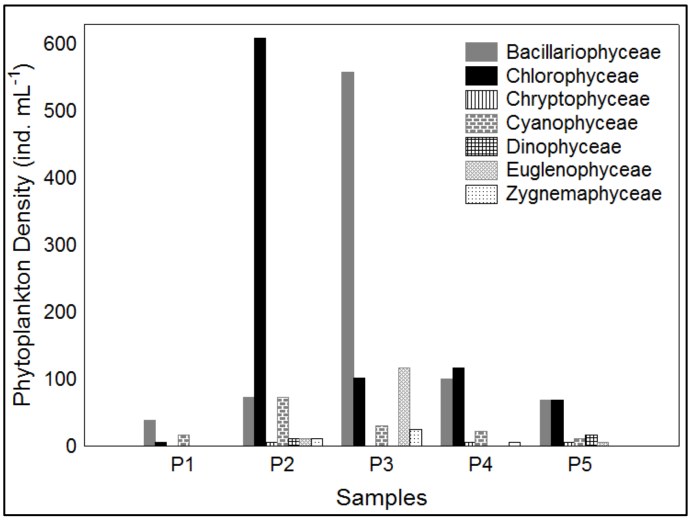
*values not allowed by CONAMA for class 2 water.
Source: The Authors.

The physicochemical analysis of temperature, turbidity and pH from all sampled points showed values within the parameters established by CONAMA for class 02 water (CONAMA 2005). However, the value of dissolved oxygen (DO) was different from that established by CONAMA at point P3. The values for electrical conductivity showed rates above 100 μS/cm^2 at points P2, P3 and P4 (Table 01).

PHYTOPLANKTON COMMUNITY ANALYSES

We found 50 phytoplankton species belonging to 07 taxonomic classes. Moreover, the species richness of phytoplankton was heterogeneous along the points (Figure 02). P2 and P3 presented moderated density. The other points presented low phytoplankton density. P2 was dominated by species of the Chlorophyceae class, with *Monoraphidium komarkovae* being the more representative species. This species is very common and is not used as a water quality indicator. In P3, there was a dominance of species of the Bacillariophyceae class, with *Eunotia sp2* being the most representative species. Species of the Cyanophyceae class registered in all sampled points of this study had low density and there are not register of toxicity in the literature for these species.

Figure 02. Density of phytoplankton species found by taxonomic class in Meia Ponte River.



Source: The Authors.

RIVER POINT'S TOXICITY

The water samples collected in all five points along the Meia Ponte River established in this work stimulated the growth of A. cepa roots in comparison to the negative control (Table 02). Corroborating the root length data, the mitotic index (MI) values for P1, P3 and P4 are significantly greater than the negative control ($p < 0.05$) (Table 02).

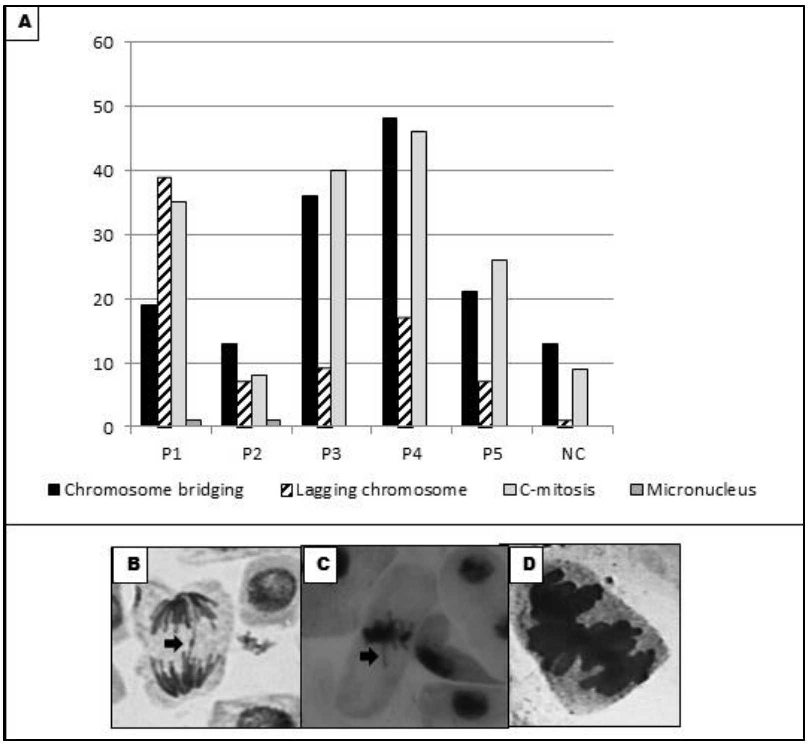
Table 02. Cytogenetic analysis of Allium cepa root tips exposed to different surface water collected from Meia Ponte river.

Samples	**Toxicity**	**Cytotoxicity**	**Genotoxicity**	
	Average root lenght ± SD (cm)	**MI (%)**	**CA**	**MN**
P1	23.7 ± 14.0	17.5*	140 b	1
P2	28 ± 12.8	2.4	38 a	1
P3	26.8 ± 11.2	27.9*	150 b	0
P4	26 ± 14.9	7.1*	149 bc	0
P5	35.7 ± 22.3*	4.7	74 b	0
NC	12.2 ± 6.0	3.9	33 a	0

NC: negative control;
*$p < 0.05$; same letter represents no significant difference using Tukey test.
Source: The Authors.

Chromosomal aberration frequency (CA) was estimated to evaluate the genotoxic potential of water samples. The results showed that CA frequencies have high incidence at P1, P3, P4 and P5 (Table 02). Among the different types of abnormalities observed (Figure 03), the most common were: lagging chromosome, c-mitosis, and chromosome bridging. Abnormalities were found more in P3 and P4.

Figure 03. Genotoxicity evaluation of Meia Ponte River water.



(A) Total number of chromosome aberrations and nuclear abnormalities on root tip cells of Allium cepa after treatment with different surface water from Meia Ponte river. **(B)** Chromosome bridge; **(C)** Lagging chromosome; and **(D)** c-mitosis.
Source: The Authors.

DISCUSSION

In different countries, regulatory limits have been established for selected water physicochemical parameters in an attempt to control the release of pollutants at harmful or dangerous levels for humans and other forms of life. Physicochemical parameters showed that the water of the Meia Ponte River is polluted and inadequate for human consumption at the urban perimeter of Goiânia (P3). In Brazil, according to resolution 357 of CONAMA, the amount of DO in the water must be equal or higher than 05 mg/l for water class 02 (CONAMA 2005). The importance of DO is related to the survival of aquatic organisms and water quality. For this reason, DO is used as an indicator of environmental pollution (Pinto, Oliveira, and Pereira 2010). Water contaminated by residues from anthropogenic activities tends to have reduced levels of DO. This occurs due to the consumption of the available oxygen in the water for respiration by bacteria decomposing organic matter (Sperling 2005). The decrease in DO has several implications for the environment, with a concentration value of 04 mg/l is usually the lower limit for fish tolerance and DO below 03 mg/l tends to be harmful to most aquatic vertebrates (Collischonn and Tassi 2008).

In addition, the result of the analysis of electrical conductivity of water collected in the urban perimeter (P3) is indicative of a high concentration of ions in the water. Although Resolution 357 of CONAMA does not establish limits for electrical conductivity, according to the Environmental Company of the State of São Paulo (CETESB 2009) levels above to 100 $\mu s/cm^{-1}$ are indicative of impacted environments. High values of electrical conductivity are indicative of high amount of solids in the water; these compounds come from industrial waste and domestic sewage(Gana et al. 2008). Carvalho and Siqueira (2011) carried out physicochemical analyzes of the water of the Meia Ponte River in the urban perimeter of Goiânia and verified that some parameters such as conductivity, true color, biochemical oxygen demand, DO, phosphorus content, nitrite and ammoniacal nitrogen were outside the standard required by resolution 357 of CONAMA (CONAMA 2005). The authors concluded that these changes are due to the discharge of numerous domestic and industrial effluents into the river (Carvalho and Siqueira 2011). The physicochemical data corroborates the phytoplankton species survey presented in this work, which highlights the predominance of the Bacillariophyceae class in P3. In fact, some species of Bacillariophyceae can indicate polluted environments (Lobo et al. 2016).

Because of the importance of water quality to health, many toxicity and genotoxicity tests have also been used in combination with these physicochemical analyses in order to evaluate both water and environmental quality (Egito et al. 2007). Plants are excellent bioindicators, because they present high sensitivity to detect cytotoxic and mutagenic agents through different genetic mechanisms, including cell cycle disorders and chromosomal aberrations (Matos et al. 2017). This way, regarding *A. cepa* bioassay, we observed a growth induction of all onion roots submitted to the Meia Ponte River water in relation to the negative control. This was expected since water from the Meia Ponte River has more organic materials and minerals than distilled water (Vujošević et al. 2008), as these compounds are removed during the distillation process.

In the cytotoxicity analysis, we observed changes in the normal pattern of cell division and mitotic index. Three sampling points (P1, P3 and P4) showed MI values higher than the negative control. The increase in MI at these points indicates that the water from the Meia Ponte River may contain substances with mitogenic activity, which can lead to uncontrolled cell proliferation (Leme and Marin-Morales 2009). Several studies have showed that changes in the pattern of cell division are related to the release of domestic, industrial and agricultural effluents into water bodies (Egito et al. 2007; Barbosa et al. 2010; Düsman et al. 2014; Costa et al. 2015). Pollutants rich in phosphorus and nitrogen, which are abundant in domestic sewage and agrochemicals, may cause mitosis stimulation (Düsman et al. 2014).

The genotoxicity analyses performed in this work showed that three samples (P1, P3 and P4) present a significant increase in chromosomal aberrations (CA). P2 showed a low CA frequency, perhaps because the number of cells in division was very low in comparison to the others points. Chromosome bridging, lagging chromosome and c-mitosis were the most common chromosomal abnormalities observed. All those aberrations may suggest genotoxic effects of Meia Ponte river water, mainly close to urban centers. In Brazil, 45% of the sewage is released into the water bodies without treatment. The chemical composition of effluents may cause DNA damage and, consequently, cause genotoxic effects. In this work, we showed that the surface water collected from the Meia Ponte River, mainly in P1, P3 and P4, is genotoxic and may result in diseases in the human population and generate environmental problems, such as: elimination of fish life stages; decrease in the number of aquatic species; cellular and tissue damage of aquatic organisms; reduction of swimming capacity of fish, and bioaccumulation (Bervoets et al. 2005; Barbosa et al. 2010; Moreno et al. 2014).

CONCLUSIONS

The results obtained in this work highlight the water toxicity of the Meia Ponte River, an important river for water supply in Goiás State. This study indicates the presence of mitogenic substances mainly at P1, P3 and P4, which represent a risk for aquatic biota and for humans. In this sense, the water utilization from Meia Ponte River without any treatment should be avoided. This work points to an urgent need to formulation and implementation of public policies to improve the water quality of this important river for Goiás State, such as the control of disposal of domestic and industrial sewage, as well as chemical residues from agricultural activities.

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Urban Occupation Increases Water Toxicity of an Important River in Central Brazil

Elisa Flávia Luiz Cardoso Bailão; Leciana de Menezes Sousa Zago; Nathan Carvalho Silva;
Karine Borges Machado; Patrícia Lima D'Abadia; Pedro Henrique Francisco de Oliveira;
João Carlos Nabout; Luciane Madureira de Almeida

Ocupação Urbana Aumenta a Toxicidade da Água de um Importante Rio no Brasil Central

RESUMO

O rio Meia Ponte fornece água para dois milhões de pessoas no estado de Goiás, Brasil. Apesar de sua importância, o rio Meia Ponte enfrenta sérios problemas ambientais, como o lançamento de efluentes domésticos e industriais, o que pode impactar a biota aquática e a saúde das pessoas que consomem sua água. Nesse sentido, neste trabalho tivemos como objetivo avaliar a qualidade e a toxicidade das águas superficiais ao longo deste rio. As análises físico-químicas da água no perímetro urbano de Goiânia apresentaram alguns parâmetros fora dos limites das regulamentações ambientais do Brasil. Em relação à diversidade de espécies, foram detectadas classes de fitoplâncton associadas a ambientes poluídos. O ensaio com *Allium cepa* sugere que este rio pode conter substâncias com atividade mitogênica. Este resultado está de acordo com a análise genotóxica, pois se observou um aumento significativo nas aberrações cromossômicas. Estes dados revelam o potencial genotóxico da água do rio Meia Ponte. Esta genotoxicidade representa um risco para a biota aquática e os seres humanos, uma vez que os agentes genotóxicos em amostras de água podem causar a perda de integridade do DNA, induzindo danos e rupturas de DNA. Nesse contexto, a utilização da água do Rio Meia Ponte, sem qualquer tratamento, deve ser evitada, e políticas públicas precisam ser formuladas e implementadas para despoluir este importante rio para o estado de Goiás.

Palavras-Chave: Aberrações Cromossômicas; Citogenotoxicidade; Rio Meia Ponte; Fitoplâncton.

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