UNIVERSIDADE FEDERAL DE UBERLÂNDIA
INSTITUTO DE BIOTECNOLOGIA
PÓS GRADUAÇÃO EM GENÉTICA E BIOQUÍMICA

O uso de espécies de peixes nativas para avaliação de ecotoxicidade dos rios da Bacia do Rio Paranaíba e Grande

Aluna: Carine de Mendonça Francisco
Orientador: Prof. Dr. Boscolli Barbosa Pereira
Co-Orientadora: Prof.ª Dr.ª Sandra Morelli

UBERLÂNDIA – MG
2021

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Tese apresentada à Universidade Federal de Uberlândia como parte dos requisitos para obtenção do Título de Doutora em Genética e Bioquímica (Área Genética).

UBERLÂNDIA – MG
2021

Ficha Catalográfica Online do Sistema de Bibliotecas da UFU
com dados informados pelo(a) próprio(a) autor(a).

F819
2021

Francisco, Carine de Mendonça, 1991-
O USO DE ESPÉCIES DE PEIXES NATIVAS PARA AVALIAÇÃO DE ECOTOXICIDADE DOS RIOS DA BACIA DO RIO PARANAIBA E GRANDE [recurso eletrônico] / Carine de Mendonça Francisco. - 2021.

Orientador: Boscolli Barbosa Pereira.
Coorientadora: Sandra Morelli.
Tese (Doutorado) - Universidade Federal de Uberlândia, Pós-graduação em Genética e Bioquímica.
Modo de acesso: Internet.
Disponível em: http://doi.org/10.14393/ufu.te.2021.8
Inclui bibliografia.
Inclui ilustrações.

1. Genética. I. Pereira, Boscolli Barbosa,1986-, (Orient.). II. Morelli, Sandra,1953-, (Coorient.). III. Universidade Federal de Uberlândia. Pós-graduação em Genética e Bioquímica. IV. Título.

CDU: 575

Bibliotecários responsáveis pela estrutura de acordo com o AACR2:

Gizele Cristine Nunes do Couto - CRB6/2091

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ATA DE DEFESA - PÓS-GRADUAÇÃO

Programa de Pós-Graduação em:	Genética e Bioquímica				
Defesa de:	Doutorado Acadêmico - nº 14/2020 PPGGB.				
Data:	Vinte e oito de janeiro de dois mil e vinte e um	Hora de início:	08:30h	Hora de encerramento:	12:40
Matrícula do Discente:	11623GBI008				
Nome do Discente:	Carine de Mendonça Francisco				
Título do Trabalho:	O uso de espécies de peixes nativas para avaliação de ecotoxicidade dos rios da bacia do Rio Paranaíba e Grande.				
Área de concentração:	Genética				
Linha de pesquisa:	Genética, Biologia e Melhoramento de Plantas e Animais.				
Projeto de Pesquisa de vinculação:	(Bio) indicadores, marcadores e monitores selecionados para estudos em Ecotoxicologia e Saúde Ambiental.				

Aos vinte e oito dias do mês de janeiro de dois mil e vinte e um, às 08:30 horas, reuniu-se via web conferência pela plataforma Google Meet, em conformidade com a Portaria nº 36, de 19 de março de 2020 da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES e Resolução de nº 06/2020 do Conselho de Pesquisa e Pós-graduação pela Universidade Federal de Uberlândia, a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Genética e Bioquímica, assim composta: Prof. Dr. Edimar Olegário de Campos Júnior, Profª. Drª. Camilla Queiroz Baesse Tolentino, Prof. Dr. Luís Paulo Pires, Profª. Drª. Celine de Melo e Prof. Dr. Boscolli Barbosa Pereira, orientador (a) do (a) candidato (a) e demais convidados presentes conforme lista de presença. Iniciando os trabalhos o (a) presidente da mesa, Prof. Dr. Boscolli Barbosa Pereira, apresentou a Comissão Examinadora e o (a) candidato (a), agradeceu a presença do público, e concedeu o (à) Discente a palavra para a exposição do seu trabalho. A duração da apresentação do (a) Discente e o tempo de arguição e resposta foram conforme as normas do Programa de Pós-graduação em Genética e Bioquímica. A seguir o (a) senhor (a) presidente concedeu a palavra, pela ordem sucessivamente, aos examinadores, que passaram a arguir o (a) candidato (a). Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu os conceitos finais. Em face do resultado obtido, a Banca Examinadora considerou o candidato (a):

APROVADO (A).

Esta defesa de Tese de Doutorado é parte dos requisitos necessários à obtenção do título de Doutor. O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU. Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata que após lida e achada conforme foi assinada pela Banca Examinadora.

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Documento assinado eletronicamente por **Luis Paulo Pires**, **Técnico(a) de Laboratório**, em 28/01/2021, às 14:06, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

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Referência: Processo nº 23117.000465/2021-91 SEI nº 2491126

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Aluna: Carine de Mendonça Francisco

COMISSÃO EXAMINADORA

Presidente: Prof. Dr. Boscolli Barbosa Pereira (Orientador)

Examinadores:

Prof. Dr.ª Celine de Melo
Prof. Dr. Edimar Olegário de Campos Júnior
Dr.ª Camilla Queiroz Baesse
Prof. Dr. Dr. Luís Paulo Pires

Suplentes:

Prof. Dr.ª Rute Magalhães Brito
Prof. Dr. Henrique Nazareth Souto

Data da Defesa: 28 de janeiro de 2021

As sugestões da Comissão Examinadora e as Normas PGGB para o formato da Tese foram contempladas

____

Boscolli Barbosa Pereira

DEDICATÓRIA

Dedico esta tese aos meus pais Moisés e Sandra, à minha irmã Camila e especialmente ao meu marido Wesley.

AGRADECIMENTOS

Agradeço primeiramente a Deus por todas as oportunidades em minha vida, bem como pela força, coragem e determinação para terminar mais uma etapa.

À Universidade Federal de Uberlândia e ao Programa de Pós-Graduação em Genética e Bioquímica por oferecerem condições para a realização deste trabalho.

Ao meu orientador Boscolli Barbosa Pereira, agradeço por ter aceitado meu pedido de orientação, por ter acreditado e confiado em mim. Um espelho de pesquisador, que sempre esteve presente e disposto a me ajudar. Obrigada pelo conhecimento, pelos ensinamentos, dedicação e inspiração.

Quero agradecer de forma especial à minha co-orientadora Sandra Morelli, pelos mais de 10 anos de ensinamento, por me possibilitar uma travessia sem pressões, com amizade, respeito e alegria. Foi você que acompanhou todo o meu desenvolvimento científico, profissional e pessoal.

Agradeço todos os demais professores e servidores, que durante toda minha vida acadêmica despertaram em mim a vontade de ser bióloga, professora e pesquisadora.

Aos professores Luiz Alfredo Pavanin e Sueli Moura Bertolino, pelo auxilio nas análises físico-químicas das amostras de água e sedimento.

Aos membros da Banca Examinadora, Dr.ª Celine de Melo, Dr. Edimar Olegário de Campos Júnior, Dr. Henrique Nazareth Souto, Prof. Dr. Dr. Luís Paulo Pires, Dr.ª Rute Magalhães Brito e Dr.ª Camilla Queiroz Baesse pela disponibilidade em participar da avaliação deste trabalho.

Agradeço aos meus pais, Moisés e Sandra, que nunca me deixaram faltar amor, carinho e educação. Eu devo tudo que sou a vocês, e se sinto orgulho de mim e do lugar onde cheguei, é porque sei que vocês vieram segurando a minha mão. À minha irmã Camila, minha eterna companheira, por todo carinho, amor e por sempre acreditar em meu crescimento.

Ao meu marido Wesley, agradeço por tudo!! Sem você, meu amor, eu não teria chegado até aqui! Obrigada por toda paciência e companheirismo, meu braço direito. Você foi um guerreiro! Não me deixou desistir! Esta conquista não é só minha, é nossa!

A todos os meus amigos e familiares, agradeço os momentos de confraternização, diálogos e desabafos. Minha família sempre foi uma fonte de momentos de alegria e descanso. Em especial, agradeço aos meus sogros, Flávio e Vanessa por toda torcida e incentivo. Sem vocês, estes anos teriam sido muito mais difíceis.

Agradeço aos colegas do laboratório de Citogenética, pela amizade, colaboração e acolhimento. E a todos aqueles que contribuíram com o desenvolvimento do trabalho, e participaram de alguma forma desse longo processo de formação profissional.

Agradeço finalmente à Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES), pelo suporte financeiro, que possibilitou-me dedicar às pesquisas aqui apresentadas.

Que venham os próximos desafios! Que venham os próximos projetos!

SUMÁRIO

APRESENTAÇÃO

A deterioração dos ambientes aquáticos tem se caracterizado como um dos maiores problemas de Saúde Pública no mundo, sendo os lançamentos de pesticidas, efluentes domésticos e industriais os maiores responsáveis pela contaminação dos recursos hídricos. Considerando que as condições de saneamento são precárias e que grande parte da população não tem acesso a água potável, monitorar a qualidade de mananciais que servem ao consumo humano é essencial. No entanto, a avaliação da qualidade da água, segundo parâmetros convencionais, baseados em indicadores físico-químicos, não consegue traduzir, por si somente, os impactos da contaminação nos organismos vivos expostos ao meio impactado. Assim, os estudos ecotoxicológicos *in situ* são usados para ampliar a sensibilidade dos estudos acerca da qualidade ambiental, complementando os resultados de caracterização do meio a partir de ensaios com organismos vivos. Este trabalho empregou espécies nativas (com aprovação da Comissão de Ética no Uso de Animais – CEUA/UFU – Análise 085/16, Protocolo 040/16 – Anexo 01) na avaliação da qualidade da água dos rios da bacia do Rio Paranaíba e Grande, direcionando o uso destas espécies *Astyanax altiparanae* e *Cichlassoma paranaense* como candidatos à organismos sentinela nos programas de biomonitoramento ambiental. Para isso, além das avaliações de parâmetros físico-químicos, foram investigados os efeitos biológicos da exposição dos peixes à amostras de água coletadas nesses afluentes, com destaque para avaliação dos danos causados ao material genético dos organismos expostos. Os resultados confirmaram a presença de contaminantes na água (Alumínio, Ferro, Manganês, Zinco e Cobre) e sedimento (Cromo, Cádmio e Níquel), sendo encontrados níveis acima do recomendado pela legislação ambiental brasileira. Além disso, nossa pesquisa revelou que a exposição à água contaminada esteve positivamente correlacionada à genotoxicidade em eritrócitos de peixes das espécies *A. altiparanae* e *C. paranaense*, popularmente conhecidas como Lambaris e Acarás, respectivamente. Os resultados reforçam a importância da realização de ensaios biológicos para detecção precoce de efeitos resultantes da exposição à contaminação por efluentes industriais e domésticos, que devem ser adotados como parâmetro complementar aos convencionais métodos físico-químicos de avaliação da qualidade da água.

Esta tese de doutorado foi elaborada de acordo com as normas estabelecidas pelo Programa de Pós-Graduação em Genética e Bioquímica da Universidade Federal de Uberlândia, e dividida em três capítulos:

CAPÍTULO I – Applications of the micronucleus assay for in situ assessment of freshwater genotoxicity using neotropical freshwater fishes. Trata-se de um artigo de revisão, o qual apresenta pesquisas e conceitos ecotoxicológicos para o desenvolvimento de experimentos de avaliação ambiental *in situ*, utilizando peixes nativos como organismos sentinelas (bioindicadores). Neste estudo, foram evidenciados modelos biológicos para avaliação da ecotoxicidade dos poluentes naturais e antropogênicos, mediante a utilização de biomarcadores de genotoxicidade.

CAPÍTULO II – Eco-genotoxic responses of the native fish species following exposure to Copper-contaminated freshwater samples Consiste num experimento *in situ*, utilizando um ciclídeo Neotropical de água doce (*Cichlasoma paranaense*) como modelo biológico para avaliação da ecogenotoxicidade de amostras de água coletadas em diferentes locais. Os resultados indicaram que a espécie testada foi sensível às amostras contaminadas por efluentes contento cobre, provenientes de descarte de agroquímicos na região, apresentando elevados níveis de alterações genotóxicas. O artigo foi submetido à revista Environmental Science and Pollution Research (Fator de impacto = 3.056), no dia 23 de maio de 2020, e encontra-se em revisão (Anexo 02).

CAPÍTULO III – Genotoxicity assessment of polluted urban streams using a native fish *Astyanax altiparanae* A pesquisa avaliou a genotoxicidade de amostras coletadas em córregos do rio Jordão, afluente do rio Paranaíba, Brasil, utilizando *Astyanax altiparanae*, que se mostrou significativamente sensível aos diferentes níveis de contaminação testados. Este estudo está no periódico Journal of Toxicology and Environmental Health, Part A (Fator de impacto = 2.7) (Anexo 03).

CAPÍTULO I

Applications of the micronucleus assay for *in situ* assessment of freshwater genotoxicity using neotropical freshwater fishes

Artigo de Revisão que será submetido,

Journal of Toxicology and Environmental Health - Part B

Applications of the micronucleus assay for *in situ* assessment of freshwater genotoxicity using neotropical freshwater fishes

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Abstract

The concern about the health of aquatic ecosystems has led to the increase in the use of biomarkers, in an attempt to assess mutagenic risks and to identify the sources and destination of contaminants. The micronucleus (MN) test as an index of genetic damage is one of the most used techniques to identify the response of organisms exposed to contaminants. The test is widely applied to aquatic species, mostly fish. The MN test has been validated and successfully applied in a large number of studies reviewed in this work. The studies that evaluated the health of aquatic ecosystems in native Neotropical species are concentrated mainly in the south and southeast regions of Brazil. Fishes are considered suitable organisms for biomonitoring studies, and regarding neotropical species, the use of native species proved to be more sensitive than species introduced into the environments, since they are adapted to the region. The performance of studies with native species, considering long-term exposures, in the form of biomonitoring programs should be encouraged, prioritized and supported, as it consists of a realistic, sensitive and reliable experimental model for the evaluation of ecogenotoxicological impacts of pollution in aquatic ecosystems.

Keywords: Biomarkers, Bioindicators, Biomonitoring, Ecotoxicology.

Introduction

The increase in urban population and the unplanned growth of cities are responsible for a series of environmental concerns, with emphasis on the loss of the natural characteristics of ecosystems, including the loss of biodiversity and changes in the structural dynamics of communities and populations (Perez-Reyes 2015). In this degradation scenario, aquatic ecosystems are the most impacted. In developing countries, a large part of waste disposal occurs in aquatic environments, receiving up to 90% of untreated sewage (UN 2019).

Industrial effluents, sewage, agricultural pesticides and household waste constitute the major contaminants in aquatic environments (Tessarolo et al. 2017). Besides the loss of biodiversity and changes in the dynamics of aquatic ecosystems, contamination of water resources is also responsible for at least a third of deaths in developing countries, which are directly associated with the consumption of non-potable water (UN 2019).

In view of the growing concern about the toxic effects of natural or synthetic contaminants, isolated or in mixture on biota, including the human species (Walker et al. 2012), Ecotoxicology has been established as an environmental natural science capable of evaluating and quantifying environmental changes and impacts at the organismic, population and community levels in ecosystems (Zagatto and Bertoletti 2008).

The assessment of the quality of the environment in aquatic ecosystems is generally performed based on physical-chemical parameters of water and sediment samples. This model is limited, either by the availability of detection methods and also by the inability to predict the toxicity of complex mixtures and their different effects (synergistic, additive, antagonistic or potentiating) on biota (Di Poi et al. 2018).

Therefore, assessment methods of biological changes induced by xenobiotic factors are essential for complementing the physical-chemical evidence (Dalzochio et al. 2016), since they can detect responses (biomarkers) at the molecular, cellular or biochemical level in sentinel organisms (bioindicators), being able to monitor effects and impacts early, thereby anticipating the possibilities of intervention in the environment (Bolognesi and Hayashi 2011).

Still from the perspective of toxicology, to assess environmental quality in aquatic ecosystems, genotoxicity biomarkers have been shown to be sensitive to chronic exposure of different organisms to the contaminated environment (Shahjahan et al. 2020; Vieira et al. 2018; do Carmo et al. 2018). Based on the use of biomarkers of environmental genotoxicity, monitoring the frequencies of micronucleated erythrocytes and other nuclear abnormalities has been a successful strategy to evaluate the impact of pollutants on the biota of aquatic ecosystems (Dixon et al. 2002; Morita, MacGregor, and Hayashi 2011; Baršienė et al. 2014; Lacerda et al. 2020).

Due to the simplicity of performance and sensitivity to detect cytogenetic damage induced by chemical and physical agents, the micronucleus (MN) test is one of the most widely applied bioassays to monitor the quality of aquatic environments (Morita, MacGregor, and Hayashi 2011; Hayashi 2016; Lemos, Oliveira, and Lemos 2011; Kushwaha et al. 2012; Baršienė et al. 2014), since it is possible to observe in small volume samples the presence of micronuclei in erythrocytes without having to sacrifice the organisms (Kushwaha et al. 2012).

The formation of micronuclei occurs in the processes of cell division, from fragments of acentric chromosomes (result of exposure to clastogenic agents) or whole chromosomes (result of exposure to aneugenic agents), which are not included in the main nucleus after anaphase (Schmid 1975; Baesse et al. 2019). The MN test, originally developed in mammalian species (Fenech 2020), is currently frequently applied to fishes and other aquatic organisms, such as sea urchins, mussels, oysters and crabs. In the aquatic environment, the majority of studies or programs on the genotoxic effect of the polluted environment have been carried out using mollusks and fish, owing to the economic and ecological importance of these organisms (Viarengo et al. 2007).

Genotoxicity assessment in neotropical freshwater fishes

The Neotropical region has the most diversified freshwater fish fauna on the planet, with approximately 5,000 known species, of which about 3,300 are found in Brazil (Froese and Pauly 2016). In a few decades, many of these species may disappear, especially endemic ones (Reis et al. 2016). Indeed, the causes of extinction are numerous due to unsustainable activities, such as mining, pesticide release and the introduction of non-native organisms. In Brazil, more than 300 freshwater species are threatened with extinction (Reis et al. 2016).

Based on the recognition that the preservation of native species of neotropical fish is crucial to ensure the maintenance and balance of aquatic ecosystems, monitoring of environmental impacts through ecogenotoxicity assessments is also required for the conservation of biota (Campos-Júnior, Pereira, and Morelli 2015; Morais et al. 2016; Kostić et al. 2016; Batista et al. 2016; Reyes et al. 2017; Francisco et al. 2019; Queiroz et al. 2019; Fasulo et al. 2015; da Silva et al. 2020).

As shown in Table 1, several studies have reported the application of the MN test in erythrocytes of neotropical fish species, hence indicating the sensitivity of the biomarker to different pollutants and in different situations, as in assessments in natural field conditions (Bogoni et al. 2014; Campos-Júnior, Pereira, and Morelli 2015; Campos-Júnior et al. 2016; Dalzochio et al. 2018a; Francisco et al. 2019; Lacerda et al. 2020), in conditions in which the specimens are inserted at experimental points by confinement (Vieira et al. 2014); and in laboratory exposures, in which an organism is exposed to different concentrations of isolated substances or effluents to assess the genotoxic effects of contamination at defined intervals (Langner et al. 2019; Baudou et al. 2019; Bianchi et al. 2019, Bocato et al. 2019; Tovar-Sánchez, Sánchez-Quiles, and Rodríguez-Romero 2019).

Also according to Table 1, among field studies, the MN test in erythrocytes was commonly performed from the extraction of blood samples from the caudal vein. Francisco et al. (2019) evaluated *in situ* a region of the Paranaíba river basin, Brazil, using *Astyanax altiparanae*. Their findings confirmed the sensitivity of the fish species and reinforced the importance of carrying out biological assays to detect effects resulting from exposure to contamination by industrial and domestic effluents, which should be adopted as a complementary parameter to conventional physical-chemical methods. In another study (Vieira et al. 2014), a general stress condition was also detected, using the same species, in the Paraná River basin. The work reported that the frequency of MN was significantly higher among individuals confined in areas with agricultural contaminants. Additionally, in this same study, other biomarkers were evaluated, revealing increased activity of glutathione-S-transferase (GST) and catalase (CAT), increased content of reduced glutathione (GSH) in liver and gills, reduced activity of acetylcholinesterase (AChE) in muscle and brain and increased erythrocytic nuclear abnormalities (ENA).

In situ assessment is a more efficient possibility to evaluate the effects of mixtures of contaminants in the environment on the tested organisms. In general, field experiments have greater ecological relevance, as they consider the interactions between biotic, physical and chemical variables in the environment, which is difficult to reproduce in laboratory tests (Vieira et al. 2016; Vieira et al. 2017; Souza-Bastos et al. 2017; Pérez et al. 2018).

Although to a lesser extent, studies performed in the laboratory from the exposure of native neotropical fishes to samples of contaminated water have confirmed the sensitivity of the evaluation of the frequency of MN as a biomarker of environmental genotoxicity. Baudou et al. (2019) exposed individuals of the species *Cnesterodon decemmaculatus* to water samples from the Reconquista River (Argentina) in laboratory tests, which detected a high frequency of micronuclei.

Accordingly, most laboratory studies have evaluated the genotoxic effects of isolated substances, mainly pesticides, on neotropical fish species (Stanley and Preetah 2016; Furley et al. 2018). Despite these studies confirmed the genotoxic potential of the chemical agents tested and the sensitivity of the species and the bioassay, the findings were unable to provide a comprehensive understanding of the real consequences of contamination in the aquatic environment, where complex mixtures occur, with numerous possibilities for interaction, with different levels of toxicity (Ashauer, Boxall, and Brown 2006; Bundschuh, Goedkoop, and Kreuger 2014). Although less than ideal, some researchers have been concerned to realistically assessing the complexity of field variables (Beliaeff and Burgeot 2002; Carriquiriborde et al. 2007; Bony et al. 2008; Moreira et al. 2010; Qu et al. 2015; Liu et al. 2016; Vieira et al. 2016; Vieira et al. 2017).

Nuclear changes in fish erythrocytes

In addition to micronuclei, several studies have described the presence of erythrocytic nuclear abnormalities (ENAs) in fish cells resulting from exposure to genotoxic, mutagenic or carcinogenic compounds (Thomé, Silva, and Santos 2016, Hussain 2017). Nuclear changes were first described in fish erythrocytes by Carrasco, Tilbury, and Myers (1990), and were classified in four categories: Blebbed: nuclei with a small evagination of the nuclear membrane, still attached to the nucleus, appearing to contain euchromatin or heterochromatin; Lobbed: nuclei with wider evaginations and not as defined as those described for blebbed; Vacuolated: nuclei with a region that resembles vacuoles absent of any visible material inside; and Notched: nuclei that have a well-defined cut in shape, usually with an appreciable depth in the nucleus. In a complementary way, Fenech (2000) suggested that Binucleated cells should be considered as another type of ENA.

Although the exact mechanisms of ENA induction are not yet elucidated (Botelho et al. 2015; Brahma et al. 2017), their increased frequency indicates exposure to contaminants (Braham et al. 2017, Ghisi, Oliveira, and Prioli 2016; Braham et al. 2017; Vieira et al. 2017) being used as valuable bioindicators (Ghisi, Oliveira, and Prioli 2016; Braham et al. 2017; Vieira et al. 2017).

In organisms exposed to heavy metal contamination, the formation of ENAs has been associated with damage to the cytoskeleton, especially in the polymerization of tubulin and actin, cytoplasmic changes, chromosomal breaks, chromatin compaction and remodeling and reduced cellular capacity to repair damage (apoptosis) (Panariti, Miserocchi, and Rivolti 2012; Ghaffar et al. 2015; Sadiqul et al. 2016; Qualhato et al. 2017; Vardavas et al. 2016; Sampaio et al. 2019). In the literature, most of the studies that evaluated the genotoxic effect in watercourses described the presence of ENAs in neotropical fish (Table 2). In particular, in comparison to other neotropical species, *Geophagus brasiliensis* has been shown to be sensitive to different environmental conditions when interacting with different pollutants (Arantes et al. 2009; Benincá et al. 2012; Osório et al. 2014; Voigt et al. 2015). Importantly, previous studies reported a similar increase in the frequency of MN and ENA, hence allowing the combined use of these biomarkers in environmental monitoring studies in Neotropical regions (Grisolia et al. 2009; Campos-Júnior, Pereira, and Morelli 2015; de Jesus et al. 2016).

Knowledge gaps and application perspectives

Fishes are considered suitable organisms for biomonitoring studies (dos Santos et al. 2020; Kumar et al. 2020), as their biological responses, such as genetic, biochemical, behavioral and morphological alterations, change, even at low levels of pollution, thus representing important biomarkers in the assessment of environmental quality (Pesce et al. 2008; Ballesteros et al. 2009; Vieira et al. 2017; da Silva et al. 2018). Notwithstanding that, even if *in vivo* exposure tests are well replicated under laboratory conditions, there is a need to encourage field evaluations to clarify possible under or overestimated results of the effects of contaminants under realistic conditions (Dalzochio et al. 2016).

The MN test provides indispensable results for a more complete assessment of water quality, as one of the biomarkers that best complements the data obtained from physical-chemical parameters, thus allowing to understand, for example, the relationship between presence and concentration of heavy metals, hydrocarbons and pesticides with the observed genotoxic damage. In spite of representing a complex biological response, the evaluation of the frequency of MN is a useful and easily interpreted index in relation to the genotoxic effects of the contaminants (Esteves 2011).

Regarding the use of Neotropical fish species, the use of native species proved to be more sensitive than species introduced into the environments, since they are adapted to the region (Langiano and Martinez 2008). Further, it is important to consider that both biological and physical-chemical parameters may vary as the climatic and hydrological characteristics of the environment change, as well as human activities, which are not always a constant (Abell et al. 2008). In this sense, the choice of fish species in genotoxicological assessment studies is of pivotal relevance, especially when performed over the long term, being preferable organisms that are neither very sensitive nor very resistant to environmental variations.

The studies herein evaluated, which use the MN test, are concentrated mainly in the south and southeast regions of Brazil. Thus, it is important and necessary to assess the genotoxicity of more neotropical freshwater regions in native fish in order to expand the database on this research strategy, to contribute towards a better interpretation of information on quality and environmental conservation.

Finally, it is worthwhile noting that the performance of studies with different native species, considering long-term exposures, in the form of biomonitoring programs should be encouraged, prioritized and supported, as it consists of a realistic, sensitive and reliable experimental model for the evaluation of ecogenotoxicological impacts of pollution in aquatic ecosystems.

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CAPÍTULO II

Eco-Genotoxic responses of the native fish species following exposure to Copper-contaminated freshwater samples

Artigo Submetido:

Environmental Science and Pollution Research

Eco-genotoxic responses of the native fish species following exposure to Copper-contaminated freshwater samples

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Abstract

Water quality has declined progressively because of the continuous pollution of aquatic resources. The use of fish genotoxicity biomarkers is an important tool to improve and complement parameters for environmental risk assessment. Therefore, the present study aimed to use *Cichlasoma paranaense* (Teleostei: Cichlidae) a Neotropical freshwater cichlid fish as a biological model for assessment of the eco-genotoxicity caused by water pollution over different stream sections in a river basin used to provide drinking water. Alarmingly, chemical analysis of water and sediments collected from different sites reported a Copper contamination gradient. After chronic exposure of the local species *Cichlasoma paranaense* to contaminated water samples, micronucleus (MN) and nuclear abnormalities (NA) frequencies were assessed in erythrocytes from the caudal and gill. Sites where the concentrations of the copper (Cu) were greater reported higher genotoxic potential. There was no significant difference between the tissues (tail and gill) regarding the observed frequencies of micronuclei and nuclear abnormalities. Data demonstrated that *Cichlasoma paranaense*, used in the test, exhibited a reliable sensitivity for detection of genotoxic consequences attributed to exposure to water samples collected near the discharge of agrochemicals.

Keywords: Ecotoxicology; Biomarkers; Water Pollution; Ciclasoma; Environmental Risk.

Introduction

The use of physicochemical parameters only not expose the realistic risk of water pollution for the aquatic biota (Furley et al. 2017). Aquatic Ecosystem Assessments for Rivers are more reliable when both physicochemical and biological parameters integrate a system of indicators. In this sense, biomarkers of genotoxicity reveal effects of pollutants nature prevailing over a long period (Costa-Silva et al. 2015; Dos Santos et al. 2016; Bianchi et al 2018; Sobrino-Figueroa 2018; Francisco et al. 2019).

Cichlids are spiny-rayed freshwater fishes widely used as model organisms in ecotoxicology due to their distinctive social hierarchies, being able to affect several physiological mechanisms, such as growth, reproduction and stress levels (Maruska and Fernald 2012).

The *Cichlasoma paranaense* (Teleostei: Cichlidae) is recognized as a Neotropical freshwater cichlid fish which can be kept in captivity under controlled environmental conditions (Nelson et al. 2016). Importantly, the species has drawn considerable attention in aquatic ecotoxicological testing, arising as an experimental model for testing the effects of different types of pollutants (Da Cuña et al. 2013; 2016; Meijide et al. 2016; Piazza et al. 2011; 2015; Vázquez et al. 2016).

The contamination of the aquatic environment by various pollutants has raised issues globally concerning the potential toxicity, abundance and persistence in the environment (Sin et al. 2001; Armitage et al. 2007; Reyes et al. 2017; Campos et al. 2016; Francisco et al. 2019). Notably, special emphasis is given to heavy metals, such as lead (Pb), chromium (Cr), zinc (Zn), copper (Cu) and mercury (Hg) (Sabale et al. 2012; Strbac et al. 2015; Campos et al. 2016; Reyes et al. 2017).

The assessment of genotoxic damages in erythrocytes through micronucleus (MN) and nuclear abnormalities (NA) tests determines the impact of pollutants on aquatic biota (Dixon et al. 2002; Baršiene et al. 2014). Accordingly, these assays are able to identify the intrinsic genotoxicity attributed to a variety of toxic substances (Francisco et al. 2019). Indeed, they are widely applied owing to the well established suitability for fish species (Çavas and Ergene-Gozukara 2005; Kushwaha et al. 2012; Praveen et al. 2014).

In the present study we aimed to use *Cichlasoma paranaense* (Teleostei: Cichlidae) a Neotropical freshwater cichlid fish as a biological model for assessment of the eco-genotoxicity caused by water pollution over different stream sections in a river basin used to provide drinking water.

Material and Methods

Study sites and sampling

Araguari is a city located in the state of Minas Gerais, in the north of the Triângulo Mineiro region. The samples (water and sediment) were collected in dry and rainy seasons in the following rivers: Paranaíba (PAR) (18°22'47.65''S 48°23'10.57''W), Araguari (ARA) (18°52'20.67''S 48°04'42.53''W) (Córrego das Araras), Tijuco (TIJ) (18°56'47.29'' S 49°01'47''W) and Grande (GRD) (19°59'14.73'' S 47°47'19.35''W) (Figure 1) with different characteristics:

PAR: The population of the Paranaíba River, with an approximate width of 50 m, was observed during a field visit in which it was detected a significant loss of native vegetation due to the expansion of agriculture, including coffee, corn and soybean plantations, apart from the presence of water bodies in the region due to the implementation of reservoirs of hydroelectric plants.

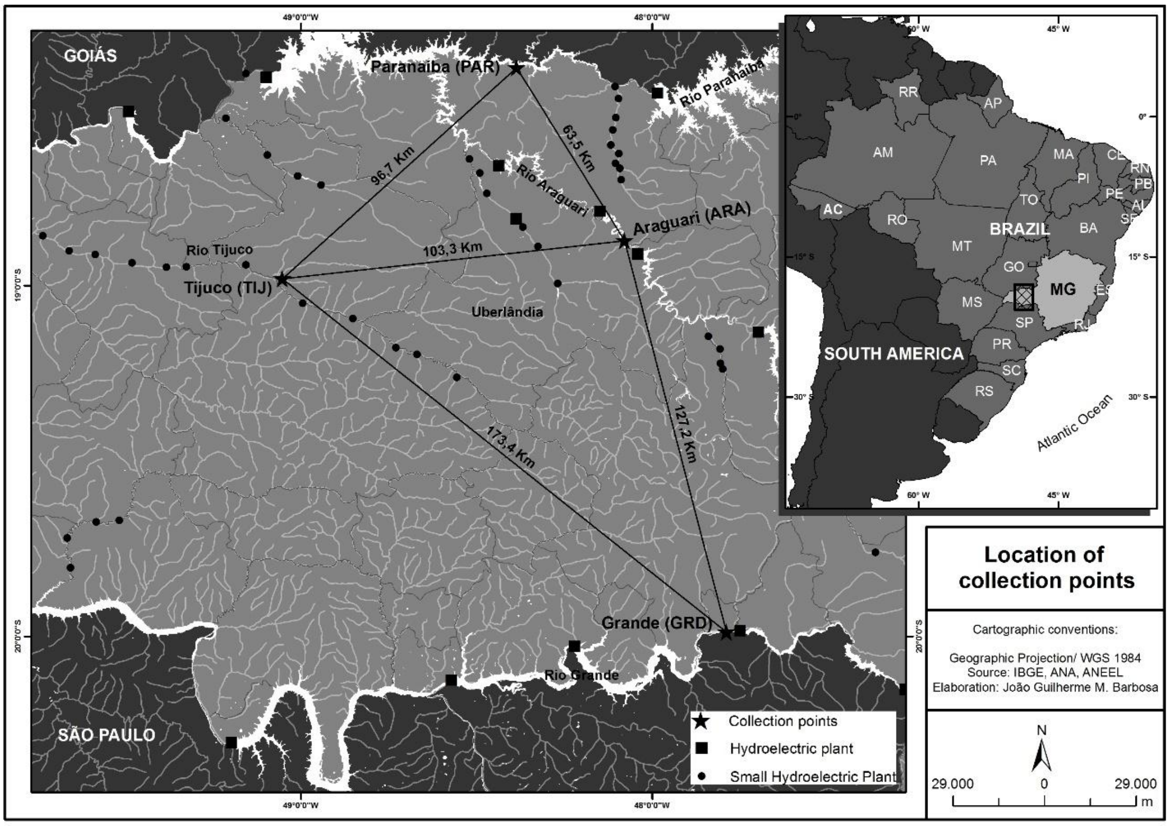
ARA: The population of the Araguari River is located upstream of the PAR population, and is about 40 m of width. In this place, it occurs the presence of riparian forest. However, very close to the sampling sites, tomato farming was evident, and a little further away was also noted coffee plantation. During the field visit, the presence of two fishes was observed near this site.

TIJ: The population of the Tijuco River (approximately 30 meters of width) exhibits a dense riparian forest on the banks of the river, and it was possible to notice the presence of nearby residents, being visibly a preserved region, and for this reason it was considered the control site.

GRD: The population of the River Grande is located near the bridge, on the border of Minas Gerais with the state of São Paulo, on the highway via Anhanguera, which is part of the BR 050, near the city of Delta, MG. The width of this location is approximately 40 meters. There is a riparian forest on the banks of the river, but with some signs of degradation due to residential buildings for leisure purposes, large sugar and ethanol plants, with an intense flow of paths. Field observations showed that there are pasture and agricultural areas nearby.

Water and sediment samples collected from the referred sites were evaluated according to Guidelines for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1998).

Figure 1: Map of sampling sites monitored. PAR (Paranaíba river), ARA (Araguari river), TIJ (Tijuco river) and GRD (Grande river).



Biological material

Fish specimens were collected in the rivers Paranaíba (PAR), Araguari (ARA), Tijuco (TIJ) and Grande (GRD), as shown in Figure 1. Conventional line fishing was employed and the samples were transported alive to laboratory. A total of 147 specimens of *Cichlasoma paranaense* of both sexes (6.0 ± 0.4 g of body mass; 7.0 ± 0.2 cm in total length, without sexual differentiation) were transported to the laboratory, where they were kept in 20L tanks. The tanks contained reconstituted water (pH 7.5, oxygen dissolved at a rate of 8mg / L and hardness of 43mg $CaCO_3$ / L), where the fish remained for 10 days before exposure under controlled conditions of temperature (25ºC), lighting (16: 8 hours light / dark cycle), daily feed with 35 mg of commercial flake feed and constant aeration. Then, the fish were fed up to 24 h before starting the test.

The techniques used in model animals and the procedures adopted to obtain tissues were approved by the Ethics Committee on the Use of Animals at the Federal University of Uberlândia, registered under protocol 040/16.

Ecotoxicity

To determine the ecotoxicity of the water samples collected at the study sites, CL 50-96h values were calculated according to the logarithmic regression model established by the Organization for Economic Cooperation and Development (OECD), Standard 203 (OECD 1992). The tests were conducted in a semi-static system, using 7 fish in each 20L aquarium. The experiments were performed in duplicate and the dilution water was the same used for acclimatizing fish. The fish were also examined daily for abnormal behavior, including erratic swimming, loss of equilibrium, lethargy and immobility.

Genotoxicity Biomarkers – Micronucleus Frequency Test (MN)

For assessment of the genotoxicity tests, peripheral blood was removed from the branchial and tail artery using sterile 1 ml heparinized syringes, one for each animal, after exposure to 168 h with the water samples from each location. Smears (blood drop sliding on the microscope slides) were made immediately, and were air dried for 24 h (Grisolia and Starling 2001). For staining, cells were fixed with absolute methanol for 10 min and stained with Giemsa and phosphate buffer (pH = 6.8) in the ratio of 1:20 for 15 min. Four slides were prepared for each animal.

Then, four thousand erythrocytes per animal were evaluated under light microscopy at a 1000-fold magnification, using immersion oil, as previously described by Schmid (1975). The criteria for identification of micronucleated erythrocytes were that the nuclear particles were required to (1) be smaller and completely separated from the main nucleus; (2) not refractory; (3) with the same shape, staining and intensity of the cell nucleus and within the cellular cytoplasm. Nuclear abnormalities (NA), such as binucleated, lobed, blebbed, notched or kariolysis, were identified and classified according to Tolbert et al. (1991) and Holland et al. (2008).

Statistical analysis

Immobility data, lethargy, erratic swimming and loss of balance were used as the evaluation criteria of the acute toxicity tests (LC15-96h) and the endpoints No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) were analyzed. The NOEC and LOEC were determined using Fisher's Exact Binomial Test with Bonferroni Correction, and Student's t-test was used for comparison between tissues (Tail and Gill). For all analyses, p values <0.05 were considered statistically significant. One-way ANOVA was used to determine the existence of differences between sites.

Results

The physicochemical parameters for water and sediments are shown in Tables 1 and 2, respectively. For all locations, temperature, turbidity, total dissolved solids and pH values were below the recommended standard established by Environmental Brazilian Council (CONAMA 2011).

The levels of iron (Fe), zinc (Zn) and copper (Cu) in the sediment samples exceeded the environmental legal limits. Regarding the copper parameter, findings were similar and all sites exceeded the environmental limit, with exception to the reference location (TIJ).

Data obtained from the acute toxicity tests performed with *Cichlasoma paranaense* exposed to different concentrations after 96h exposure are depicted in Table 3. According to OECD guidelines (2004), for all tests the mortality of the controls did not exceed 10%.

The ecotoxicological parameters NOEC and LOEC were also evaluated with reference to the acute toxicity tests. Accordingly with results, the endpoint immobility was not observed during exposure time, but the fish exposed to water samples from Paranaíba, Araguari and Grande Rivers exhibited loss of equilibrium, erratic swimming and lethargy, hence indicating effects of contamination on behavioral parameters. As given in Table 4, there was no significant difference between the tissues (peripheral and gill blood) regarding the observed frequencies of micronuclei and nuclear abnormalities according to Student's t-test, suggesting that both methods of sampling cell did not differ in their responses to environmental pollution.

The frequency of micronuclei varied among the fish exposed to the different samples obtained at the monitoring sites, with the highest frequencies being observed in individuals submitted to exposure to water samples from River Grande> Araguari> Paranaíba.

There was no significant difference between the frequencies of nuclear abnormalities for individuals exposed to different water samples from the monitored sites.

Discussion

The water quality of rivers that surround the Triângulo Mineiro region faces several challenges, such as the incidence of copper in the sediments, as reported by this study. Notably, the hurdles are caused predominantly by the agricultural discharge of pesticides, as the region has an expressive area for plantation.

Industrial and domestic effluents are frequently released in environment and represent the major cause of water pollution (Aich et al 2015). Notwithstanding, in Brazil, water quality parameters (CONAMA 2011) do not establish standards for a wide range of contaminants, including pesticides and drug residues, hence unveiling the need of a revision. Organic matter is a remarkable source of disease-causing microorganisms, being domestic sewage one of the main contributors to the reduction of environmental quality. In this context, considering that metals are retained in sediments, their assessment is required. It is worthwhile noting that, depending upon various biotic and abiotic factors, the pollutants become resuspended in the water column.

Heavy metals are essential elements for living organisms, including humans, but in excess they are toxic. Indeed, small amounts of Cu exert a pivotal role in environment under natural conditions. Although the heavy metal is an essential trace element, excessive amounts may be toxic to fish, microorganisms and humans (Nemery and Banza 2018).

The levels of Cu and other metals in river water may have increased risen owing to anthropogenic activities in nearby regions (GRD), soybean and coffee farming processes near to the Araguari River (ARA) and industrials sites (PAR).

Despite the importance in maintaining normal physiology and the functions of different biological mechanisms, copper is frequently detected at high concentrations in aquatic environments and, when excessive, the heavy metal is toxic (Xiao et al 2018). In this sense, the unregulated level of Cu may be associated with oxidative damage for the production of reactive oxygen species (ROS), and is also related to a negative impact in energy reserves and glycolytic and lipogenic enzymes in many fish (Azqueta and Collins 2013).

Apart from being simple, sensitive and reliable, the micronucleus (MN) test provides a rapid result for the examination of genetic damage caused by the presence of chemical agents in a specific environment (Pollo et al. 2015). The MN has been used to investigate the initial effects of chronic exposure to xenobiotic substances in target species, either in laboratory or field, thus being an important marker for environmental biomonitoring (Udroiu et al. 2015).

Although the micronucleus test has been applied for several decades, the evaluation of nuclear abnormalities (NA) is also relevant as a complementary approach to MN analysis. According the results, a significant increase was not observed for binucleated cells, notched nucleus, lobed nucleus and blebbed nucleus. Among these, cells with two nuclei are known as binucleate, and occur due to the blockade of cytokinesis by an abnormal cell division (Çavas¸ Ergene-Gozükara, 2005; Mahboob et al. 2014). Previous research reported that the origin of binucleate cells along with the origin of MN is associated with cell division, while other abnormalities may be related to DNA amplification (Pollo et al. 2015).

The assessment of micronuclei frequency in gill and in peripheral erythrocytes of fish species indicates that this biomarker offers sensitive results in monitoring the pollution (Çakal et al. 2015).

Cichlasoma paranaense was considered an efficient candidate to sentinel specie for biomonitoring Cu contamination, because survives in environments with contamination and, even at sublethal concentrations, water pollution had a detrimental effect on its swimming performance and induces high MN frequencies.

In this sense, monitoring eco-genotoxic responses of native fish species in contaminated freshwater, using behavioral changes and genotoxic parameters offer a rapid, sensitive and biologically significant tool to risk assessment of river water pollution.

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CAPÍTULO III

Genotoxicity assessment of polluted urban streams using a native fish *Astyanax altiparanae*

Artigo Publicado

Journal of Toxicology and Environmental Health, Part A

Genotoxicity assessment of polluted urban streams using a native fish *Astyanax altiparanae*

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Abstract

Water quality has declined globally notably due to increased contamination of aquatic ecosystems. The use of fish genotoxicity biomarkers may improve and complement parameters for environmental risk assessment. The aim of this study was to assess the genotoxicity of samples collected from streams of the Jordão River, a tributary of the Paranaíba River, Brazil with different levels of metal contamination, utilizing a native fish species to determine the sensitivity and viability of implementing a useful, reliable technique for routine biomonitoring programs. Chemical analysis of water and sediments collected from different sites indicated that a gradient of contamination existed as evidenced by different concentrations of metals detected. After chronic exposure to contaminated samples, micronucleus (MN) frequencies in fish erythrocytes were measured and correlation with environmental parameters determined. Sites where the water concentrations of the metals aluminum (Al), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were high indicating a greater genotoxic potential of these elements. At the samples collected from the urban zone, a gradual increase was found for chromium (Cr), cadmium (Cd) and nickel (Ni) indicative of adverse impacts of discharge of urban effluents. Data demonstrated that *Astyanax altiparanae*, used in the test, exhibited a reliable sensitivity for detection of genotoxic consequences attributed to exposure to water samples collected near the discharge of industrial and domestic waste.

Keywords: Micronucleus; Biomonitoring; Contaminants; Metals; Toxicology

Introduction

Water quality and aquatic biodiversity have declined significantly due to the exploitation by several human activities, which have altered the aquatic environment (Yuan et al. 2012; DiGiulio and Clark, 2015; Tessarolo et al. 2017; Awange 2018). Discharges of industrial and domestic effluents, combined with adjacent agricultural and urban flows, constitute a serious threat to aquatic ecosystems and, consequently, to human health (Lemos et al. 2008; Reyes et al 2017). A large amount of chemicals, released in aquatic environments due to intense urbanization processes and industrial activities (Srebotnjak et al. 2012; Su et al. 2013; Campos et al 2016; Pereira et al, 2017; Reyes et al 2017) have contributed to contamination of streams and rivers (Araújo and Dallos 2006; Pereira et al,2017). Among the main pollutants are metals, with potential to accumulate in living organisms (Vaz et al. 2016).

The contamination of the aquatic environment by metals has raised concerns globally due to potential toxicity, abundance and persistence (Sin et al. 2001; Armitage et al. 2007; Reyes et al 2017; Campos et al 2016) and special attention is given to metals as lead (Pb), chromium (Cr), zinc (Zn), copper (Cu) and mercury (Hg) (Sabale et al. 2012; Strbac et al. 2015; Campos et al 2016; Reyes et al 2017). These contaminants are not only harmful to the health of aquatic organisms, but also to humans through the consumption of water and fish (Rocha et al. 2009).

Genotoxic contaminants present mutagenic and / or clastogenic effects and their damage are transmitted to the next generations (Bolognesi and Hayashi 2011). DNA damage may be expressed as induction of mutations, hereditary defects, teratogenic effects and uncontrolled cell proliferation (Mitchelmore and Chipman

1998). Therefore, there is a growing interest in using biomarkers to improve rapid assessment of the genotoxicity consequences in aquatic fauna (Russo et al. 2004).

Local and global environmental agencies establish the acceptable levels of residues of various compounds that are used in domestic, agricultural, livestock and industrial processes, based upon the interference that these compounds may produce alterations in the physical and chemical parameters of aquatic ecosystems. However, pollutant sources may present diverse forms and behaviors in terms of interaction, mobility, biological availability and toxicity potential when combined (Sundaray et al. 2011). Thus, assessing only chemical contaminants isolated using physicochemical assays may not adequately estimate potential adverse toxic effects on organisms. Thus, in addition to employing physicochemical tests, it is also important to evaluate potential toxicological interactions by utilizing complementary biological assays (EFSA 2013; Raies and Bajic 2016).

Bioassays using fish provide information on the bioavailability of pollutants that contribute to metal biomagnification processes (Marcon et al. 2010). Bioassays employ organisms of different trophic levels, isolated or combined with chemical analyses to assess toxic potential of aquatic contaminants. Freshwater fish of the genus *Astyanax*, employed in environmental monitoring studies, were found to present with high sensitivity as a bioindicator for contaminants (Silva and Martinez 2007; Trujillo-Jiménez et al. 2011; Vieira et al. 2014; Yamamoto et al. 2016). This fact favors the choice of these organisms, as observed in the species *Astyanax altiparanae* (Ramsdorf et al. 2012) in field and lab investigations in temperate regions (Vieira et al. 2014; Bettim et al. 2016; Dourado et al. 2017).

Among the predominant biomarkers of environmental genotoxicity, the micronucleus (MN) frequency test constitutes a promising method in assessing cytogenetic damage that is regularly used to monitor water quality (Lemos et al. 2011; Kushwaha et al. 2012). The measurement of cytogenetic damage by NM frequency evaluates the stress of pollutants on aquatic ecosystems (Dixon et al. 2002; Baršiene et al. 2014) and consequent aneugenic and clastogenic effects. This test has the ability to identify the genotoxicity attributed to a wide range of toxic compounds (Heddle et al. 1991) and is widely applied because of its established suitability for fish species (Çavas and Ergene-Gozukara 2005; Kushwaha et al. 2012; Praveen et al. 2014). Although there are a considerable number of studies in Brazil, few data are available on effluents in the Triangulo Mineiro region, MG, and no reports in the Jordão River sub-basin for toxicological analyzes. The aim of the present study was to determine the genotoxicity of samples collected at different sites from streams located along a Brazilian river basin with varying levels of contamination. In particular a native fish species was employed to examine viability as well as sensitivity of biomarkers of genotoxicity as a complementary parameter to conventional physical-chemical methods of water quality assessment.

Material and Methods

Study locations

Araguari is a city located in the state of Minas Gerais, in the north of the Triângulo Mineiro region, near the Jordão River, a tributary of the Paranaíba River.

The collections of samples (water and sediment) were carried out in November 2016 (rainy season) in 8 sample sites (Figure 1), with differing characteristics.

Site 1 (18º44'9.36 "S and 47º57'49.08" W) - Located at Jordão River spring. Field observations showed that the river´s spring is not protected by ciliary forest, being surrounded by corn and soybean cultivation and landing strip for agricultural aircraft.

Site 2 (18º36'51.91 "S and 48º5'56.28" W) - The second collection site is located in the Jordão River before the confluence with the Brejo Alegre Stream. Located near the highway 050 bridge, at the exit to the city of Catalão - GO. At this site the presence of ciliary forest occurs.

Site 3 (18º36'51.91 "S and 48º5'56.28" W) - The third site is centered in the urban area of the city of Araguari, MG, near the John Kennedy Forest, a public reserve of the city. Its geographical coordinates are 18º38'50.70 "S and 48º10'52.49" W. At this site there is occurrence of high levels of pollution, with open sewage disposal and domestic effluent discharges.

Site 4 (18º39'8.54 "S and 48º10 ' 2.60 "O) - The fourth collection site is located in the Brejo Alegre Stream, near to a slaughterhouse, an old tannery, a juice company and an old dump, also centered in the city of Araguari. The situation of this site is critical, with remarkable degradation, presenting strong odor and dark coloration of water. On the field visit, a residual waste of white color was observed, most probably from the plastic bag industry, located in the proximity.

Site 5 (18º37'36.35 "S and 48º8'55.36" W) - Site located near to a large slaughterhouse in the city, other than that mentioned in site 4, and to the landfill. This collection site also presents a high degree of environmental degradation, surrounded by tomato crops, and discharges of sewage from nearby districts, which are distant from site 3. It is worth mentioning that near this site the sewage treatment plant of the city is being built.

Site 6 (18º35'25.62 "S and 48º7'43.39" W) - The sixth sampling site is located in the Jordão River, after the drainage of the Brejo Alegre Stream. At this site there is a ciliary forest, not as dense as in site 2, surrounded by the cultivation of maize, livestock, residences and a rural restaurant.

Site 7 (18º25'43.58 "S and 48º5'58.46" O) - Located upstream Paranaíba River. It precedes the drainage of the Jordão River, so it is considered the control site. There is a ciliary forest on the river banks, a large restaurant nearby, and it is located on BR 050, at the frontier of MG and GO states. It was possible to note the presence of nearby residents, including those on platforms at river banks. There also was personal fishing for their own consumption and consumption of the restaurant along the highway.

Site 8 (18º25'27.31 "S and 48º3'58.69" W) - Located on the Paranaíba River, downstream Jordão River tributary. There is a dense ciliary forest and a large number of fishermen in this region ingesting fish for own consumption and recreation. It is noteworthy that at this point dredges were observed for sand extraction.

Biological material

The fish were obtained from aquarist shops in the city of Araguari-MG. A total of 287 specimens of *Astyanax altiparanae* of both sexes (5 ± 0.4 g of body weight, 7.2 ± 0.2 cm of total length) were transported to the Cytogenetic Laboratory of the

Federal University of Uberlândia, where they were kept in tanks of 20L. The tanks contained reconstituted water (pH 7.5, dissolved oxygen at a rate of 8mg / L and hardness of 43mg $CaCO_3$ / L), where fish remained for 10 days before exposure under controlled conditions of temperature (25ºC), lighting (16: 8 hr light / dark cycle), fed daily with 35 mg of flaky commercial feed and constant aeration. The fish were fed up to 24 hr prior to starting the experiment.

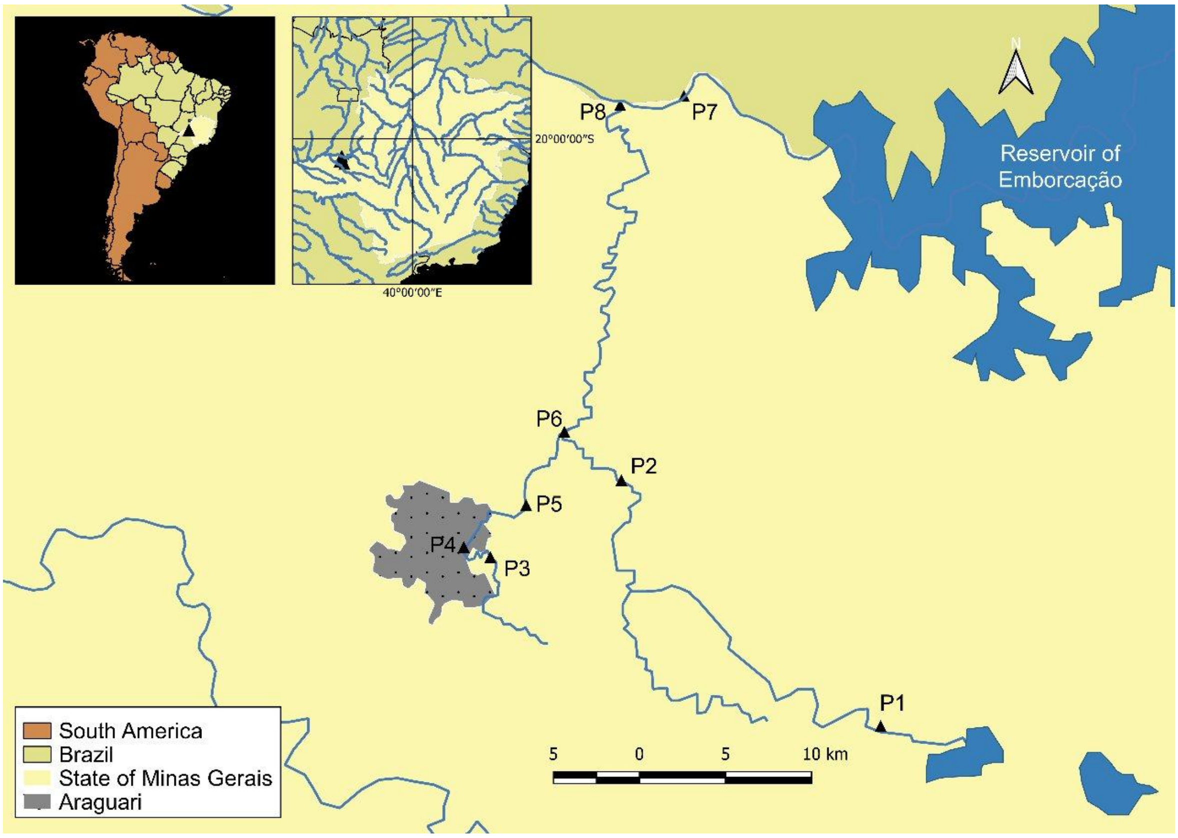
Water and Sediment Samples

In order to evaluate the physicochemical characteristics, the water at the 8 locations were sampled and analyzed according to the procedures in Standard Methods for Examination Water and Wastewater (1998). The parameters analyzed for water and sediment were carried out in collaboration with the Laboratory of Environmental Quality of the Institute of Agrarian Sciences of the Federal University of Uberlândia.

Genotoxicity Biomarkers - Micronucleus frequency test (MN)

In order to carry out the genotoxicity tests, fish were kept on display in a semi-static system, with renewal of the medium every 48 for 168hr. For each site, 5 concentrations were prepared, and 7 fish were subjected in each dilution, including a negative control, totaling a sample of 287 fish. After contact of 168 hr with the water samples from each location, peripheral blood was removed from the branchial artery using sterile 1 ml heparinized syringes, one for each animal. Immediately the smears (blood drop sliding on the microscope slides) were made, which were air dried for 24 hr (Grisolia and Starling 2001). The cells were then fixed with absolute methanol for 10 min, and subjected to the staining process with Giemsa and phosphate buffer (pH = 6.8) in the ratio of 1:20 for 15 min. Four slides were prepared for each animal.

Figure 1: Map of the state of Minas Gerais showing the sites evaluated and the reference site. Brejo Alegre Stream (3, 4 and 5), Jordão river (1, 2 and 6) and Paranaíba river (7 and 8). Reference site: P7.



Four thousand erythrocytes per animal were analyzed under light microscopy (1000x magnification - using immersion oil), according to Schmid (1975). The criteria for identification of micronucleated erythrocytes were that the nuclear particles were required to (1) be smaller and completely separated from the main nucleus;(2) not refractory; (3) with the same shape, staining and intensity of the cell nucleus and within the cellular cytoplasm.

Statistical analysis

One-way ANOVA was used to determine the existence of differences between sites. The sensitivity of the MN frequency test to the presence and concentration of Cr, Ni, Cd, Cu, Zn and Pb was tested using Pearson's correlation. All tests were performed with a minimum significance level of $p<0.01$.

Results and Discussion

The physicochemical parameters for water and sediments are presented in Tables 1 and 2, respectively. For all locations, temperature and pH were below the values established as normal for Environmental Brazilian Council (CONAMA 2005). However, values for turbidity exhibited rates above 100UNT, at sites 1 and 2. For total dissolved solids, sites 2 and 6 presented rates above 500mg / L values that exceeded the parameters. These environmental parameters altered at sites 2 and 5 were found to affect the MN rate suggesting a genotoxic effect of the metal pollutants present in the samples.

Human activities that affect Brejo Alegre Stream water quality at sites 3, 4 and 5 were predominantly based upon untreated sewage discharge from the city of Araguari, MG, and agricultural discharge of pesticides in the Jordão River (locations 1, 2 and 6). Domestic sewage is one of the primary contributors to reduction of environmental quality, since organic matter is the source and input of disease-causing microorganisms. Further, it was necessary to investigate the sediment, since the metals are retained in this material and, depending upon several factors (biotic and abiotic) become resuspended in the water column. Thus it is necessary to monitor and implement sewage networks in order to maintain water quality with high environmental standards.

The levels of aluminum (Al), iron (Fe), manganese (Mn), Zn and Cu metals in the water samples exceeded the permitted limit at different sites (CONAMA 2005). Al and Fe displayed high levels at all analyzed locations except in the Paranaíba River (sites 7 and 8). This may be attributed to sources that are diverse in origin. Aluminium is considered a micro environmental contaminant, and Fe sources include natural rock erosion (Suslick 1998) resulting from activities such as mining, or fertilizers (Sharma et al. 2005), and may be found in municipal and industrial sewage effluents (Klauck et al. 2013). High values of Al and Fe linked to the increase of suspended solids may be associated with the process of soil loss, which was noted downstream and upstream of the Brejo Alegre Stream in the Jordão River, where clear signs of environmental degradation were observed (Personal communication).

For Cu parameter, results were similar and all sites exceeded the environmental limit, except for the reference location (site 7). It is known that small amounts of Cu are essential for the environment under natural conditions, however, excessive amounts may be toxic to fish, microorganisms and humans (Stern et al 2007). The levels of Cu and other elements in stream and river water may have risen due to anthropogenic activities, such as sewage discharge in the urban area of the Brejo Alegre Stream (3 and 4), soybean and coffee farming processes nearby to the Jordão River (1, 2 and 6) and industrials sites, such as slaughterhouses and sanitary landfills (4 and 5).

Manganese levels in the water samples were high, but for the sites of the Jordão River (1, 2 and 6) and Paranaíba River (7 and 8) the values were higher (> 1 mg / ml), when compared to concentrations of the Brejo Alegre Stream (3, 4 and 5) (<1mg / ml). Zinc also exceeded the established limits at the sites of the Jordão River, with levels greater than 0.18mg / ml. The locations 3 and 4 were within the limit for this parameter. It has been Segura Munoz et al (2003) reported that Zn negatively affected the bioavailability of Cu and altered the metabolism of Fe, an essential component of DNA repair proteins and cell maintenance.

In the analysis of metals in sediments, Pb, Zn and Cu presented below the acceptable parameters established by CONAMA Resolution 344/04, at all sites, according to values in Table 2. Lead concentrations were below limits both in water andsediment in this region but, only at site 2 was the level near the limit, which may be attributed to proximity to the industrial urban region, indicating that this may be a possible site of disposal of toxic contaminants. All locations, except control, presented Cr rates above the pre-established values, and Ni exceeded levels at sites 2, 4, 5 and 6. Evidence thus indicated that at the intersection between the Brejo Alegre Stream and the Jordão River there might be an accumulation of metal clusters in this central region of the study. Figure 2 depicts the sites that indicate where the parameters exceeded the environmental limits of safety.

ANOVA was used to assess the different genotoxic responses in *A. altiparanae*. According to the results indicated in Table 3, the tests employing the MN frequency (at all concentrations) demonstrated distinct genotoxic responses at the monitored sites, and the frequencies of MN observed between the concentrations were similar. The incidence of MN at site 2 was significant when compared to other sites referring to all concentrations. It may also be noted that there was a discrepancy at site 5, especially at concentrations of 100 and 25% (Table 3).

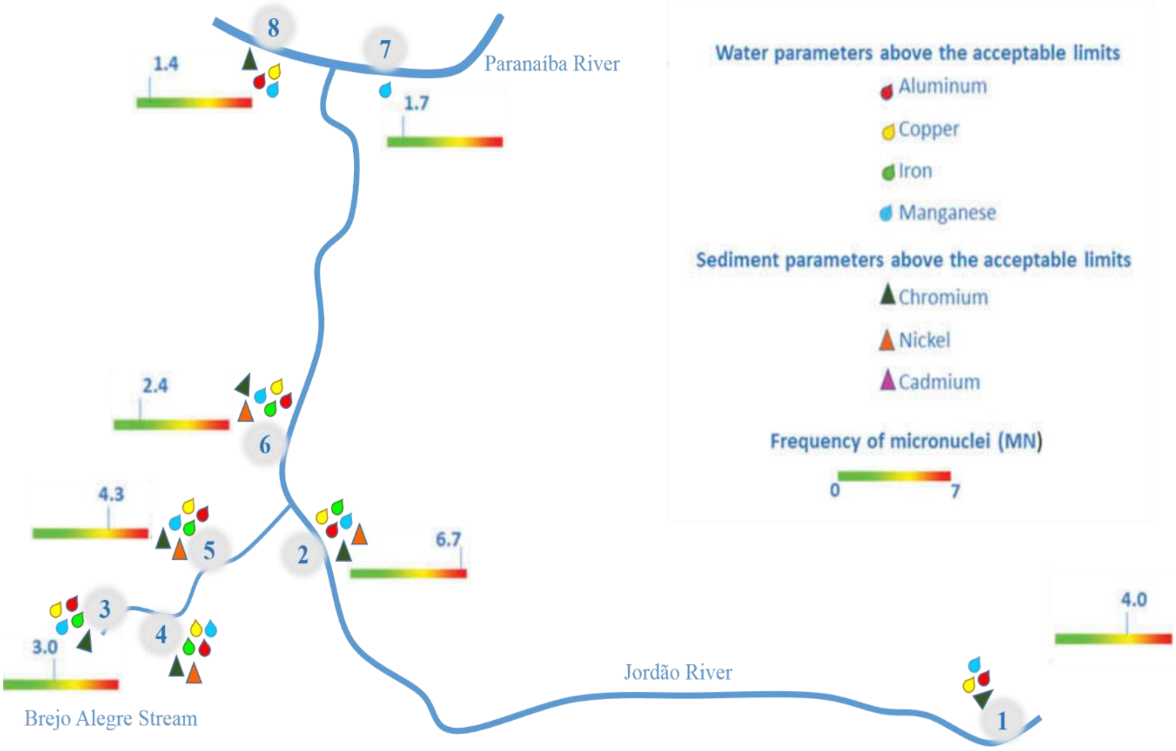
Site 2 presented a higher incidence in the MN rate followed by site 5, which are geographically close. Sites 3 and 4 also showed high MN rates, and are located in the Brejo Alegre stream, a tributary of the Jordão River, upstream of site 5. High values of metals and high MN count were detected at location 1 which is the spring of the Jordão River. The site 8, located on the Paranaíba River, the final receptor site, downstream of the Jordão River, presented lower values of both water and sediment parameters and the amount of MN.

In order to verify the sensitivity of the *Astyanax altiparanae* species found in the Jordão River sub-basin, correlations were made between the observed MN rates (concentration 100%) and levels of Cr, Ni, Cd, Cu, Zn and Pb detected in water and sediment. The results of these correlations indicated that the MN frequency values were moderately sensitive to Cr levels increases followed by Ni in sediments. For water, a weak correlation was found, but not negligible to Cu concentration elevations (Table 4).

The genotoxic potential of Cu was reported in hamster cell lines (Grillo et al. 2009), bacterial strains (Siddiqui et al. 2011), plant cells (Wasi et al. 2013) and animal cells (Erbe et al. 2011). It is believed that Cu contributes to significant toxicogenetic changes , since this metal modifies the activity of antioxidant enzymes, which induces and aggravates oxidative stress (Stern et al 2007;

Lushchak 2011). Fish exposed to Cu showed a rise in the primary and secondary activities of the oxidative enzymes (Hansen et al 2006). In addition, other investigators noted that Cu enhanced cytotoxicity and reactive oxygen species (ROS) production, resulting in increased breaks in DNA chain (Bopp et al. 2008).

Figure 2. Representative map showing the sub-basin of the Jordan River, indicating with different colors the sites with parameters above that pre-established by the CONAMA legislation. In detail, the 8 sites where the experiments were carried out: Brejo Alegre Stream (3, 4 and 5), Jordão river (1, 2 and 6) and Paranaíba river (7 and 8).



Klobucar et al (2003) reported an increase in MN frequency in vertebrate aquatic species of contaminated water compared to control sites. . In this study, data demonstrated that the highest frequency of MN was found at sites 1, 2 and 5, considered to be underdeveloped areas, but surrounded by agricultural activities. The lowest values were found in the Paranaíba River (sites 7 and 8) located in rural areas, not urbanized and geographically distant from the other sites. The highly urbanized and industrially impacted sites 3 and 4 exhibited evident environmental degradation. Although not the sites with the highest MN rates, the Brejo Alegre Stream was considered to be the most contaminated of Araguari due to the reception of wastewater, containing high concentrations of pollutants, which accumulate in sediments, and consequently no fish were found in these locations. The significant cytotoxicity of the pollutants in this area induced high mortality rates which may explain the observed low frequency in MN rates due to the absence of fish.

In conclusion data demonstrated that *Astyanax altiparanae* constituted a sensitive, reliable species to be used in in detecting genotoxic effects resulting from exposure to the water samples collected near the discharge of industrial and domestic waste. These findings reinforce the importance of using biomarkers of genotoxicity with tropical species as a complementary parameter to conventional physical-chemical methods of water quality assessment.

Acknowledgements

Financial support is acknowledge to “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (Capes).

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CONSIDERAÇÕES FINAIS

Ao chegarmos ao final deste trabalho, concluímos, a partir dos experimentos *in situ*, que os peixes empregados nos bioensaios responderam sensivelmente às diferentes condições de exposição aos contaminantes testados.

No primeiro estudo (capitulo 2), as variações na frequência de micronúcleo (MN) e anormalidades nucleares (ENAs) foram significativamente maiores nos peixes expostos às amostras de água coletadas em locais que recebiam efluentes contaminados, especialmente com resíduos de agroquímicos. Assim, locais onde as concentrações de cobre (Cu) foram maiores foram positivamente correlacionados a maior potencial genotóxico. Os dados demonstraram que o *Cichlasoma paranaense*, utilizado no teste, exibiu sensibilidade confiável e que não houve diferença significativa entre os tecidos (cauda e brânquia) analisados.

Posteriormente, no segundo experimento (capitulo 3), foi determinado que áreas contaminadas por Al, Fe, Mn, Zn e Cu, presentes na água e no sedimento de diferentes locais do rio Jordão e do córrego Brejo Alegre, afluentes do rio Paranaíba, que abastecem a cidade de Araguari, localizada no estado de Minas Gerais, induziram respostas biológicas em *Astyanax altiparanae*, de forma provocar danos genotóxicos e mutagênicos.

Em conjunto, estes estudos representam uma pesquisa pioneira na região de Araguari, MG, que não apresentava uma estação de tratamento de esgoto (ETE) até agosto de 2019. Agora, após início do funcionamento da ETE, esta pesquisa poderá servir de referência para o desenvolvimento de pesquisas futuras, voltadas para a produção de conhecimentos sobre a qualidade da água de rios e córregos da região e em todo o Brasil.

Ainda, foi possível concluir que os peixes neotropicais, tais como os Acarás (*Cichlasoma paranaense*) e Lambaris (*Astyanax altiparanae*), podem ser usados como organismos sentinela em programas de biomonitoramento ambiental.

Finalmente, reiteramos que a realização de programas de biomonitoramento ambiental com base em parâmetros ecotoxicológicos, *in situ,* usando peixes como organismos sentinela, em complemento às análises físico-químicas, oferece ao observador-pesquisador uma maior aproximação à realidade ambiental dos ecossistemas aquáticos, uma vez que os peixes respondem rápida e sensivelmente aos contaminantes, especialmente quando biomarcadores de genotoxicidade são incluídos ao conjunto de testes.

TABELAS

CAPÍTULO I

Table 01. Representation of the individuals analyzed, indicating: reference of the study, geographic location, source of pollution, native species, means and standard deviations of the amount of micronucleus (MNs) recorded in 1000 erythrocyte cells of the peripheral blood of the studied fish species.

REFERENCE	LOCATION	POLLUTANTS	NATIVE FISH SPECIES	MICRONUCLEI (‰)	
				In refence site	In polluted site
Bianchi, et al. 2019	29º48'59.08"S, 50º30'49.97"W	Urban wastewater discharges	*Astyanax jacuhiensis*	0.15 ± 0.24	0.25 ± 0.26
	29º39'30.27"S, 50º30'49.97"W			0.15 ± 0.24	0.00 ± 0.00
	29º37'42.40"S, 50º49'43.57"W			0.15 ± 0.24	0.15 ± 0.34
	29º40'37.49"S, 51º03'24.96"W			0.15 ± 0.24	0.25 ± 0.35
	29º51'11.16"S, 51º10'39.27"W			0.15 ± 0.24	0.05 ± 0.16
Campos Júnior et al., 2016	18°52′37.632″S,48°17′38.04″W	Urban waste and irregular/clandestine domestic sewage discharge	*Rhamdia quelen*	0.05 ± 0.03	0.19 ± 0.10*
			Geophagus brasiliensis	0.04 ± 0.02	0.18 ± 0.07*
	18°59′12.624″S,48°12′41.61″W	Industrial activities, including textile, food, metallurgical, tanning, and chemical manufacturing	*Rhamdia quelen*	0.05 ± 0.04	0.70 ± 0.32*
			Geophagus brasiliensis	0.04 ± 0.02	0.63 ± 0.22*
			18°54′54.216″ S - 48°18′36.504″ W (Reference site)		
Campos Júnior et al., 2015	18º43'63.03"S, 47º29'56.04" W	Area of residential flow and little industrial activity	*Rhamdia quelen*	0.08 ± 0.01	0.24 ± 0.06*
			Geophagus brasiliensis	0.06 ± 0.01	0.28 ± 0.09*
	18º41'93.07"S, 47º29'41.07"W	Region of residential flow and intense industrial pottery activity	*Rhamdia quelen*	0.07 ± 0.01	0.66 ± 0.22*
			Geophagus brasiliensis	0.06 ± 0.02	0.59 ± 0.12*
			18º44'39.9" S – 47º29'93.6" W. (Reference site)		
de Jesus, et al., 2016	13º52'10.63"S, 40º13'38.93"W	Domestic effluents			0.12 ± 0.07
	13º51'59.61"S, 40º04'38.61"W	Urban effluents and untreated wastes of artisanal tanneries			0.00 ± 0.00
	13º53'49.39"S, 40º02'29.58"W	Small human population. The margins are surrounded by natural vegetation;	*Serrasalmus brandtii*	0.11 ± 0.06	0.17 ± 0.13
	13º55'40.21"S, 40º01'19.73"W	Rural area			0.17 ± 0.17
	14º11'43.37"S, 39º39'31.32"W	Rural area and river margins are deforested. Region of nickel mining.			0.00 ± 0.00

	14º13'05.23"S, 39º31'10.48"W	Domestic effluents. Sand is extracted for civil construction.			0.14 ± 0.14
	13º52'10.63"S, 40º13'38.93"W	Domestic effluents			0.33 ± 0.33
	13º55'40.21"S, 40º01'19.73"W	Rural area	*Hoplias malabaricus*	0.14 ± 0.08	0.75 ± 0.43
	14º11'43.37"S, 39º39'31.32"W	Region of nickel mining.			0.25 ± 0.18
	14º13'05.23"S, 39º31'10.48"W	Domestic effluents.			0.13 ± 0.13
	13º53'49.39"S, 40º02'29.58"W	Natural vegetation;	*Geophagus brasiliensis*	–	0.07 ± 0.07
	13º55'40.21"S, 40º01'19.73"W	Rural area			0.14 ± 0.06
			13º51'56.07"S - 40º14'10.19"W - (Reference site)		
			Rhamphichthysmarmoratus	0.013 ± 0.023	
			Steatogenys elegans	0.006 ± 0.015	
			Sternopygus macrurus	0.019 ± 0.030	
	03°01'41.80"S, 64°51'16.60"W		*Parapteronotus hasemani*	0.033 ± 0.023	
			Gymnotus mamiraua	0.007 ± 0.022	
			Gymnotus arapaima	0.005 ± 0.012	
Melo, et al., 2013			*Brachyhypopomus beebei*	0.023 ± 0.030	
			Brachyhypopomus n. sp	0.164 ± 0.258	
	01°15'38,20"S, 49°28'42,20"W			0.017 ± 0.019	
	01°45'49,80"S, 49°43'53,04"W		*Sternopygus macrurus*	0.013 ± 0.030	
	03°07'09,04"S, 64°47'24,30"W			0.077 ± 0.106	
	01°37'23,49"S, 48°55'33,00"W	Influence of a bauxite mining region of many aluminum industries.			0.048 ± 0.065
Vieira et al., 2014	23°09'39.2 "S, 50º35'52.4"W	Artificial impoundment used for aquaculture.		0.50 ± 0.18	2.85 ± 0.69*
	23°10'5.2 "S, 50°33'18.3"W	Close to wheat and corn fields	*Astyanax altiparanae*	0.50 ± 0.18	1.25 ± 0.25*
	23°09'38.4"S, 50°31'27"W	Corn and wheat crops.		0.50 ± 0.18	2.37 ± 0.32*
	23°09'48.5"S, 50°30'08.9"W	Proximity to wheat and corn crops		0.50 ± 0.18	1.66 ± 0.28*

	23°09’59.0”S, 50°28’57.0”W	The region of the stream is characterized by intensive agricultural activity.		0.50 ± 0.18	3.62 ± 0.37*
			23°09’23.6”S 50°34’13.8”W (Reference site)		
Dalzochio et al., 2018 a					0.45 ± 0.37*
	29°71′63.31″ S, 50°71'49.34″W	Agricultural activities			0.05 ± 0.16
					0.30 ± 0.35
					0.22 ± 0.37
					0.15 ± 0.33
					0.00 ± 0.00
	29°69′18.48″S, 50°74′67.42″W	Agricultural activities	*Bryconamericus iheringii*		0.11 ± 0.22
					0.50 ± 0.46
					0.15 ± 0.33
					0.09 ± 0.20
	29°68′62.29″S, 50°85′09.88″W	Urban area and industrial effluents (mainly leather and footwear).			0.15 ± 0.28
					0.07 ± 0.14
Dalzochio et al., 2018 b			*Bryconamericus iheringii*		0.33 ± 0.34
	29°40'56.42"S, 50°44'22.86"W	Agricultural inputs (mainly cattle farming and rice fields)	*Diapoma alburnus*		0.18 ± 0.32
			Hyphessobrycon luetkenii		0.05 ± 0.15
			Bryconamericus iheringii		0.08 ± 0.15
	29°41'9.77"S, 50°48'35.14"W	Domestic and industrial effluents (logging, leather and footwear industries)	*Diapoma alburnus*		0.16 ± 0.28
			Hyphessobrycon luetkenii		0.06 ± 0.22
Francisco et al., 2019	18°44'9.36“S, 47°57'49.08”W				4.00 ± 0.70*
	18°36'51.91“S, 48°5'56.28”W				6.07 ± 0.80*
	18°36'51.91“S, 48°5'56.28”W		*Astyanax altiparanae*	1.4 ± 0.8	3.00 ± 0.70
	18°39'80.54“S, 48°10‘2.60“W				2.02 ± 1.00
	18°37'36.35“S, 48°8'55.36”W				4.03 ± 0.70*

	18°35'25.62“S, 48°7'43.39”W				2.04 ± 1.10
	18°25'27.31“S, 48°3'58.69”W				1.07 ± 1.00
			18°25'43.58 “S, 48°5'58.46”W (Reference site)		
Bogoni et al., 2014	27º04'41.40"S, 52º08'12.8"W	Pig farming and urban sewage.	*Astyanax bimaculatus*	0.022 ± 0,04	0.065 ± 0.10
	27º15'14.23"S, 52º19'35.96"W			0.022 ± 0,04	0.050 ± 0.11
Dalzochio et al., 2017	29º40’56.42”S, 50º44’22.86”W	Agricultural area	*Bryconamericus iheringii*	0.08 ± 0.41	0.15 ± 0.47
				0.08 ± 0.15	0.33 ± 0.34*
			29º40’56.42”S and 50º44’22.86”W (Reference site)		
Morais et al., 2016	18º43'36.7"S, 47º29'35.9"W	Domestic sewage	*Geophagus brasiliensis*	1.02 ± 0.83	03.33 ± 00.77
	18º42'86.3"S, 47º29'67.6"W	Domestic and industrial sewages			11.50 ± 02.23*
	18º41'24.6"S, 47º28'98.1"W	Agricultural activities			09.41 ± 02.27*
			18º44'19.7"S, 47º29'47.6"W (Reference site)		
Silva et al., 2016	18°10’58.69”S, 47°54’28.46”W	Fertilizer industry	*Astyanax fasciatus*	0.01 ± 0.01	0.08 ± 0.02*
			Astyanax altiparanae	0.01 ± 0.01	0.08 ± 0.03*
			Characidium fasciatum	0.01 ± 0.01	0.04 ± 0.01*
	18°11’24.18”S, 47°55’17.45”W	Urban perimeter	*Astyanax fasciatus*	0.01 ± 0.01	0.08 ± 0.03*
			Astyanax altiparanae	0.01 ± 0.01	0.12 ± 0.03*
			Characidium fasciatum	0.01 ± 0.01	Not determined
	18°14’04.46”S,47°49’18.78” W	Urban perimeter	*Astyanax fasciatus*	0.01 ± 0.01	0.03 ± 0.01
			Astyanax altiparanae	0.01 ± 0.01	0.07 ± 0.02*
			Characidium fasciatum	0.01 ± 0.01	0.07 ± 0.02*
			18°11’37.63”S, 47°53’52.18”W (Reference site)		

* Indicates sensitivity.

Table 02. Representation of the individuals analyzed, indicating: study reference, geographical location, source of pollution, native species, means and standard deviations of nuclear abnormalities (ENAs) recorded in 1000 erythrocyte cells from the peripheral blood of the studied fish species.

REFERENCE	LOCATION	POLLUTANTS	NATIVE FISH SPECIES	MICRONUCLEI (‰) In refence site	MICRONUCLEI (‰) In polluted site
Bianchi., et al. 2019	29º48'59.08"S, 50º30'49.97"W	Urban wastewater discharges	*Astyanax jacuhiensis*	3.80 ± 2.44	4.60 ± 4.48
	29º39'30.27"S, 50º30'49.97"W			3.80 ± 2.44	2.75 ± 1.90
	29º37'42.40"S, 50º49'43.57"W			3.80 ± 2.44	4.00 ± 3.03
	29º40'37.49"S, 51º30'24.96"W			3.80 ± 2.44	3.80 ± 2.57
	29º51'11.16"S, 51º10'39.27"W			3.80 ± 2.44	3.20 ± 1.90
Campos Júnior et al., 2015	18º43’63.3” S, 47º29’56.4”W	Area of residential flow and little industrial activity	*Rhamdia quelen*	0.09 ± 0.04	2.12 ± 0.05*
			Geophagus brasiliensis	0.08 ± 0.05	1.76 ± 0.05*
	18º41’93.7”S, 47º29’41.7”W	Region of residential flow and intense industrial pottery activity	*Rhamdia quelen*	0.09 ± 0.04	5.15 ± 4.43*
			Geophagus brasiliensis	0.08 ± 0.05	3.96 ± 2.89*
			18º44’39.9” S – 47º29’93.6” W. (Reference site)		
de Jesus, et al., 2016	13º52'10.63"S, 40º13'38.93"W	Domestic effluents	*Serrasalmus brandtii*	1.63 ± 0.32	1.85 ± 0.37
	13º51'59.61"S, 40º04'38.61"W	Urban effluents and untreated wastes of artisanal tanneries			8.92 ± 2.20*
	13º53'49.39"S, 40º02'29.58"W	The margins are surrounded by natural vegetation;			4.83 ± 1.48*
	13º55'40.21"S, 40º01'19.73"W	Rural area			4.33 ± 1.05*
	14º11'43.37"S, 39º39'31.32"W	Region of nickel mining.			3.72 ± 1.01
	14º13'05.23"S, 39º31'10.48"W	Domestic effluents. Sand is extracted for civil construction.			2.57 ± 0.92
	13º52'10.63"S, 40º13'38.93"W	Domestic effluents	*Hoplias malabaricus*	5.00 ± 0.79	2.33 ± 0.33
	13º55'40.21"S, 40º01'19.73"W	Rural area			29.50 ± 8.71*

	14º11'43.37"S, 39º39'31.32"W	Rural area and river margins are deforested. Region of nickel mining.			8.75 ± 2.93
	14º13'05.23"S, 39º31'10.48"W	Domestic effluents. Sand is extracted for civil construction.			6.13 ± 1.26
	13º53'49.39"S, 40º02'29.58"W	The margins are surrounded by natural vegetation;	*Geophagus brasiliensis*	–	7.20 ± 1.16
	13º55'40.21"S, 40º01'19.73"W	Rural area			3.00 ± 0.56
			13º51'56.07"S - 40º14'10.19"W - (Reference site)		
Melo, et al., 2013	03°01’41.8”S, 64°51’16.6”W		*Rhamphichthysmarmoratus*	0.02 ± 0.02	
			Steatogenys elegans	0	
			Sternopygus macrurus	0.13 ± 0.18	
			Parapteronotus hasemani	0.14 ± 0.13	
			Gymnotus mamiraua	0.03 ± 0.04	
			Gymnotus arapaima	0.06 ± 0.09	
			Brachyhypopomus beebei	0.08 ± 0.13	
			Brachyhypopomus n. sp	0.15 ± 0.14	
	01°15'38,2 ”S, 49°28'42,2”W		*Sternopygus macrurus*	0.04 ± 0.04	
	01°45'49,8 ”S, 49°43'53,4”W			0.15 ± 0.08	
	03°07'09,4”S, 64°47'24,3”W			0.25 ± 0.37	
	01°37'23,49”S, 48°55'33”W	Influence of a bauxite mining region of many aluminum industries.			0.50 ± 0.24*
Vieira et al., 2014	23°09’39.2 ”S, 50º35’52.4”W	Artificial impoundment used for aquaculture.	*Astyanax altiparanae*	1.57 ± 0.29	3.71 ± 0.83*
	23°10’5.2 ”S, 50°33’18.3”W	Close to wheat and corn fields		1.57 ± 0.29	2.87 ± 0.89*
	23°09’38.4”S, 50°31’27”W	Corn and wheat crops.		1.57 ± 0.29	4.50 ± 0.56*
	23°09’48.5”S, 50°30’08.9”W	Proximity to wheat and corn crops		1.57 ± 0.29	3.57 ± 0.84*
	23°09’59.0 ”S, 50°28’57.0”W	The region of the stream is characterized by intensive agricultural activity.		1.57 ± 0.29	6.14 ± 0.85*
			23°09’23.6”S 50°34’13.8”W (Reference site)		

Dalzochio et al., 2018a	29°71′63.31″S,50°71'49.34″W	Agricultural activities	Bryconamericus iheringii	3.55 ± 2.33
				2.40 ± 1.31
				4.55 ± 3.13
				3.52 ± 2.36
	29°69′18.48″S,50°74′67.42″W	Agricultural activities		3.05 ± 3.53
				3.00 ± 2.83
				2.35 ± 0.92
				2.78 ± 1.38
	29°68′62.29″S,50°85′09.88″W	Urban area and industrial effluents (mainly leather and footwear).		2.85 ± 2.62
				4.23 ± 2.45
				2.89 ± 2.95
				3.43 ± 2.26
Dalzochio et al., 2018 b	29°40'56.42"S,50°44'22.86"W	Agricultural inputs (mainly cattle farming and rice fields)	*Bryconamericus iheringii*	3.19 ± 3.22
			Diapoma alburnus	2.18 ± 1.25
			Hyphessobrycon luetkenii	4.50 ± 2.31
	29°41'9.77"S, 50°48'35.14"W	Domestic and industrial effluents (logging, leather and footwear industries)	*Bryconamericus iheringii*	2.28 ± 1.59
			Diapoma alburnus	3.25 ± 2.77
			Hyphessobrycon luetkenii	3.95 ± 2.32
Baudou et al., 2019	34°41′03.5″S, 58°51′15.5″W	Domestic, agricultural and industrial sewages	*Cnesterodon decemmaculatus*	2.58 ± 0.83

Dalzochio et al., 2017	29º40’56.42”S, 50º44’22.86”W	Agricultural area	*Bryconamericus iheringii*	3.50 ± 7.43 1.50 ± 1.34	3.70 ± 4.20 3.19 ± 3.22*
				29º40’56.42”S - 50º44’22.86”W (Reference site)	

* Indicates sensitivity

CAPÍTULO II

Table 1. General characteristics of the sampling sites. (* Dry period; ** Rainy period).

Physicochemical parameters of water	COLLECTION SITES										
	PAR		GRD		TIJ		ARA		LEGAL PARAMETERS		
	*	**	*	**	*	**	*	**	C1	C2	C3
True color (Pt/L)	19	26	56	89	12	201	17	49	-	-	-
Turbidity (UNT)	5,4	5,9	5,1	11,3	0,59	12,2	1,19	11,5	40.0	100.0	100.0
pH	6,65	6,23	6,71	7,2	6,79	7,1	5,74	7,2	6.0 - 9.0	6.0 - 9.0	6.0 - 9.0
DQO (mg/L)	ND	ND	ND	71	ND	ND	ND	ND	-	-	-
DBO (mg/L)	1	6,8	1	6,7	1	6,8	1	7,2	-	-	-
Total Dissolved Solids (mg/ml)	ND	63	15	ND	115	71	36	43	500	500	500
Total Phosphorus (lotics) (mg/L)	0,5	0,1	0,2	ND	0,3	ND	0,2	ND	0.01	0.1	0.15
Oils and greases (mg/L)	82	186	335	602	55	40	266	21	-	-	-
Chlorides (mg/L)	2,94	3,92	3,92	4,9	1,96	2,94	2,94	3,92	250	250	250
Residual chlorine (mg/L)	0,07	ND	0,05	ND	0,05	ND	0,06	ND	-	0.01	0.01
Iron (mg/L)	0,07	0,69	0,47	0,75	0,42	3,5	0,25	0,83	0.3	0.3	5.0
Nitrate (mg/L)	ND	ND	0,37	ND	0,24	ND	0,075	ND	10.0	10.0	10.0
Nitrites (mg/L)	ND	ND	ND	ND	4	ND	ND	ND	1.0	1.0	1.0
Sulfates (mg/L)	ND	ND	ND	ND	ND	ND	5	ND	250	250	250

*Above the values established by the resolution CONAMA430/11. ND = Not detected.

Table 2. Physical parameters of sediments. (* Dry period; ** Rainy period).

PARAMETERS	COLLECTION SITES								LEGAL PARAMETERS	
	PAR		GRD		TIJ		ARA		Minimum value	Maximum value
	*	**	*	**	*	**	*	**		
Moisture %	35,56	52,8	31,97	22,4	56,57	27,3	50,31	32,5		
Volatile Solids%	6,63	14,7	6,65	4,7	4,29	8,4	9,16	10,5		
Fixed Solids%	93,37	85,3	93,35	95,3	95,71	91,6	90,34	89,5		
SiO_2%	34,47	11,82	62,63	36,85	28,31	35,63	34,33	23,7		
Sodium (mg/Kg ppm)	14,55	2021	113,7	2664	73,17	1896	129,78	2757		
Manganese (mg/Kg ppm)	27,21	72,1	700,82	722,4	75,89	148,6	237,69	989,3		
Copper (mg/Kg ppm)	95,15	118	214,98	237	50,43	113	155,75	261	35.7	197.0
Iron (mg/Kg ppm)	859,77	2058	2356,12	2751	745,61	2086	1757,57	3131		
Zinc (mg/Kg ppm)	26,48	95,2	76,27	102	NO	ND	17,63	131	123.0	315.0
Silver (mg/Kg ppm)	ND	ND	ND	ND	45,64	ND	264,97	ND		

*Above the values established by the resolution CONAMA 344/04. ND = Not detected.

Table 3. $LC_{15\text{-}96h}$ and behavioral changes of *Cichlasoma paranaense* exposed to different concentrations of water samples from monitored sites during 96hr.

Site	$LC_{15,96h}$	LOSS OF EQUILIBRIUM		ERRATIC SWIMMING		LETARGY		IMMOBILITY	
		NOEC	LOEC	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC
PAR	117.4 (57.7-238.7)	100	-	100	-	12.5	25	100	-
ARA	135.1 (66.4-274.8)	100	-	25	50	100	-	100	-
GRD	144.6 (71.1-294.1)	0	6.25	100	-	100	-	100	-
TIJ	154.5 (76.0-314.3)	100	-	100	-	100	-	100	-

Table 4. Nuclear Abnormalities and Micronucleus in Erythrocytes of *Cichlasoma paranaense* exposed to water samples of monitored sites.

	Nuclear Abnormalities											
Site	Micronuclei		Notched		Lobbed		Blebded		Binucleated cells		Kariolysis	
	Tail	Gill	Tail	Gill	Tail	Gill	Tail	Gill	Tail	Gill	Tail	Gill
PAR	2.0 ± 1.9b	0.7 ± 0.9ab	0.2 ± 0.4a	0 ± 0a	0 ± 0a	0.2 ± 0.63a	0.8 ± 1.03a	0.7 ± 1.33a	0.1 ± 0.31a	0 ± 0a	0.1 ±0.3a	0.3 ± 0.9a
ARA	1.7 ± 1.8b	1.1± 1.4ab	0 ± 0a	0 ± 0a	0.9 ± 1.3a	0.4 ± 0.9a	0.3 ± 0.4a	0.4 ± 0.6a	0.5 ± 1.6a	0 ± 0a	0.2 ± 0.6a	0 ± 0a
GRD	2.4 ± 1.9b	2.1 ± 1.2b	0 ± 0a	0.1 ± 0.3a	1.1 ± 0.7a	1.1 ± 1.2a	1.4 ± 2.0a	1.2 ± 1.5a	0.6 ± 1.3a	0.3 ± 0.6a	0.3 ± 0.7a	0.1 ± 0.3a
TIJ	0.3 ± 0.7a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0.1 ± 0.3a	0.1 ± 0.3a	0 ± 0a	2.3 ± 5.3a	1.1 ± 1.9a	0.2 ± 0.6a	0 ± 0a

Student's t-test for comparison between tissues. *indicates significant difference ($p<0.05$). ANOVA – one way; Tukey for comparisons between sites. Significant differences are indicated by different letters.

CAPÍTULO III

Table 1. General characteristics of the 8 sampling sites.

Physicochemical parameters of water	COLLECTION SITES										
	1	2	3	4	5	6	7	8	CONAMA 357/05		
									C1	C2	C3
Temperature (°C)	23.63	24.05	18.81	19.18	16.59	19.44	22.77	22.68	-	-	-
Turbidity (UNT)	136.0	204.0	0.0	1.9	12.1	59.2	3.9	4.9	40.0	100.0	100.0
pH	7.34	7.43	9.0	7.66	7.1	8.27	7.24	7.12	6.0 - 9.0	6.0 - 9.0	6.0 - 9.0
Dissolved Oxygen (mg/ml)	2.6	2.58	2.85	3.59	4.49	3.62	_	_	> 6.0	5.0	4.0
Tot Sol. Dis.. (mg/ml)	450.0	532.0	4.0	127.0	24.0	641.0	49.0	52.0	500.0	_	500.0
Aluminum (mg/ml)	0.521*	0.425*	0.303*	0.230*	0.283*	0.271*	0.051	0.401	0.1	0.1	0.2
Barium (mg/ml)	0.041	0.058	0.021	0.015	0.029	0.080	0.012	0.025	0.7	0.7	1.0
Cadmium (mg/ml)	0.0001	0.0001	ND	ND	ND	ND	0.0001	0.0002	0.001	0.001	0.01
Chromium (mg/ml)	0.009	0.0028	0.0018	0.0038	0.0016	0.0016	0.0008	0.0019	0.05	0.05	0.05
Cobalt (mg/ml)	0.0015	0.0014	0.0009	0.0006	0.0015	0.0014	0.0004	0.0014	0.05	0.05	0.2
Copper (mg/ml)	0.0429*	0.0271*	0.0253*	0.0291*	0.0292*	0.0275*	0.0016	0.0220*	0.009	0.009	0.013
Iron (mg/ml)	0.6053*	1.0982*	0.1806*	0.4482*	0.6523*	1.5090*	0.0385	0.1452	0.3	0.3	5.0
Lead (mg/ml)	0.0006	0.0044	0.0044	ND	0.0007	0.0037	0.0027	ND	0.01	0.01	0.033
Manganese (mg / ml)	1.5162*	1.8869*	0.1918*	0.4312*	0.5358*	1.8957*	1.3903*	1.5385*	0.1	0.1	0.5
Nickel (mg / ml)	0.0048	0.0101*	0.0012	0.0055	0.0050	0.0035	0.0053	0.0059	0.025	0.025	0.025
Silver (mg / ml)	0.0032	0.0006	0.0022	0.0041	0.0020	0.0008	0.0019	0.0035	0.01	0.01	0.05
Vanadium (mg / ml)	0.0073	0.0075	0.0063	0.0071	0.0074	0.0077	0.0070	0.0072	0.1	0.1	0.1
Zinc (mg / ml)	0.4497*	0.3025*	0.1468	0.1509	0.1017	0.1873*	0.0311	0.0996	0.18	0.18	5.0

Beryllium (mg / ml)	0.0001	0.0002	0.0001	0.0001	0.0002	0.0002	0.0002	0.0002
Bismuth (mg / ml)	ND	0.0002	ND	ND	ND	ND	ND	0.0008
Calcium (mg / ml)	10.2859	8.2249	2.4502	1.5115	2.2206	8.0832	2.9566	3.6720
Gallium (mg / ml)	0.0008	0.0008	0.0027	0.0008	0.0012	0.0026	0.0013	0.0029
Potassium (mg / ml)	11.4890	6.9770	0.3520	0.5840	0.5160	7.2980	1.0740	1.1830
Lithium (mg / ml)	0.0050	0.0050	0.0040	0.0040	0.0040	0.0040	0.0040	0.0040
Molybdenum (mg/ml)	0.0421	0.0040	0.0020	0.0020	0.0020	0.0020	0.0010	0.0020
Sodium (mg / ml)	40.9090	37.1760	0.6210	0.7970	2.3220	27.3240	1.3190	1.4540
Rubidium (mg / ml)	0.0110	0.0180	0.0040	0.0030	0.0030	0.0130	0.0050	0.0030
Strontium (mg / ml)	0.0470	0.0400	0.0100	0.0080	0.0120	0.0460	0.0200	0.0230

*Above the values established by the resolution CONAMA357/05. ND = Not detected.

Table 2. Metal analysis of sediments.

PARAMETERS	COLLECTION SITES								CONAMA 344/04(mg kg-1)	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Minimum value	Maximum value
Cadmium	3.0	3.0	0.3	4.9*	3.2	3.2	1.2	2.2	0.6	3.5
Chromium e	110.7*	113.3*	149.4*	181.4*	130.9*	152.5*	83.5	104.8*	37.3	90.0
Copper	179.8	172.9	36.0	131.4	143.0	157.2	37.7	79.7	35.7	197.0
Nickel	30.6	45.2*	18.0	140.8*	42.0*	44.9*	25.8	29.1	18.0	35.9
Lead	18.0	14.4	10.8	20.3	20.5	16.4	27.5	19.5	35.0	91.3
Zinc	124.4	112.0	28.2	93.4	133.2	123.8	67.6	115.6	123.0	315.0

*Above the values established by the resolution CONAMA344/04.

Table 3. Frequency of Micronuclei (MN) for *A. altiparanae* obtained at sites 1-8.

	Frequency *MN (mean + SD)*							
Concentrations	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***
100%	4.0* ± 0.79	6.7* ± 0.88	3.0 ± 0.77	2.2 ± 1.09	4.3* ± 0.77	2.4 ± 1.16	1.4 ± 0.85	1.7 ± 1.0
50%	2.1 ± 0.85	5.0* ± 0.62	1.4 ± 1.13	1.1 ± 0.75	2.4 ± 1.12	0.6 ± 0.68	0.9 ± 0.84	0.9 ± 0.75
25%	2.1 ± 0.83	3.1* ± 1.23	1.4 ± 0.90	1.0 ± 0.79	3.1* ± 1.23	1.0 ± 0.95	0.7 ± 0.74	1.4 ± 1.08
12,5%	1.4 ± 0.87	3.9* ± 1.39	0.7 ± 0.80	0.6 ± 0.71	1.5 ± 1.37	0.4 ± 0.68	0.4 ± 0.74	1.0 ± 0.98
6,25%	0.6 ± 0.88	3.5* ± 0.65	0.3 ± 0.74	0.4 ± 0.70	1.6 ± 1.43	0.6 ± 0.73	0.3 ± 0.74	1.5 ± 1.15
0%	0.2 ± 0.81	0.2 ± 0.81	0.2 ± 0.81	0.2 ± 0.81	0.2 ± 0.81	0.2 ± 0.81	0.2 ± 0.81	0.2 ± 0.81

* $p<0.01$

Table 4. Pearson correlation coefficient between metal concentrations and Micronucleus test in fish cells.

	Sediments		**Water**	
	r^2	p	r^2	p
Chromium	0.390*	<0.0001	0.007	0.506
Nickel	0.270*	<0.0001	0.010	0.426
Cadmium	0.144	0.002	_	_
Copper	0.059	0.054	0.161*	0.001
Zinc	0.021	0.253	0.017	0.303
Lead	0.027	0.195	0.017	0.300

* p<0.01

ANEXOS

ANEXO 01

Universidade Federal de Uberlândia

– Comissão de Ética na Utilização de Animais –

Comissão de Ética na Utilização de Animais CEUA

CERTIFICADO

Certificamos que o projeto intitulado "Biomonitoramento da qualidade da água e análises ecotoxicológicas de peixes da bacia do rio Paranaíba e Grande", protocolo nº 040/16, sob a responsabilidade de **Sandra Morelli** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata, para fins de pesquisa científica – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **APROVADO** pela COMISSÃO DE ÉTICA NA UTILIZAÇÃO DE ANIMAIS (CEUA) da UNIVERSIDADE FEDERAL DE UBERLÂNDIA, em reunião de **06 de maio de 2016.**

(We certify that the project entitled "Biomonitoramento da qualidade da água e análises ecotoxicológicas de peixes da bacia do rio Paranaíba e Grande", protocol 040/16, under the responsibility of Sandra Morelli - involving the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata, for purposes of scientific research - is in accordance with the provisions of Law nº 11.794, of October 8th, 2008, of Decree nº 6.899 of July 15th, 2009, and the rules issued by the National Council for Control of Animal Experimentation (CONCEA) and it was approved for ETHICS COMMISSION ON ANIMAL USE (CEUA) from FEDERAL UNIVERSITY OF UBERLÂNDIA, in meeting of May 06th, 2016).

Vigência do Projeto	Início: 01/08/2016 Término: 21/08/2020
Espécie / Linhagem / Grupos Taxonômicos	*Astyanax sp.* – Lambari; *Cichlasoma sp.* - Acará
Número de animais	14
Peso / Idade	10 g / -
Sexo	Machos e Fêmeas
Origem / Local	Ambientes aquáticos naturais
Número da Autorização SISBIO	-
Atividade(s)	-

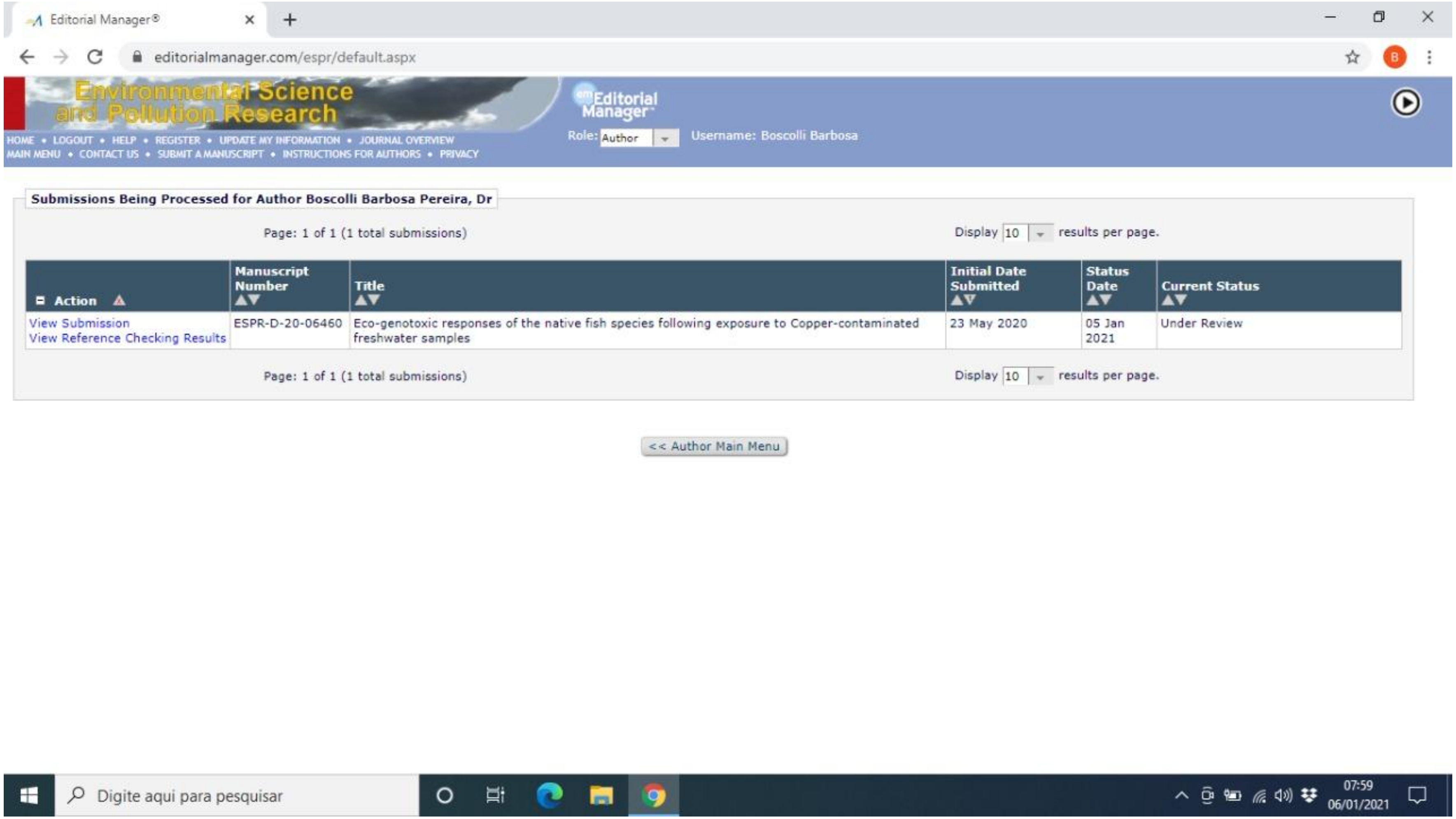
Uberlândia, 09 de maio de 2016.

Prof. Dr. César Augusto Garcia

Coordenador da CEUA/UFU

ANEXO 02

Submissão do artigo intitulado: ***"Eco-genotoxic responses of the native fish species following exposure to Copper-contaminated freshwater samples"*** na revista: *Environmental Science and Pollution Research* (Fator de impacto = 3.056)**.**



ANEXO 03

Artigo Publicado: Francisco, C. D. M., Bertolino, S. M., De Oliveira Junior, R. J., Morelli, S., & Pereira, B. B. (2019). Genotoxicity assessment of polluted urban streams using a native fish *Astyanax altiparanae*. Journal of Toxicology and Environmental Health, Part A, 82(8), 514-523.

Taylor & Francis
Taylor & Francis Group

Journal of Toxicology and Environmental Health, Part A
Current Issues

ISSN: 1528-7394 (Print) 1087-2620 (Online) Journal homepage: https://www.tandfonline.com/loi/uteh20

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To cite this article: Carine De Mendonça Francisco, Sueli Moura Bertolino, Robson José De Oliveira Júnior, Sandra Morelli & Boscolli Barbosa Pereira (2019) Genotoxicity assessment of polluted urban streams using a native fish *Astyanax altiparanae*, Journal of Toxicology and Environmental Health, Part A, 82:8, 514-523, DOI: 10.1080/15287394.2019.1624235

To link to this article: https://doi.org/10.1080/15287394.2019.1624235

Published online: 29 May 2019.

JOURNAL OF TOXICOLOGY AND ENVIRONMENTAL HEALTH, PART A
2019, VOL. 82, NO. 8, 514–523
https://doi.org/10.1080/15287394.2019.1624235

Genotoxicity assessment of polluted urban streams using a native fish *Astyanax altiparanae*

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ABSTRACT

Water quality has declined globally due to increased contamination of aquatic ecosystems. The use of fish genotoxicity biomarkers may improve and complement parameters for environmental risk assessment. The aim of this study was to assess the genotoxicity of samples collected from streams of the Jordão River, a tributary of the Paranaíba River, Brazil with different levels of metal contamination, utilizing a native fish species to determine the sensitivity and viability of implementing a useful, reliable technique for routine biomonitoring programs. Chemical analysis of water and sediments collected from different sites indicated that a gradient of contamination existed as evidenced by different concentrations of metals detected. After chronic exposure to contaminated samples, micronucleus (MN) frequencies in fish erythrocytes were measured and correlation with environmental parameters determined. Sites where the water concentrations of the metals aluminum (Al), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were high indicating a greater genotoxic potential of these elements. At the samples collected from the urban zone, a gradual increase was found for chromium (Cr), cadmium (Cd) and nickel (Ni) indicative of adverse impacts of discharge of urban effluents. Data demonstrated that *Astyanax altiparanae*, used in the test, exhibited a reliable sensitivity for detection of genotoxic consequences attributed to exposure to water samples collected near the discharge of industrial and domestic waste.

KEYWORDS
Micronucleus; biomonitoring; contaminants; metals; toxicology

Introduction

Water quality and aquatic biodiversity have declined significantly due to the exploitation by several human activities, which have altered the aquatic environment (Awange 2018; DiGiulio and Clark 2015; Tessarolo et al. 2017; Yuan et al. 2012). Discharges of industrial and domestic effluents, combined with adjacent agricultural and urban flows, constitute a serious threat to aquatic ecosystems and, consequently, to human health (Lemos et al. 2008; Reyes et al. 2017). A large amount of chemicals, released in aquatic environments due to intense urbanization processes and industrial activities (Campos et al. 2016; Pereira et al. 2017; Reyes et al. 2017; Srebotnjak et al. 2012; Su et al. 2013) have contributed to contamination of streams and rivers (Araujo and Dallos 2006; Pereira et al. 2017). Among the main pollutants are metals, with potential to accumulate in living organisms (Vaz S. Silva et al. 2016).

The contamination of the aquatic environment by metals has raised concerns globally due to potential toxicity, abundance and persistence (Armitage, Bowes, and Vincent 2007; Campos et al. 2016; Reyes et al. 2017; Sin et al. 2001) and special attention is given to metals as lead (Pb), chromium (Cr), zinc (Zn), copper (Cu) and mercury (Hg) (Campos et al. 2016; Reyes et al. 2017; Sabale et al. 2012; Strbac et al. 2015). These contaminants are not only harmful to the health of aquatic organisms, but also to humans through the consumption of water and fish (Rocha et al. 2009).

Genotoxic contaminants present mutagenic and/or clastogenic effects and their damage are transmitted to the next generations (Bolognesi and Hayashi 2011). DNA damage may be expressed as induction of mutations, hereditary defects, teratogenic effects and uncontrolled cell proliferation (Mitchelmore and Chipman 1998). Therefore, there

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is a growing interest in using biomarkers to improve rapid assessment of the genotoxicity consequences in aquatic fauna (Russo et al. 2004).

Local and global environmental agencies establish the acceptable levels of residues of various compounds that are used in domestic, agricultural, livestock and industrial processes, based upon the interference that these compounds may produce alterations in the physical and chemical parameters of aquatic ecosystems. However, pollutant sources may present diverse forms and behaviors in terms of interaction, mobility, biological availability and toxicity potential when combined (Sundaray et al. 2011). Thus, assessing only chemical contaminants isolated using physicochemical assays may not adequately estimate potential adverse toxic effects on organisms. Thus, in addition to employing physicochemical tests, it is also important to evaluate potential toxicological interactions by utilizing complementary biological assays (EFSA 2013; Raies and Bajic 2016).

Bioassays using fish provide information on the bioavailability of pollutants that contribute to metal biomagnification processes (Marcon et al. 2010). Bioassays employ organisms of different trophic levels, isolated or combined with chemical analyses to assess toxic potential of aquatic contaminants. Freshwater fish of the genus *Astyanax*, employed in environmental monitoring studies, were found to present high sensitivity as a bioindicator for contaminants (Silva and Martinez 2007; Trujillo-Jiménez et al. 2011; Vieira et al. 2014; Yamamoto et al. 2016). This fact favors the choice of these organisms, as observed in the species *Astyanax altiparanae* (Ramsdorf et al. 2012) in field and lab investigations in temperate regions (Bettim et al. 2016; Dourado et al. 2017; Vieira et al. 2014).

Among the predominant biomarkers of environmental genotoxicity, the micronucleus (MN) frequency test constitutes a promising method in assessing cytogenetic damage that is regularly used to monitor water quality (Kushwaha et al. 2012; Lemos, Oliveira, and Lemos 2011). The measurement of cytogenetic damage by NM frequency evaluates the stress of pollutants on aquatic ecosystems (Baršienė et al. 2014; Dixon et al. 2002) and consequent aneugenic and clastogenic effects. This test has the ability to identify the genotoxicity attributed to a wide range of toxic compounds (Heddle et al. 1991) and is widely applied because of its established suitability for fish species (Çavaş and Ergene-Gözükara 2005; Kushwaha et al. 2012; Praveen et al. 2014). Although there are a considerable number of studies in Brazil, few data are available on effluents in the Triangulo Mineiro region, Minas Gerais, and no apparent reports in the Jordão River sub-basin for toxicological analyzes. The aim of the present study was to determine the genotoxicity of samples collected at different sites from streams located along a Brazilian river basin with varying levels of contamination. In particular, a native fish species was employed to examine viability as well as sensitivity of biomarkers of genotoxicity as a complementary parameter to conventional physical-chemical methods of water quality assessment.

Material and methods

Study locations

Araguari is a city located in the state of Minas Gerais, in the north of the Triângulo Mineiro region, near the Jordão River, a tributary of the Paranaíba River. The collections of samples (water and sediment) were carried out in November of 2016 (rainy season) in 8 sample sites (Figure 1), with differing characteristics.

Site 1 (18°44′9.36 "S and 47°57′49.08" W) – Located at Jordão River spring. The width of this location is 8 m. Field observations showed that the river´s spring is not protected by ciliary forest, being surrounded by corn and soybean cultivation and landing strip for agricultural aircraft.

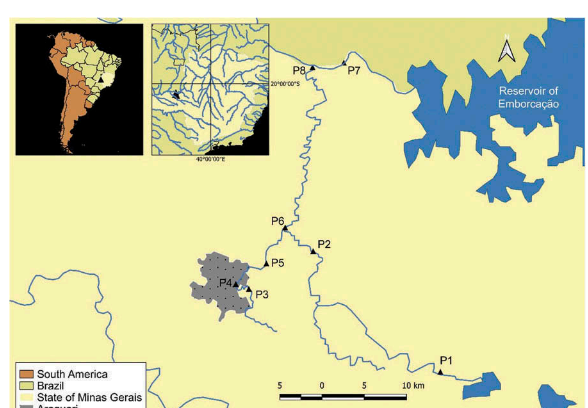

Figure 1. Map of the state of Minas Gerais showing the sites evaluated and the reference site.

Site 2 (18°36′51.91 “S and 48°5′56.28” W) – The second collection site is located in the Jordão River (with 25 m of width) before the confluence with the Brejo Alegre Stream. Located near the highway 050 bridge, at the exit to the city of Catalão – GO. At this site the presence of ciliary forest occurs.

Site 3 (18°36′51.91 “S and 48°5′56.28” W) – The third location is centered in the urban area of the city of Araguari, MG, near the John Kennedy Forest, a public reserve of the city. At this site there is occurrence of high levels of pollution, with open sewage disposal and domestic effluent discharges. The width of this location is 8 m.

Site 4 (18°39′8.54 “S and 48°10 ‘ 2.60 “O) – The fourth collection site is located in the Brejo Alegre Stream (with 9 m of width), near to a slaughterhouse, an old tannery, a juice company and an old dump, also centered in the city of Araguari. The situation of this site is critical, with remarkable degradation, presenting strong odor and dark coloration of water. On the field visit, a residual waste of white color was observed, most probably from the plastic bag industry, located in the proximity.

Site 5 (18°37′36.35 “S and 48°8′55.36” W) – Site located near to a large slaughterhouse in the city, other than that mentioned in site 4, and to the landfill. The width of this location is 15 m. This collection site also presents a high degree of environmental degradation, surrounded by tomato crops, and discharges of sewage from nearby districts, which are distant from site 3. It is worth mentioning that near this site the sewage treatment plant of the city is being built.

Site 6 (18°35′25.62 “S and 48°7′43.39” W) – The sixth sampling site is located in the Jordão River (with 25 m of width), after the drainage of the Brejo Alegre Stream. At this site there is a ciliary forest, not as dense as in site 2, surrounded by the cultivation of maize, livestock, residences and a rural restaurant.

Site 7 (18°25′43.58 “S and 48°5′58.46” O) – Located upstream Paranaíba River (with 120 m of width). It precedes the drainage of the Jordão River, so it is considered the control site. There is a ciliary forest on the river banks, a large restaurant nearby, and it is located on BR 050, at the frontier of MG and GO states. It was possible to note the presence of nearby residents, including those on platforms at river banks. In addition, there was personal fishing for their own consumption and consumption of the restaurant along the highway.

Site 8 (18°25′27.31 “S and 48°3′58.69” W) – Located on the Paranaíba River (with 150 m of width), downstream Jordão River tributary. There is a dense ciliary forest and a large number of fishermen in this region. It is noteworthy that at this point dredges were observed for sand extraction.

Biological material

The fish were obtained from aquarist shops in the city of Araguari-MG. A total of 287 specimens of *Astyanax altiparanae* of both sexes (5 ± 0.4 g of body weight, 7.2 ± 0.2 cm of total length) were transported to the Cytogenetic Laboratory of the Federal University of Uberlândia, where they were kept in tanks of 20L. The tanks contained reconstituted water (pH 7.5, dissolved oxygen at a rate of 8mg/L and hardness of 43mg $CaCO3$/L), where fish remained for 10 days before exposure under controlled conditions of temperature (25°C), lighting (16: 8 hr light/dark cycle), fed daily with 35 mg of flaky commercial feed and constant aeration. The fish were fed up to 24 hr prior to starting the experiment.

Water and sediment samples

In order to evaluate the physicochemical characteristics, the water at the 8 locations were sampled and analyzed according to the procedures in Standard Methods for Examination Water and Wastewater (1998). The parameters analyzed for water and sediment were carried out in collaboration with the Laboratory of Environmental Quality of the Institute of Agrarian Sciences of the Federal University of Uberlândia.

Study design

The tests were performed in a semi-static system, with renewal of the medium every 48 for 168hr, using 7 fish in each 20L aquarium. Five dilutions (100, 50, 25, 12.5 and 6.25%) in dilution water and a negative control (dilution water only) were employed for each study location. The tests were performed in duplicate and dilution water was the same utilized for acclimatization of fish. The techniques employed in the animals during the test and procedures adopted to obtain tissue samples were approved by the Ethics Committee on Animal Use of the Federal University of Uberlândia, registered with protocol 085/16.

Genotoxicity biomarkers – micronucleus frequency test (MN)

In order to carry out the genotoxicity tests, after exposure of 168 hr with the water samples from each location, peripheral blood was removed from the branchial artery using sterile 1 ml heparinized syringes, one for each animal. Immediately the smears (blood drop sliding on the microscope slides) were made, which were air dried for 24 hr (Grisolia and Starling 2001). The cells were then fixed with absolute methanol for 10 min, and subjected to the staining process with Giemsa and phosphate buffer (pH = 6.8) in the ratio of 1:20 for 15 min. Four slides were prepared for each animal.

Four thousand erythrocytes per animal were analyzed under light microscopy (1000x magnification – using immersion oil), according to Schmid (1975). The criteria for identification of micronucleated erythrocytes were that the nuclear particles were required to (1) be smaller and completely separated from the main nucleus;(2) not refractory; (3) with the same shape, staining and intensity of the cell nucleus and within the cellular cytoplasm.

Statistical analysis

One-way ANOVA was used to determine the existence of differences between sites. The sensitivity of the MN frequency test to the presence and concentration of Cr, Ni, Cd, Cu, Zn and Pb was tested using Pearson's correlation. All tests were performed with a minimum significance level of $p < .01$.

Results and discussion

The physicochemical parameters for water and sediments are presented in Tables 1 and 2, respectively. For all locations, temperature and pH were below the values established as normal for Environmental Brazilian Council (CONAMA 2005). However, values for turbidity exhibited rates above 100UNT, at sites 1 and 2. For total dissolved solids, sites 2 and 6 presented rates above 500mg/L values that exceeded the parameters. These environmental parameters altered at sites 2 and 5 were found to affect the MN rate suggesting a genotoxic effect of the metal pollutants present in the samples.

Human activities that affect Brejo Alegre Stream water quality at sites 3, 4 and 5 were predominantly based upon untreated sewage discharge from the city of Araguari, MG, and agricultural discharge of pesticides in the Jordão River (locations 1, 2 and 6).

Due absence or improper wastewater treatment, domestic and industrial effluents are frequently discharged in environment and remain the major cause of water pollution. In Brazil, water quality parameters (CONAMA 2005) do not establish standards for a variety of contaminants such as pesticides and drug residues and therefore need to be revised. Domestic sewage is one of the primary contributors to reduction of environmental quality, since organic matter is the source and input of disease-causing microorganisms. Further, it was necessary to investigate the sediment, since the metals are retained in this material and, depending upon several factors (biotic and abiotic) become resuspended in the water column. Thus it is necessary to monitor and implement sewage networks in order to maintain water quality with high environmental standards.

The levels of aluminum (Al), iron (Fe), manganese (Mn), Zn and Cu metals in the water samples exceeded the permitted limit at different sites (CONAMA 2005). Al and Fe displayed high levels at all analyzed locations except in the Paranaíba River (sites 7 and 8). This may be attributed to sources that are diverse in origin. Aluminum is considered a micro environmental contaminant, and Fe sources include natural rock erosion (Suslick 1998) resulting from activities such as mining, or fertilizers (Sharma et al. 2005), and may be found in municipal and industrial sewage effluents (Klauck, Rodrigues, and Basso Da Silva 2013). High values of Al and Fe linked to the increase of suspended solids may be associated with the process of soil loss, which was noted downstream and upstream of the Brejo Alegre Stream in the Jordão River, where evident signs of environmental degradation were observed (Personal communication).

Table 1. General characteristics of the 8 sampling locations.

	COLLECTION SITES										
									CONAMA 357/05		
Physicochemical parameters of water	1	2	3	4	5	6	7	8	C1	C2	C3
Temperature (°C)	23.63	24.05	18.81	19.18	16.59	19.44	22.77	22.68	-	-	-
Turbidity (UNT)	136.0	204	ND	1.9	12.1	59.2	3.9	4.9	40	100.0	100
pH	7.34	7.43	9	7.66	7.1	8.27	7.24	7.12	6–9.	6–9	6–9
Dissolved Oxygen (mg/ml)	2.6	2.58	2.85	3.59	4.49	3.62	_	_	> 6	5.0	4
Total Dissolved Solids (mg/ml)	450	532	4	127	24	641	49	52	500	_	500
Aluminum (mg/ml)	0.521*	0.425*	0.303*	0.230*	0.283*	0.271*	0.051	0.401	0.1	0.1	0.2
Barium (mg/ml)	0.041	0.058	0.021	0.015	0.029	0.080	0.012	0.025	0.7	0.7	1
Cadmium (mg/ml)	0.0001	0.0001	ND	ND	ND	ND	0.0001	0.0002	0.001	0.001	0.01
Chromium (mg/ml)	0.009	0.0028	0.0018	0.0038	0.0016	0.0016	0.0008	0.0019	0.05	0.05	0.05
Cobalt (mg/ml)	0.0015	0.0014	0.0009	0.0006	0.0015	0.0014	0.0004	0.0014	0.05	0.05	0.2
Copper (mg/ml)	0.0429*	0.0271*	0.0253*	0.0291*	0.0292*	0.0275*	0.0016	0.0220*	0.009	0.009	0.013
Iron (mg/ml)	0.6053*	1.0982*	0.1806*	0.4482*	0.6523*	1.509*	0.0385	0.1452	0.3	0.3	5
Lead (mg/ml)	0.0006	0.0044	0.0044	ND	0.0007	0.0037	0.0027	ND	0.01	0.01	0.033
Manganese (mg/ml)	1.5162*	1.8869*	0.1918*	0.4312*	0.5358*	1.8957*	1.3903*	1.5385*	0.1	0.1	0.5
Nickel (mg/ml)	0.0048	0.0101*	0.0012	0.0055	0.005	0.0035	0.0053	0.0059	0.025	0.025	0.025
Silver (mg/ml)	0.0032	0.0006	0.0022	0.0041	0.002	0.0008	0.0019	0.0035	0.01	0.01	0.05
Vanadium (mg/ml)	0.0073	0.0075	0.0063	0.0071	0.0074	0.0077	0.007	0.0072	0.1	0.1	0.1
Zinc (mg/ml)	0.4497*	0.3025*	0.1468	0.1509	0.1017	0.1873*	0.0311	0.0996	0.18	0.18	5.0
Beryllium (mg/ml)	0.0001	0.0002	0.0001	0.0001	0.0002	0.0002	0.0002	0.0002			
Bismuth (mg/ml)	ND	0.0002	ND	ND	ND	ND	ND	0.0008			
Calcium (mg/ml)	10.2859	8.2249	2.4502	1.5115	2.2206	8.0832	2.9566	3.6720			
Gallium (mg/ml)	0.0008	0.0008	0.0027	0.0008	0.0012	0.0026	0.0013	0.0029			
Potassium (mg/ml)	11.489	6.977	0.352	0.584	0.516	7.298	1.0740	1.183			
Lithium (mg/ml)	0.005	0.005	0.004	0.004	0.004	0.004	0.0040	0.004			
Molybdenum (mg/ml)	0.0421	0.004	0.002	0.002	0.002	0.002	0.0010	0.002			
Sodium (mg/ml)	40.909	37.176	0.621	0.797	2.322	27.324	1.3190	1.454			
Rubidium (mg/ml)	0.011	0.018	0.004	0.003	0.003	0.013	0.0050	0.003			
Strontium (mg/ml)	0.047	0.04	0.01	0.008	0.012	0.046	0.0200	0.023			

*Above the values established by the resolution CONAMA357/05. ND = Not detected

Table 2. Metal analysis of sediments.

PARAMETERS	COLLECTION SITES 1	2	3	4	5	6	7	8	CONAMA 344/04(mg kg-1) Minimum value	Maximum value
Cadmium	3	3	0.3	4.9*	3.2	3.2	1.2	2.2	0.6	3.5
Chromium	110.7*	113.3*	149.4*	181.4*	130.9*	152.5*	83.5	104.8*	37.3	90
Copper	179.8	172.9	36	131.4	143	157.2	37.7	79.7	35.7	197
Nickel	30.6	45.2*	18	140.8*	42*	44.9*	25.8	29.1	18	35.9
Lead	18	14.4	10.8	20.3	20.5	16.4	27.5	19.5	35	91.3
Zinc	124.4	112	28.2	93.4	133.2	123.8	67.6	115.6	123.	315.

*Above the values established by the resolution CONAMA 344/04.

For Cu parameter, results were similar and all sites exceeded the environmental limit, except for the reference location (site 7). It is known that small amounts of Cu are essential for the environment under natural conditions; however, excessive amounts may be toxic to fish, microorganisms and humans (Stern et al. 2007). The levels of Cu and other elements in stream and river water may have risen due to anthropogenic activities, such as sewage discharge in the urban area of the Brejo Alegre Stream (3 and 4), soybean and coffee farming processes nearby to the Jordão River (1, 2 and 6) and industrials sites, such as slaughterhouses and sanitary landfills (4 and 5).

Manganese levels in the water samples were high, but for the sites of the Jordão River (1, 2 and 6) and Paranaíba River (7 and 8) the values were higher (> 1 mg/ml), when compared to concentrations of the Brejo Alegre Stream (3, 4 and 5) (<1mg/ml). Zinc also exceeded the established limits at the sites of the Jordão River, with levels greater than 0.18mg/ml. The locations 3 and 4 were within the limit for this parameter. It has been Segura-Muñoz et al. (2003) reported that Zn negatively affected the bioavailability of Cu and altered the metabolism of Fe, an essential component of DNA repair proteins and cell maintenance.

In the analysis of metals in sediments, Pb, Zn and Cu presented below the acceptable parameters established by CONAMA Resolution 344/04, at all sites, according to values in Table 2. Lead concentrations were below limits both in water and sediment in this region but, only at site 2 was the level near the limit, which may be attributed to proximity to the industrial urban region, indicating that this may be a possible site of disposal of toxic contaminants. All locations, except control, presented Cr rates above the pre-established values, and Ni exceeded levels at sites 2, 4, 5 and 6. Evidence thus indicated that at the intersection between the Brejo Alegre Stream and the Jordão River there might be an accumulation of metal clusters in this central region of the study. Figure 2 depicts the sites that indicate where the parameters exceeded the environmental limits of safety and genotoxicity level (MN frequency) observed in *Astyanax altiparanae*.

ANOVA was used to assess the different genotoxic responses in *A. altiparanae*. According to the results indicated in Table 3, the tests employing the MN frequency (at all concentrations) demonstrated distinct genotoxic responses at the monitored sites, and the frequencies of MN observed between the concentrations were similar. The incidence of MN at site 2 was significant when compared to other sites referring to all concentrations. It may also be noted that there was a discrepancy at site 5, especially at concentrations of 100 and 25% (Table 3).

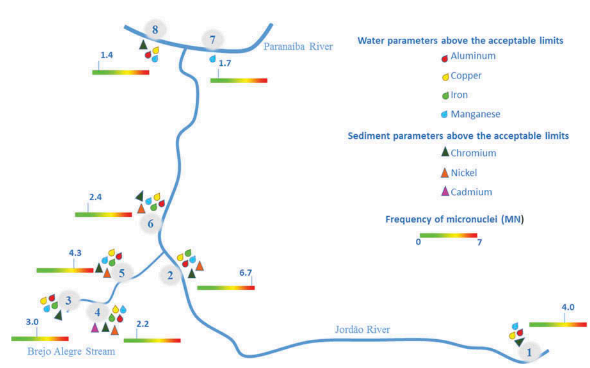

Figure 2. Representative map showing the sub-basin of the Jordan River, indicating with different colors the sites with parameters above that pre-established by the CONAMA's legislation and genotoxicity level (MN frequency) observed in *Astyanax altiparanae*. In detail, the 8 sites where the experiments were carried out: Brejo Alegre Stream (3, 4 and 5), Jordão river (1, 2 and 6) and Paranaíba river (7 and 8).

Table 3. Frequency of Micronuclei (MN) for *A. altiparanae* obtained at sites (S1-8).

Concentrations	Frequency *MN (mean + SD)*							
	S1	S2	S3	S4	S5	S6	S7	S8
100%	4.0* ± 0.7	6.7* ± 0.8	3.0 ± 0.7	2.2 ± 1.0	4.3* ± 0.7	2.4 ± 1.1	1.4 ± 0.8	1.7 ± 1.0
50%	2.1 ± 0.8	5.0* ± 0.6	1.4 ± 1.1	1.1 ± 0.7	2.4 ± 1.1	0.6 ± 0.6	0.9 ± 0.8	0.9 ± 0.7
25%	2.1 ± 0.8	3.1* ± 1.2	1.4 ± 0.9	1.0 ± 0.7	3.1* ± 1.2	1.0 ± 0.9	0.7 ± 0.7	1.4 ± 1.0
12,5%	1.4 ± 0.8	3.9* ± 1.3	0.7 ± 0.8	0.6 ± 0.7	1.5 ± 1.3	0.4 ± 0.6	0.4 ± 0.7	1.0 ± 0.9
6,25%	0.6 ± 0.8	3.5* ± 0.6	0.3 ± 0.7	0.4 ± 0.7	1.6 ± 1.4	0.6 ± 0.7	0.3 ± 0.7	1.5 ± 1.1
0%	0.2 ± 0.1	0.5 ± 0.5	0.4 ± 0.2	0.2 ± 0.1	0.8 ± 0.4	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.1

* $p < 0.01$

Site 2 presented a higher incidence in the MN rate followed by site 5, which are geographically close. Sites 3 and 4 also showed high MN rates, and are located in the Brejo Alegre stream, a tributary of the Jordão River, upstream of site 5. High values of metals and high MN count were detected at location 1 which is the spring of the Jordão River. The site 8, located on the Paranaíba River, the final receptor site, downstream of the Jordão River, presented lower values of both water and sediment parameters and the amount of MN.

In order to verify the sensitivity of the *Astyanax altiparanae* species found in the Jordão River sub-basin, correlations were made between the observed MN rates (concentration 100%) and levels of Cr, Ni, Cd, Cu, Zn and Pb detected in water and sediment. The results of these correlations indicated that the MN frequency values were moderately sensitive to Cr levels increases followed by Ni in sediments. For water, a weak correlation was found, but not negligible to Cu concentration elevations (Table 4).

The genotoxic potential of Cu was reported in hamster cell lines (Grillo, Reigosa, and Mele 2009), bacterial strains (Siddiqui, Tabrez, and Ahmad 2011), plants (Wasi, Tabrez, and Ahmad 2013) and animal cells (Erbe et al. 2011). It is believed that Cu contributes to significant toxicogenetic changes, since this metal modifies the activity of antioxidant enzymes, which induces and aggravates oxidative stress (Lushchak 2011; Stern et al. 2007). Fish exposed to Cu showed a rise in the primary and secondary activities of the oxidative enzymes (Hansen et al. 2007). In addition, other investigators noted that Cu enhanced cytotoxicity and reactive oxygen species (ROS) production, resulting in increased breaks in DNA chain (Bopp, Abicht, and Knauer 2008).

Klobucar et al. (2003) reported an increase in MN frequency in vertebrate aquatic species of contaminated water compared to control sites. . In this study, data demonstrated that the highest frequency of MN was found at sites 1, 2 and 5, considered to be underdeveloped areas, but surrounded by agricultural activities. The lowest values were found in the Paranaíba River (sites 7 and 8) located in rural areas, not urbanized and geographically distant from the other sites. The highly urbanized and industrially impacted sites 3 and 4 exhibited evident environmental

Table 4. Pearson correlation coefficient between metal concentrations and frequency of micronucleus in fish cells.

	Sediments		Water	
	r^2	p	r^2	p
Chromium	0.390*	<0.0001	0.007	0.506
Nickel	0.270*	<0.0001	0.010	0.426
Cadmium	0.144	0.002	_	_
Copper	0.059	0.054	0.161*	0.001
Zinc	0.021	0.253	0.017	0.303
Lead	0.027	0.195	0.017	0.300

* $p < 0.01$

degradation. Although not the sites with the highest MN rates, the Brejo Alegre Stream was considered to be the most contaminated of Araguari due to the reception of wastewater, containing high concentrations of pollutants, which accumulate in sediments, and consequently no fish were found in these locations. The significant cytotoxicity of the pollutants in this area induced high mortality rates which may explain the observed low frequency in MN rates due to the absence of fish.

In conclusion data demonstrated that *Astyanax altiparanae* constituted a sensitive, reliable species to be used in in detecting genotoxic effects resulting from exposure to the water samples collected near the discharge of industrial and domestic waste. These findings reinforce the importance of using biomarkers of genotoxicity with tropical species as a complementary parameter to conventional physical-chemical methods of water quality assessment.

Acknowledgments

Financial support is acknowledge to “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (Capes).

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