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

MONITORAMENTO BIOLÓGICO DE RECURSOS HÍDRICOS NA REGIÃO DO TRIÂNGULO MINEIRO-MG, POR MEIO DO TESTE DE MICRONÚCLEO E SMART

Aluno: Edimar Olegário de Campos Júnior

Orientador: Profa. Dra. Sandra Morelli

UBERLÂNDIA - MG
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Tese apresentada à Universidade Federal de Uberlândia como parte dos requisitos para obtenção do Título de Doutor em Genética e Bioquímica (Área Genética)

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ALUNO: Edimar Olegário de Campos Júnior

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_____
Profa. Dra. Sandra Morelli

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LISTA DE ABREVIATURAS

ACEMC	Associação Ceramista de Monte Carmelo
cm	Centímetros
CMN	Célula Micronucleada
CONAMA	Conselho Nacional do Meio Ambiente
DBO	Demanda Bioquímica de Oxigênio
DESVPAD	Desvio Padrão
DL	Limite de Detecção
DQO	Demanda química de Oxigênio
DXR	Doxorrubicina
ENA	Alterações nucleares em eritrócitos
FAAS	Espectometria de Absorção Atômica de Chama
H	Hora
HB	Cruzamento de alta bioativação
IGAM	Instituto Mineiro de Gestão das Águas
IQA	Índice de qualidade de água
L	Litro
mg	Miligrama
Min	Minuto
mL	Mililitro
mM	Milimolar
MN	Micronúcleo
ºC	Graus Celsius
pH	Potencial Hidrogeniônico
R. quelen	*Rhamdia quelen*
SMART	Teste de Mutação e recombinação somática
ST	Cruzamento padrão
UpH	Unidades de Potecial Hidrogeniônico

Sumário

RESUMO

A exposição à poluentes pode induzir uma série de alterações no DNA, as quais podem causar efeitos genotóxicos / mutagênicos nos indivíduos expostos. É de responsabilidade do Conselho Nacional do Meio Ambiente, nos termos da Resolução 357/2005, a regulação dos limites de compostos químicos permitidos em águas superficiais. O presente estudo teve como objetivo monitorar o potencial genotóxico, mutagênicas e recombinogênico e, consequentemente, a qualidade da água, em dois córregos da bacia do rio Paranaíba (região Triângulo Mineiro), além de avaliar o potencial genotóxico e consequente qualidade da água do córrego Mumbuca, que é utilizado como recurso de abastecimento da cidade de Monte Carmelo, em Minas Gerais, Brasil, utilizando dois peixes bioindicadors com respostas variáveis (*Rhamdia quelen* e *Geophagus brasiliensis*). Os locais avaliados, o Córrego do Óleo apresentou qualidade da água intermediária, com um aumento significativo na frequência de micronúcleos em comparação com o local de referência, assim como o Córrego Liso que mostrou um elevado potencial de toxicidade, com a presença de cádmio e de chumbo, que resultou em altas taxas de micronúcleos que foram semelhantes ao controle positivo. Nos trechos avaliados nos efluentes na bacia do rio Paranaíba, houve indução de mutação e recombinação nas asas de *Drosophila melanogaster*, indicando uma possível presença de compostos potencialmente tóxicos. Os dados sugerem que a descarga de efluentes industriais em trechos específicos dos córregos interfere na biota.

Palavras-Chave: Micronúcleo; Biomonitoramento; SMART; *R. quelen*; *G. brasiliensis*

ABSTRACT

Exposure to pollutants can induces a series of alterations in DNA that can cause genotoxic/mutagenic effects in the exposed individuals. It is the responsibility of the National Council for the Environment, under resolution 357/2005, to regulate the limits of chemical compounds permitted in surface waters. The present study aimed to monitor the genotoxic, mutagenic and recombinogenic potential and consequently the water quality, in two streams in the Paranaíba River basin (Minas Triangle region) and evaluate the genotoxic potential and consequent quality of the water from the Mumbuca stream, which supplies the city of Monte Carmelo in Minas Gerais state, Brazil, using two bioindicator fish with variable responses (*Rhamdia quelen* and *Geophagus brasiliensis*). Within the sites assessed, the Córrego do Óleo presented intermediate water quality, with a significant increase in micronucleus frequency compared to the reference site and similar to the Córrego Liso that showed a high toxic potential, with the presence of cadmium and lead, that resulted in high micronucleus rates that were similar to the positive control. At the sites assessed in the effluents in the Paranaíba River basin, there was inducement to somatic mutation and recombination in the wings of *Drosophila melanogaster*, indicating a possible presence of potentially toxic compounds. Data suggest that discharge of industrial effluents in specific stretch of the stream interfered with biota.

***Keywords**: Micronucleus; Biomonitoring; SMART;* R. quelen*;* G. brasiliensis

APRESENTAÇÃO

A contaminação da água de grandes cidades em todo o mundo é fator de preocupação para os órgãos responsáveis pela preservação dos reservatórios hídricos. Os rios que representam, ao mesmo tempo, pontos de captação de água para abastecimento de áreas metropolitanas, servem de receptores para os lançamentos de lixo, esgotos urbanos, e efluentes agrícolas/industriais. Os biomonitores, ou organismos sentinela são amplamente utilizados no que diz respeito a alertar sobre ambientes poluídos. Para acessar as respostas de tais indivíduos é necessário lançar mão de metodologias como os testes de monitoramento biológico. Nesse contexto, o teste do micronúcleo tem sido utilizado, pois baseia-se na caracterização de células que sofrem alterações na distribuição de suas cromátides (efeito sobre o fuso no ciclo celular) ou eventos clastogênicos. Aliado a tal teste, a avaliação química da água, e consequente determinação do índice de qualidade de água, a quantificação de agentes tóxicos em biomonitores e a aplicação do teste de mutação e recombinação somática em *Drosophila melanogaster*, são metodologias que podem ser utilizadas para melhor caracterização da área de estudo, e aumento da especificidade dos agentes químicos ativos nos trechos avaliados e seu potencial de interferência para os organismos locais. O trabalho objetivou realizar o monitoramento biológico das espécies *Rhamdia quelen* e *Geophagus brasiliensis*, as quais apresentam níveis de sensibilidade distintas (repostas variáveis). A área de estudo, na região do Triângulo Mineiro, apresenta grande importância econômica para a região, além de despertar interesse na área de saúde e de vigilância ambiental, para os gestores e

órgãos responsáveis locais, haja visto o grande fluxo de pessoas, alta demanda de consumo de água e alimentos e as elevadas taxas de degradação dos reservatórios hídricos nas bacias do estado. De forma conclusiva, fica evidente a necessidade de monitorar esses locais que são alvo de interferências antrópicas, objetivando a manutenção de reservatórios hídricos ecologicamente equilibrados. O trabalho foi escrito conforme descrito pelas normas de formatação do Programa de Pós-Graduação em Genética e Bioquímica da Universidade Federal de Uberlândia.

Estruturação dos Capítulos: O **Capítulo I**, trata-se da avaliação literária a que se propõe o trabalho, abordando os requisitos legais, caracterização de ambientes poluídos e os meios dos quais o presente trabalho fez uso, para efetivação do monitoramento biológico.

Em acordo com o que foi evidenciado no primeiro capítulo, o **Capítulo II** discute os resultados da avaliação de dois córregos, no município de Uberlândia, os quais são afluentes do Rio Uberabinha, que é um conhecido reservatório hídrico poluído da região, e que merece destaque, por ser o principal reservatório de distribuição de água do departamento municipal local responsável.

Sob o mesmo enfoque, o **Capítulo III** apresenta os resultados de um estudo que avaliou os danos genéticos no córrego Mumbuca, na região de Monte Carmelo, local que possui como característica particular, a indústria ceramista, como principal atividade econômica.

Capítulo I

FUNDAMENTAÇÃO TEÓRICA

1. FUNDAMENTAÇÃO TEÓRICA

1.1 Qualidade Ambiental dos Recursos Hídricos

A região sudeste do Brasil, é a maior contribuinte para o desenvolvimento do polo industrial nacional, e tal fato, aliado com a alta densidade urbana nessa área, promove a existência de restrição à utilização de água para consumo, mesmo com o país apresentando cerca de 20% do volume de água doce disponível no mundo (BRITO et al., 2012).

Os processos de urbanização podem ser considerados como responsáveis ativos pela degradação de ecossistemas aquáticos, como, córregos, rios e lagos, devido a não existência ou falta de cumprimento do plano diretor municipal, já que, este seria capaz de regular o isolamento dos reservatórios hídricos em seus cursos naturais, facilitando a manutenção de qualidade desses locais (FITZHUGH e RICHTER, 2004). Em atenção à essa situação, Galvan (2011) observou que a urbanização, quando ocorre de forma desordenada, causa problemas ambientais nos ambientes aquáticos de forma direta ou indireta.

Nos países em desenvolvimento, em especial no Brasil, os reservatórios hídricos, que representam na legislação um bem difuso e de uso comum, têm apresentado um aumento progressivo nas taxas de deterioração da água. Esse fato tem ocorrido devido à introdução de compostos químicos, xenobióticos, ou presentes em concentrações elevadas, causando efeitos imediatos no local, em meio à contaminação (THOMPSON et al. 1995).

Compostos estranhos ao meio se dispersam em ecossistemas aquáticos por vias diversas, como efluentes de atividades antrópicas, ou mesmo de forma

natural, através da contribuição biótica. A contaminação, então causada, é originada em sua grande maioria por atividade antrópica direta, através da produção e direcionamento de substâncias que interagem entre si, formando compostos com potencial danoso à saúde ambiental e à saúde do homem (LIVINGSTONE, 1993, 1998; GUPTA et al., 2014).

Machado et al. (2004), afirmam que os municípios brasileiros, de média ou alta densidade populacional, possuem no mínimo uma fonte de contaminação em seus reservatórios hídricos, e ainda aletam que essa situação causa um descompasso econômico municipal, ao passo que o gestor público deverá se incumbir dos gastos com um tratamento mais complexo da água, afim de viabilizar a distribuição nos centros de abastecimento.

1.2 Avaliação Físico-química da Qualidade da Água

A poluição de reservatórios de água naturais ocorre devido à presença de poluentes decorrentes de atividades biológicas ou químicas (TELLES, 1999). Infelizmente os recursos hídricos representam a forma mais fácil de despejo de qualquer tipo de resíduo, e portanto, se tornam alvos fáceis para a ocorrência de impactos ambientais. Dentre as atividades no perímetro urbano de maior impacto ecológico, enquadram-se a produção de efluentes domésticos e industriais (COSTA, 2004).

Para Orssato (2008) o despejo de efluentes nos reservatórios hídricos, está associado com a presença de alta carga de compostos orgânicos, e consequente aumento dos níveis bacterianos na água, influenciando diretamente a demanda química e biológica de oxigênio local. Efluentes domésticos apresentam elevadas taxas de nitrogênio (N), sódio (Na), magnésio

(Mg), sulfatos e cloretos. Esses compostos interferem diretamente na qualidade da água, quando em desacordo com os parâmetros legais permitidos, assim como alguns metais pesados derivados de atividades residenciais, como o alumínio (Al), ferro (Fe), cobre (Cu) e cromo (Cr) (HIRATA, 2001; FERNANDES et al., 2009).

O recurso hídrico ainda pode sofrer alterações físico-químicas, em decorrência da produção de efluente agrícola, e essa interação entre químicos diversos, ocasiona a incerteza da ação desses compostos, tornando-os potenciais tóxicos ativos, capazes de lesar a integridade genética e bioquímica, e consequente reposta fisiológica dos organismos presentes de forma isolada em um único recurso natural (BEGUM, 2004; MACEDA et al. 2015), ou mesmo de forma integrada em um ecossistema (PARVEZ; RAISUDDIN, 2005).

De acordo com Cestari et al. (2004), alguns compostos já se mostraram determinantes para a indução de atividade genotóxica e mutagênica, como os metais pesados e Hidrocarbonetos Aromáticos Policíclicos (HAP's). Além da extensa gama de inseticidas e pesticidas orgânicos, que se acumulam nas áreas sedimentares, potencializando sua atividade de degradação.

Os metais pesados, mais recentemente chamados de elementos traço, são, de acordo com as considerações de Jain (2004), muito estáveis, acumuláveis e podem se complexar em compostos de alta toxicidade, além de se concentrarem em sedimentos (CHEN e WHITE, 2004; HORTELLANI et al., 2013; SIQUEIRA; APRILE, 2013). Devido a isso, são um dos principais problemas ambientais em reservatórios hídricos. Esses compostos e complexos, mesmo que em pequenas quantidades, podem causar danos

celulares e em tecidos de indivíduos expostos (WHITE e RASMUSSEM, 1998; MANSOURI et al., 2012).

O chumbo, de acordo com Johnson (1998) é um composto químico, largamente utilizado em processos industriais (processos de produção de roupas, composição de vernizes, além de ser um constituinte de explosivos e baterias). Este elemento químico pode causar danos na reprodução e crescimento, ou mesmo, ocasionar a morte de espécies presentes em recursos hídricos (BURDENA et al. 1998).

A exposição prolongada aos poluentes previamente citados, ocasiona efeitos genotóxicos e mutagênicos, declínio populacional (devido a interferência reprodutiva) e efeitos carcinogênicos, promovendo desordem na cadeia trófica (MICTHELMORE e CHIPMAN, 1998; RIBEIRO et al., 2003).

Reservatórios aquáticos, portanto**,** são caracterizados por constantes alterações nas variáveis físico-químicas da água, como no potencial hidrogeniônico (pH) e alcalinidade, temperatura, demanda bioquímica de oxigênio (DBO) e demanda química de oxigênio (DQO), as quais podem influenciar na determinação da avaliação de danos celulares em modelos biológicos (WITTERS, 1998; MONSERRAT et al., 2007).

A avaliação e parametrização da qualidade de água fica a cargo dos órgãos ambientais responsáveis, como o Conselho Nacional do Meio Ambiente – CONAMA (2005), o qual determina que, poluição aquática representa qualquer modificação das variáveis físico-químicas e/ou biológicas na água, seja de forma direta ou indireta. Esse órgão da esfera federal, é o responsável por legislar sobre os limites e padrões aceitáveis de compostos químicos no meio, através da Resolução n° 357/ 2005.

O Índice de qualidade de água (IQA), pode ser utilizado como determinante final da qualidade de água de qualquer reservatório hídrico, visto que é um índice que pondera as variáveis físico-químicas para classificação da qualidade do recurso. Tem sido utilizado em avaliações toxicológicas para categorizar reservatórios utilizados no abastecimento de água municipal (BOLLMANN; EDWIGES, 2008; ARRUDA et al., 2014).

A toxicologia ambiental pode ser utilizada para determinação dos efeitos de substâncias tóxicas no meio, ou presentes em algum organismo modelo, além de avaliar o impacto dessas interações com a saúde humana (SILVA, 2005).

A caracterização físico-química pode ser complementada com o uso de monitores ambientais, como por exemplo, os monitores aquáticos, que são ferramentas importantes para a avaliação da qualidade do recurso hídrico, visto que, qualificam e até mesmo quantificam o efeito dos poluentes no ecossistema aquático, e consequente potencial tóxico (JESUS; CARVALHO, 2008), além disso devido à alta sensibilidade dos modelos biológicos, QUEIROZ et al. (2000) afirmam que o monitoramento biológico é mais vantajoso que a mera avaliação físico-química.

Ainda avaliando a situação em território nacional, as regiões sul e sudeste, que representam grandes contribuintes para o processo de industrialização no país, segundo Brito et al. (2012) têm empregado programas de monitoramento biológico nos últimos anos. Essa experiência tem revelado, que a utilização de peixes e demais organismos aquáticos é capaz de direcionar aos locais com maior interferência de atividade tóxica,

estabelecendo assim, a possibilidade de intervenção do poder público para a fiscalização e identificação de riscos à saúde humana nesses locais.

O monitoramento ambiental aquático precisa ser reconhecido como importante para a gestão de recursos hídricos diversos, pois proporciona o acompanhamento de dados *in loco*, capazes de facilitar o gerenciamento correto das águas superficiais, e sua distribuição quando em condições mínimas para o uso da população (MAGALHÃES JÚNIOR, 2000).

1.3 Espécies Biomonitoras/bioindicadoras

Biomonitores, também conhecidos como monitores biológicos, são quaisquer organismos, capazes de indicar algum tipo de resposta quando ocorre algum dano ambiental. Biomonitores aquáticos, respondem, portanto, à presença de poluição da água, de forma integrada à essa alteração ambiental. Dessa forma, esses organismos podem ser utilizados para complementação em um sistema de monitoramento de recursos hídricos, afim de qualificar a presença de poluentes no meio (GRISOLIA; CORDEIRO, 2000; BATZIAS; SIONTOROU, 2006; SOUSA et al., 2013).

Para Cristaldi et al. (2004) muitas espécies podem ser utilizadas no biomonitoramento, ou mesmo para a bioindicação (resposta pontual, sem o acompanhamento temporal, em contrapartida ao que ocorre no biomonitoramento), objetivando avaliar o efeito de agentes químicos alvo em ambiente experimental controlado (laboratório), ou mesmo para o acesso a populações naturais (*in situ*).

Dentre os modelos biológicos para avaliação de danos ambientais, estão as espécies aquáticas, tais como, microcrustáceos, anfíbios, moluscos e peixes (COTELLE; FERARD, 1999; RAMSDORF et al. 2012).

Os peixes são modelos experimentais eficientes, devido sua rápida resposta frente aos agressores ambientais, e a possibilidade de transposição de efeitos para grandes vertebrados, além da extrapolação de efeitos à saúde humana (RIBEIRO et al., 2014). Em concordância com tal aspecto, diversos autores já descreveram o uso de peixes em programas de bioindicação/ biomonitoramento, por se tratarem de modelos experimentais com alta capacidade de sensibilidade quando na presença de agentes químicos ativos com potencial genotóxico (AL-SABTI; METCALFE 1995; GRISOLIA; STARLING 2001; RABITTO et al., 2011; MELO et al., 2014).

Para Larcher (2000), quando os modelos biológicos são expostos, eles alteram seu padrão comportamental, devido às variações de respostas fisiológicas e bioquímicas, além da interferência no genoma desses indivíduos.

Dentre os indivíduos que podem ser usados para experimentação em programas de monitoramento, o gênero *Rhamdia* (Siluriformes: Heptapteridae), tem sido utilizado por estar amplamente distribuído nos reservatórios hídricos brasileiros (BOCKMANN, 2007; MELA et al., 2013). O jundiá (*Rhamdia quelen*), apresentado na figura 1, é um peixe teleósteo de atividade noturna nativo da América do Sul, que habita águas profundas e se esconde na lama, ou mesmo em troncos, representando assim, um peixe com contato direto com o sedimento. A espécie é onívora, e o tempo de vida e tamanho são variáveis entre os gêneros (GOMES et al., 2000; BALDISSEROTTO, 2004).

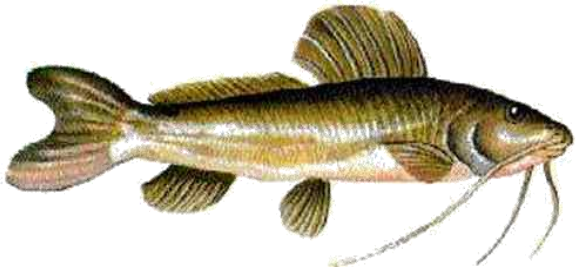

Fig. 1. Peixe da espécie *Rhamdia quelen*.
Fonte: http://www.geocities.ws/diversidad_animal/Paginas_peces/Rhamdia_sapo.htm

O peixe *Geophagus brasiliensis* (figura 2), de nome popular acará, é outro indicador biológico da família Cichlidae, e assim como o *Rhamdia quelen* é comum no território brasileiro. Os indivíduos dessa espécie têm hábito diurno e se posicionam mais superficialmente nos corpos d'água (SCHWANTES; BARTLETI; SCHWANTES, 1991; BENFICA, 2006). Calza et al. (2004) e Silvano (2003), avaliaram a indução de danos por metais tóxicos, aliada a capacidade de bioacumulação em espécimes de *Geophagus Brasiliensis,* caracterizando a espécie como um monitor ambiental eficaz.

Fig. 2. Peixe da espécie *Geophagus brasiliensis*.
Fonte:http://petssubmersos.blogspot.com.br/2011/06/peixes-amazonicos-parte-1.html

1.4 Testes de Monitoramento Biológico

Os bioensaios, são as ferramentas utilizadas para determinar os efeitos nos organismos vivos avaliados. Os testes são capazes de caracterizar diversos efeitos na estrutura biológica dos organismos sentinela, sejam eles histológicos, citológicos ou de comportamento (RIBEIRO et al., 2003).

Diversos testes podem ser utilizados, e a escolha correta, deve ser realizada de acordo com a substância a ser testada, e do efeito esperado. Além disso existe a possibilidade de utilizar dois ou mais bioensaios, afim de aumentar o grau de confiança das amostragens (SILVA, 2005). Na genética ecotoxicológica é possível, portanto, avaliar o efeito mutagênico, clastogênico e/ou aneugênico no DNA de organismos vivos (KENDALL *et al*., 2001).

Dentre as metodologias para investigação de genotoxicidade, o Teste de micronúcleos (MN) tem se mostrado eficiente (AL-SABIT; METCALFE, 1995). O teste do micronúcleo (figura 3) é um teste que detecta atividade clastogênica e aneugênica (RIBEIRO et al., 2003; VILLELA et al., 2003). Essas alterações são resultantes da perda de fragmentos de cromossomos, e/ou cromossomos inteiros durante os eventos da divisão celular (KIRSCH-VOLDERS et al., 2003). Para a realização do teste, faz-se necessário a utilização de uma pequena amostra de eritrócitos, a qual, possui células uniformes para a avaliação dos supostos efeitos, após avaliação técnica simples (HOOFMAN; RAAT, 1982; NEPOMUCENO et al. 1997; PALHARES; GRISOLIA, 2002).

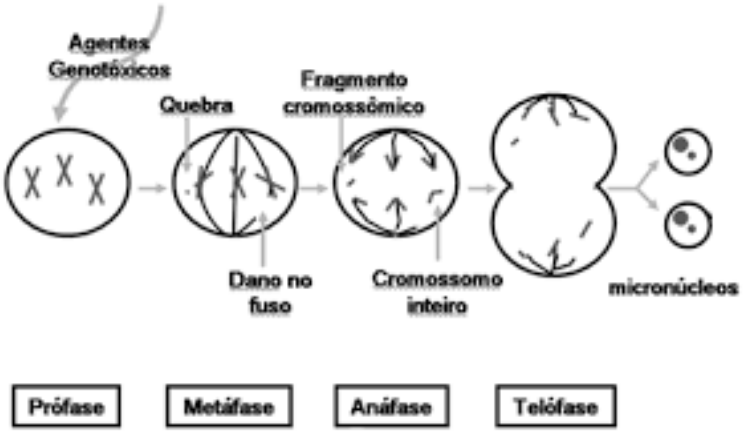

Fig. 3: Esquema para formação de micronúcleo durante a divisão celular.
Fonte: Kappes, 2010.

Em uma célula podem ser encontrados um ou mais micronúcleos, promovendo atrasos e/ou erros nos estágios da divisão celular. Para indicar a frequência de alterações por célula, além da frequência de Micronúcleos (MN), a frequência de Células Micronucleadas (CMN) também pode ser avaliada (SCHMID, 1975; FENECH, 2000).

Micronúcleos são pequenas massas nucleares, e que possuem mesma cor e intensidade do núcleo principal, do qual são proporcionais (WINTER et al., 2007).

Além da avaliação da frequência de micronúcleos, Ergene et al. (2007) e Costa et al. (2008) determinam que, as anormalidades nucleares de eritrócitos, podem ser utilizadas como uma variável do teste padrão (MN), e portanto, se trata de uma metodologia complementar. Dentre as anormalidades conhecidas, são definidas as seguintes variações nucleares: evaginações pequenas, evaginações maiores, e núcleos entalhados (cortados). Tais alterações já foram avaliadas por diversos autores (AYLLÓN et al. 2000; LEMOS et al. 2008), os quais determinaram o efeito de compostos genotóxicos, e consequente formação de micronúcleos e alterações nucleares diversas.

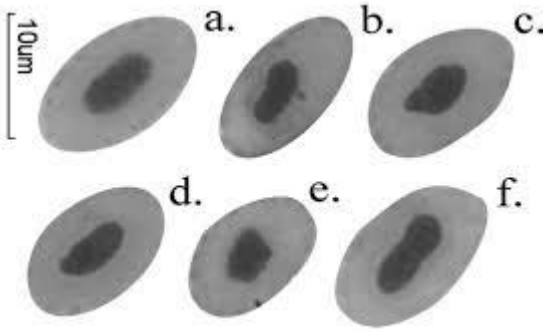

Fig. 4. Alterações nucleares em células animais. Tipos - a: núcleo normal, b: Micronucleado e entalhado, c, d, e: evaginações, f: Vacuolado. Fonte: Cort, Ghisi, 2014.

Outro teste que pode ser utilizado como bioensaio, é através da utilização de *Drosophila melanogaster*, conhecida como mosca da fruta, e que possui curto ciclo reprodutivo, garantindo assim, a facilidade para produção e manutenção da progênie (GRAF, 2006).

O Teste de Mutação e Recombinação Somática (SMART), utiliza linhagens de *D. melanogaster* para a detecção de efeitos genotóxicos e/ou capacidade de regulação desses eventos, e possui vantagens, por ser um teste rápido que identifica a atividade de químicos isolados e compostos complexos. (Graf et al., 1998).

O SMART, pode detectar a presença de evento mutagênico (mutação pontual), mutação cromossômica e recombinação mitótica (GRAF et al., 1984; WURGLER; VOGEL, 1986; REZENDE et al. 2013).

As linhagens utilizadas, são caracterizadas como mwh, flr^3 e ORR;flr^3. Elas proporcionam a realização do cruzamento padrão (ST), com utilização de fêmeas da linhagem flr^3 e machos mwh, que possuem nível basal de enzimas metabólicas do tipo citocromo P450 - CYP, P450; além do cruzamento de alta capacidade de bioativação (HB), com utilização de fêmeas ORR;flr^3 e machos mwh, que possuem linhagens com alto nível de CYP, P450. Tais cruzamentos permitem a avaliação de genotoxinas de ação direta e indireta (GUZMÁN-RINCON; GRAF, 1995; REZENDE et al. 2013).

1.5 Caracterização dos Sítios de Estudo

Em relação à gestão das bacias hidrográficas, é importante considerar a responsabilidade quanto à fiscalização do Instituto Mineiro de Gestão das águas – IGAM, que promove na esfera estadual a aplicação dos parâmetros

legais permitidos para a manutenção da qualidade dos recursos hídricos (ANA, 2009).

A bacia hidrográfica do rio Paranaíba está localizada na região central do Brasil, e possui área total de 222,6 mil km², representando uma das principais bacias do território brasileiro. Seus cursos hídricos abrangem os estados do Distrito Federal (DF), Mato Grosso do Sul (MS), Minas Gerais (MG), além de Goiás (GO), local de maior abrangência da bacia. A origem do nome e principal recurso hídrico da bacia, é o rio Paranaíba, localizado no município de Rio Paranaíba/MG, e que possui abrangência interestadual (IGAM, 2014). Segue a descrição dos cursos hídricos avaliados na bacia hidrográfica do Rio Paranaíba:

Córrego Mumbuca, localizado na Região de Monte Carmelo - Minas Gerais, situado a 18°44’29.30” de Latitude Sul e 47°29’55.45” de Longitude a Oeste. A população é estimada em 50.694 habitantes. De acordo com ACEMC (2000), a principal atividade econômica da cidade é a produção de telhas, tijolos e artefatos cerâmicos e também é destaque na produção de curtume e de embalagens, além da produção de café.

A outra população avaliada, tem influência direta na área da sub-bacia do Rio Uberabinha, a qual possui cerca de 2000 Km^2, e abrange os municípios de Uberaba, Uberlândia e Tupaciguara. O rio apresenta trechos afetados por atividades antrópicas, sejam elas, agrícolas, domésticas ou industriais. O Rio é um local conhecido da população por apresentar atividade de agentes poluentes, e possui vários efluentes menores, o Córrego Liso e o Córrego do Óleo, os quais também podem apresentar potencial tóxico devido à proximidade, desses córregos com a área urbana (BRITES; RANTIN, 2004).

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Assessment of the genotoxic mutagenic and recombinogenic potential of water resources in the Paranaíba River basin, Minas Triangle area, Brazil

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ABSTRACT

Exposure to pollutants can induces a series of alterations in DNA that can cause genotoxic/mutagenic effects in the exposed individuals. It is the responsibility of the National Council for the Environment, under resolution 357/2005, to regulate the limits of chemical compounds permitted in surface waters. The present study aimed to monitor the genotoxic, mutagenic and recombinogenic potential and consequently the water quality, in two streams in the Paranaíba River basin in Minas Gerais state, Brazil, using two bioindicator fish with variable responses (*Rhamdia quelen* and *Geophagus brasiliensis*) and the micronucleus test and the somatic mutation and recombination test (SMART). Within the two sites assessed, the Córrego do Óleo (S2) presented intermediate water quality, with a significant increase in micronucleus frequency compared to the reference site (S1) and similar to the Córrego Liso (S3) that showed a high toxic potential, with the presence of cadmium and lead, that resulted in high micronucleus rates that were similar to the positive control. At the sites assessed, there was inducement to somatic mutation and recombination in the wings of *Drosophila melanogaster*, indicating a possible presence of potentially toxic compounds in the effluents in the Paranaíba River basin Sites.

Keywords: Micronucleus; Biomonitoring; SMART; *R. quelen*; *G. brasiliensis*

1. INTRODUCTION

The Uberabinha River, located in the Triangle Area of the state of Minas Gerais, Brazil, belongs to the Paranaíba River basin and is very important for the municipality of Uberlândia because it is a capture reservoir for water distribution. In the urban area, the Uberabinha River has some smaller tributaries, such as Córrego do Óleo and Córrego Liso that are in strategic positions because the first is located within urban activities and receives residential effluents and the second is located in the industrial sector of the municipality and therefore is the target of effluents with great pollution potential (Carrijo and Baccaro, 2000).

Water pollution reflects the presence of chemical compounds, derived from human activities that can cause problems in public health and imbalances in ecosystems (White and Rasmussen, 1998; Codd, 2000). In the urban perimeter, discharging effluents from industrial activity is the greatest contributor to environmental contamination, followed by the disposal of untreated residential effluents and contamination from agricultural activities (Amaral et al., 2006).

The National Council for the Environment (CONAMA), under resolution 357/2005 (Brasil, 2005), regulates the limits of chemical compounds permitted for surface waters and the legal parameters for releasing effluents into water bodies, in agreement with the determinations of resolution 430/2011 (Brasil, 2011).

Heavy metals are a key class of compounds for the occurrence of environmental problems, as they are persistent toxic substances, capable of forming complexes with high capacity to degrade the environment. Cadmium

(Cd), mercury (Hg), lead (Pb), copper (Cu), chromium (Cr) and zinc (Zn) stand out as contaminants of the water environment (Gurcu et al., 2010; Matsumoto et al., 2006; Chatterjee et al., 2010).

To assess water quality and its effluents is extremely important to determine the physical chemical parameters of the water resources and in addition in certain countries it is compulsory to carry out cell damage tests (genotoxicity/mutagenicity) in live organisms (Kim et al., 2008). The use of bioindicators, such as fish, allows the presence of potentially toxic components to be assessed in water reservoirs (Bolognesi and Hayashi, 2011). Therefore, water organisms accumulate in their tissues the pollutants present in contaminated waters that, due to pollution, alter their physical chemical characteristics and consequently their quality standard (Hammer, 2004).

The micronucleus (MN) test is one of the assay models for *in vivo* biological monitoring, using as resource, erythrocyte samples of peripheral blood from bioindicator species. With this, it is possible to analyze damage in the genetic material (caused by chromosome break or errors during mitosis) signaling the general toxic potential of environments in which the individuals are exposed (Fenech, 2000). The application of the fish micronucleus test is well-known, due to its ability to indicate the presence of isolated chemical compounds or complex mixtures in the water environment (Al-Sabit and Metcalfe, 1995 and Udroiu, 2006).

Another test, capable of indicating the presence of polluted water environments, is the somatic mutation and recombination test (SMART). SMART is a test that uses different *Drosophila melanogaster* lines to assess

mutations and recombinations in the wings of individuals exposed to samples of contaminated waters (Amaral et al., 2005; Pantaleão et al., 2007).

Uberlândia is a large municipality that has various water resources and many residential/industrial activities, capable of interfering in the quality of the watercourse in these locations. Thus the objective of the present study was to monitor the water quality of two streams in the Paranaíba River basin, in the Minas Triangle Area, using the Micronucleus Test, using two bioindicator fish (*Rhamdia quelen* and *Geophagus brasiliensis)* and somatic mutation and recombination test (SMART) in *Drosophila melanogaster*.

2. MATERIALS AND METHODS

2.1 Collection sites and sampling

Three collection sites were assessed (Figure 1) to deter mine the study: site 1 (designated reference site) is located on the headspring of the Uberabinha River (geographic coordinates 18° 54′ 54.216″S and 48° 18′ 36.504″W) about 10 km from the Uberlândia municipal perimeter (Southeast Brazil); this site has a conservation area with riverbank vegetation without interference from any human activities; site 2 (Córrego do Óleo) is located within the urban perimeter (geographic coordinates 18° 52′ 37.632″S and 48° 17′ 38.04″W) in an area with the presence of urban waste and irregular/clandestine domestic sewage discharge; site 3 (Córrego Liso) is located within the municipal area (geographic coordinates: 18° 59′ 12.624″S and 48° 48° 12′ 41.616″W) and is greatly influenced by the development of industrial activity (textile, food, metallurgical, tanning and chemical industries). In this location there is also a small interference from domestic effluents, but no description of agricultural activity.

2.2 Physicochemical assessment and biological material collection

To determine the mean rates of the physicochemical parameters between January and June 2015, five water sample collections were made per site, following the standards recommended by Awwa, Apha and Wpcf (2005).

The water quality index (WQI) was calculating using the data obtained in the physicochemical parameters, according to the recommendations of the local authorities, the Minas Water Management Institute (IGAM, 2005). In addition to the variables regarding the WQI, the presence was also assessed of trace elements in the sampled sites.

The collection of biological material was approved by the National Council of Control of Animal Experimentation, process number 090/2014. Twelve fish were collected of each species at each sampling site, also considering the positive control, totaling 96 individuals of both sexes of the *Rhamdia quelen* and *Geophagus brasiliensis* species. Forty-eight fish were used of the species that inhabits deep waters (*Rhamdia quelen*) that have 500g average weight and are about 18 cm long, in addition to 48 specimens of the fish that inhabit surface waters (*Geophagus brasiliensis*) that have 28g average weight and are 12 cm long.

2.3 Micronucleus Test

The fish micronucleus test was carried out according to the criteria established by Countryman and Heddle (1976) and Fenech (1993). A peripheral blood sample (about 30 µl) was extracted from each fish and immediately placed on cytological slides. The slides were dried at room temperature and fixed in ethanol for 20 minutes, then stained with 4% Giemsa. To determine the micronucleus frequency (MN) and the micro-nucleated cells (CMN) four

thousand erythrocyte were assessed per fish on each slide under an optical light microscope (1000x).

The positive control group was formed by 12 fish, collected at the reference site (S1) where more individuals were available and kept under acclimatization for a week in an aquarium after collection. The 0.4 mg cyclophosphamide was applied per fish gram in the specimens and after 96 hours blood puncture was carried out to determine the micronucleus.

2.4 Stock strains and crosses of SMART Test

The somatic mutation and recombination test (SMART) was carried out using three mutant *Drosophila melanogaster* lines: mwh, flr^3 and *ORR*, carriers of genetic markers multiple wing hairs *multiple wing hairs* (*mwh*, 3-0,3) and *flare-3* (*flr^3*, 3-38,8). The following crossings were made: (1) standard cross (ST) in which female virgin *flr^3/In(3LR)TM3, ri p^p sep I(3)89Aa bx^{34e}* and *Bd^s* were crossed with male *mwh/mwh* (Graf et al., 1989); (2) and the high bioactivation cross (HB) in which female virgin *ORR/ORR; flr^3 /In(3LR)TM3, ri p^p sep I(3)89Aa bx^{34e}* and *Bd^s* were crossed with male *mwh/mwh* (Graf e Van Schaik, 1992). In this last cross, especially, there were high levels of cytochrome P-450 enzyme metabolism in contrast to the ST crossing that had basal metabolism levels of these enzymes (Saner et al., 1996). Both the crosses (ST and HB) produced in their descent: marked trans-heterozygote flies (MH - *mwh* +/+ *flr^3*) with wild wings and balanced heterozygote flies (BH - *mwh* +/+ *TM3, Bd^S*) with serrated wings.

2.4.1 Experimental and slide procedures

After crossing, the couples were transferred to an egg laying environment and the eggs were collected during eight hours, in flasks containing 3% agar

base covered by a layer of biological yeast and supplemented with sucrose. The larvae were collected 72 hours after egg laying started, washed and transferred to glass flasks with 1.5 instant potato purée medium (HIKARI® brand) with the water samples from sites S1, S2 or S3. The water from S1 was considered as the *in situ* negative control and doxorubicyn (DXR 0.125mg/mL) was used for the positive control. Third instar larvae were submitted to chronic treatment for about 48 hours. After this period they climbed the walls of the flasks and went to the pupa phase (Orsolin et al., 2012). The adult flies were stocked in 70% ethanol. To mount the material, the wings were removed with pincers and fixed in pairs on glass slides with Faure solution and covered with a slide cover. They were analyzed under an optical light microscope with 400x magnification. The quantity of mutant hairs, the type of spot (single or twin) and the position of these mutant hairs on the wings were recorded.

2.5 Statistical analysis

The frequencies found in the micronucleus test were compared using parametric statistical evaluation by the analysis of variance (one way ANOVA) followed by the Tukey test. Regarding the SMART test, the frequency of spots per fly at each site assessed was compared to the negative control (water from site S1) using the binomial conditional test by Kastenbaum and Bowman described by Frei and Wurgler (1998) to assess the possible mutagenic and/or recombinogenic effects. The positive diagnoses obtained (for the total number of spots) were confirmed by the nonparametric U-tests by Wilcoxon and Mann-Whitney (Frei and Wurgler, 1995). The level of significance was p=0.05 in all the tests.

3. RESULTS AND DISCUSSION

The CONAMA states that the surface waters of water bodies should be monitored and inspected according to the maximum limits permitted for each parameter (Brasil, 2005). According to the considerations by Eggen and Suter (2007) chemical monitoring of an ecosystem should aim to maintain its integrity. Table 1 shows the mean values and respective standard deviations of the chemical variables analyzed at the source of the Uberabinha River (S1), Córrego do Óleo (S2) and Córrego Liso (S3). The S1 stretch was designated as reference site because of its good water quality because all its variables presented values according to the limits established by resolution number 357 of deliberative and consultative organ, CONAMA (Brasil, 2005).

In the state of Minas Gerais, the organ responsible for executing the environmental guidelines created by CONAMA regarding the conditions and standards for discharging effluents, according to the particularity of the water body, is the IGAM. This state inspection organ establishes a water quality index (WQI) that has five classification levels for the stretch assessed, on a scale from 0 to 100.

S2 was characterized as intermediate for water quality (Table 1), because some variables were above the permitted limit, such as the biochemical oxygen demand (BOD), dissolved oxygen rates (DO), total solids and fecal coliforms. All these parameters were also violated at site S3 that in addition showed high levels of total phosphorus and turbidity that meant that this site was highly degraded. As described by Mitteregger-Junior et al. (2006), the chemical variables that violate the current legal parameters are associated

with industrial and domestic waste, linked to the effluent production and the particularity of each stretch assessed.

WQI can be used to associate water quality monitoring and reporting methods (Bharti and Katyal, 2011). The chemical assessments indicated that site S2, that receives residential effluents, has a high WQI (60), as observed by Udonchoke et al. (2010). These authors assessed an Asian river, that was greatly influenced by organic load from human activity, derived from domestic sewage and consequently with polluting impact. The WQI was lower at site S3, reflecting a lower water quality, due to its proximity with the industrial complex in the municipality of Uberlândia and the contribution of organic load from domestic effluents, that are also present in the area of influence of this river stretch.

Allied to the chemical parameters of the streams in question, heavy metals were also present, known as trace elements, in the sites sampled (S2 and S3) but only at site S3 the presence of heavy metals (Pb= 0.046± 0.32 e Cd= 0.16± 0.31) was above the permitted legal limit (table 2). Pack et al. (2014) stated that urbanization activities in developing countries trigger increase in the levels of trace elements in water environments.

The presence of heavy metals is common in some stretches of watercourses bearing in mind the type of anthropic activity carried out in their surroundings (Yilmaz, 2009). These compounds can have genotoxic effect on the organisms present in the environment, causing increases in the micronucleus frequencies of the indicators assessed. Ololade et al., (2011) reported that Cd and Pb are metals with high toxic potential and directly influence the local biota.

The MN test was used in fish collected at the sample sites to assess the possible genotoxic effect. Regarding the micronucleus test, it was observed that at site S3, Córrego Liso (site of greatest heavy metal incidence), there were higher MN rates and consequently increased rates of micronucleated cells (CMN). Significant MN frequencies compared to the Reference Site (S1) were observed by Hoshina et al. (2008), indicating the presence of pollutants in the effluents produced in petroleum refining activities. This parameter indicates that in diverse situations and different anthropic activities, the micronucleus test is considered sensitive and efficient (Udroiu, 2006) to detect genotoxic events.

In site S3, according to the data in Table 3, significant increase in MN and CMN was found compared to the reference site (S1). This site (S3) was similar to the results found in the positive control (cyclophosphamide) for the two indicators (*Rhamdia quelen* and *Geophagus brasiliensis*), according to the Tukey statistical test with significance at 0.05. The increases in these frequencies in the animals in situ indicated prolonged exposure of the monitored individuals to a polluted environment (Ohe et al., 2004; de Campos Junior et al., 2014).

Regarding the Córrego do Óleo, site S2, considerable statistically significant MN and CMN rates were observed compared to S1, but they differed from the positive control. Therefore, this stretch presents intermediate toxicity potential, indicated by the WQI of the location. A previous assessment of this stretch described the presence of waste, riverbank vegetation decharacterization, the presence of farms in the permanent preservation area in addition to contamination of the reservoir by clandestine deviation of the sewage network. Thus, even though classified as an intermediate site regarding

water quality, the stretch in question requires monitoring by the municipal management, to consider the coexistence of the residential area and maintaining the water quality of this water resource.

Starting from the principle that fish species present different responses regarding sensitivity to pollution and different accumulation capacity (Henry et al., 2004; Ellesat et al., 2011; Omar et al. 2012) is important to consider that in the present research there were no statistically significant differences regarding the response of the indicators assessed (*R. quelen* and *G. brasiliensis*). Thus, even in different habitats, the species presented similar degrees of sensitivity, as there are various characteristics that can influence the response pattern, such as nutritional conditions, physiological and ecological particularities of each species (Ellesat et al., 2011).

The assessment by the SMART showed (Table 4) that all the water samples (positive control, water from site S2 and water from site S3) induced a statistically significant increase in mutant spots on the wings of the *Drosophila melanogaster* trans-heterozygote descendants (MH) from the standard cross (ST).

The positive correlation between the increase in mutant spot frequency and the presence in the environment of a chemical agent with toxic potential was reported by Pantaleão et al. (2007) and the impossibility of determining conclusively which was the conditioning compound for the occurrence of these events. Groten et al. (2001) stated that the chemical compound interaction and consequent complex formation hinders discussion regarding the origin of the pollutant effects and their respective action mechanisms in the environment and exposed organisms.

Site S1, considered as reference site, presented a low number of mutant spots on the descendants from both the crosses that emphasizes its designation as negative control in the test.

The statistically significant increase in mutant spots on the individuals exposed to water samples from site S3 is in line with the assessment of the chemical characterization of the location that was reflected in a lower WQI, due to the presence of Cd and Pb. According to DFG (2006) and Cambier et al. (2010) this metal has mutagenic and recombinogenic action. Another common agent in Brazilian water reservoirs is Pb that, according to Cestari et al. (2004), has high potential to induce various types of genetic damage. These metals (Cd and Pb), along with mercury (Hg) are considered toxic even at low concentrations (Alloway, 1995) in contrast to the others that become noxious at high concentrations (Vries et al., 2007).

Table 5 shows the results observed in the individuals derived from the HB cross, exposed to water samples from sites S2 and S3. There was a slight increase in the number of mutant spots for all the categories, compared to the individuals from the standard cross (ST). Research carried out in the central western region of Brazil (Pimenta et al., 2008) demonstrated that the greater number of spots in the HB crosses, not only correlated with the presence of chrome (Cr) in the water samples, with high toxic potential, but also, the influence of activities in that region.

The balanced heterozygote (BH) individuals, mwh/TM3, were analyzed for all the treatments because there was positive diagnosis for increase in mutant spots in the individuals derived from both crosses (ST and HB) in all the assessments. The data showed that for all the treatments there were mutation

and recombination type inductions (Figure 2). The mutation rates of the individuals derived from the ST cross ranged from (19.44 to 38.68%) and the recombination rates (61.32 to 80.56%). Regarding the individuals derived from the high bioactivation cross (HB) the mutation rates ranged from (24.39 to 35.67%), and the highest rates were associated to the recombinations (64.33% to 75.61%).

The main effect in all the collection sites and crosses carried out was for recombinogenic activity. Some in vivo assessments (Jacociunas et al., 2010) with *Drosophila melanogaster* have already shown exclusive recombinogenic activity, a fact that was associated to high frequency of twin spots in all the groups treated.

Thus as pointed out by Amaral et al. (2005), assessment of the SMART was shown to be sensitive in detecting toxic activity in water samples in collection sites close to industrial activity. In agreement with the data obtained in the present research, there were events associated with somatic mutation/recombination in the different stretches of the watercourses assessed.

For Fent (2003) the action of isolated compounds or their association with other chemical agents and consequently interaction with live organisms in the environment cannot be assessed by physicochemical characterization alone and therefore the SMART is an alternative for monitoring water environments. Generally, assessment by the SMART showed that, as in the micronucleus test, site S3, the stretch of the Córrego Liso, presented damaged DNA in the species sampled, as a result of the high presence and activity of toxic compounds of industrial origin in the location

4. CONCLUSION

The increase in the micronucleus rates, along with the variable chemical parameters and respective WQI, related the fact of site S3 characterized by a stretch of the Córrego Liso (located in an area with high toxic potential). Site S2 was characterized by an intermediate water quality, with medium MN and CMN induction rate. Both the treated sites (S2 and S3) presented a significantly different total number of spots compared with the negative control (site S1), thus, there was a positive correlation between Micronucleus Test and SMART. Therefore the interference of toxic agents in the water samples was shown both in the Córrego do Óleo and the Córrego Liso, in the stretches assessed, and consequent interference in the assessed monitored species (*Rhamdia quelen* and *Geophagus brasiliensis*).

5. CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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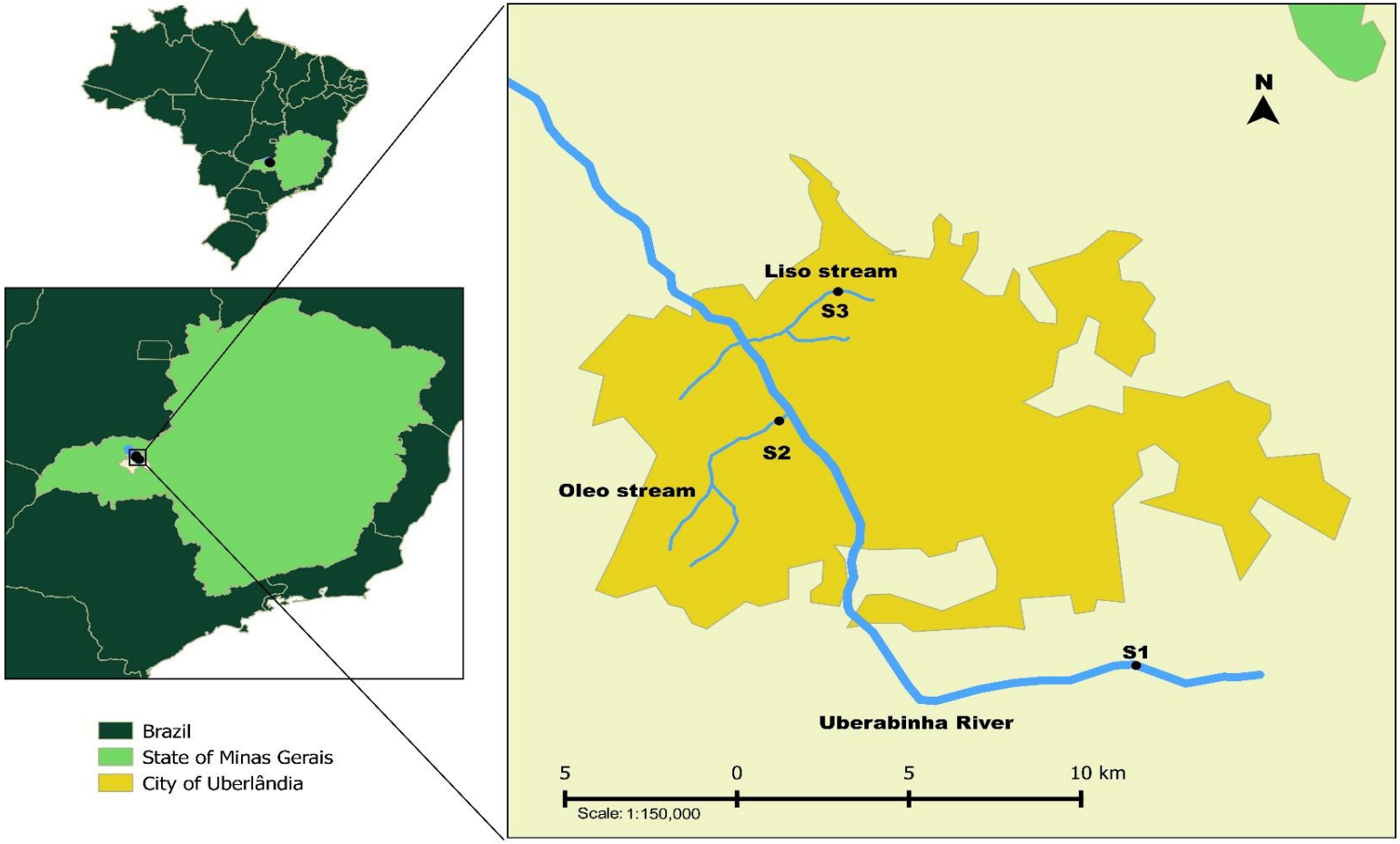

Fig. 1 Location of the sites assessed (Site 1, Site 2 and Site 3) in the Paranaíba River basin, Uberlândia, MG.

Table 1. Physical-chemical variables and Water Quality Index (WQI) of Paranaíba River basin.

Variable	Units	Sampling Sites		
		Site 1 (Mean ±SD)	**Site 2** (Mean ±SD)	**Site 3** (Mean ±SD)
Temperature	°C	22.34 ±1.66	23.07 ±1.23	22.81 ±2.12
pH	UpH	6.75 ±1.96	6.24 ±1.17	6.38 ±1.32
Biochemical Oxygen Demand	mg L^{-1}	1.18 ±0.25	6.13 ±2.34*	10.45 ±2.95*
Dissolved Oxygen	mg L^{-1}	8.03 ±1.16	5.02 ±1.76*	4.24 ±1.13*
Nitrates	mg L^{-1}	0.52 ± 0.46	3.52±2.77	4.18 ± 3.61
Total Phosphorus	mg L^{-1}	0.01 ±0	0.031 ±0.012	0.059 ±0.10*
Total Solids	mg L^{-1}	48 ±22	326 ±337*	831 ±456*
Turbidity	UNT	3 ±5	50 ±13	139 ±23*
Fecal Coliform	NMP/100 ml	12.18 ±4.53	421 ±264*	870 ±316*
WQI **Classification****		**90** **Excellent**	**60** **Regular**	**44** **Poor**

*Values above the allowed level as determined by CONAMA Resolution no. 357 (2005).

**WQI Classification: 90<IQA≤100 (excellent); 70<IQA≤90 (Good); 50<IQA≤70 (Regular), 25<IQA≤50 (poor); 0<IQA≤25 (very poor).

Table 2. Trace elements frequency of Paranaíba River basin sites (S1, S2 and S3).

Collection Sites	Trace elements (mg/L)			
	Pb	**Cr**	**Mn**	**Cd**
Site 1	<DL	0.01± 0	0.09 ± 0.23	<DL
Site 2	0.012± 0.22	0.02± 0.01	0.25± 0.14	0.07± 0.21
Site 3	0.046± 0.32*	0.04± 2.74	0.47± 0.66	0.16± 0.31*

*Values above the allowed level as determined by CONAMA Resolution no. 357 (2005) / DL (Detection Limit)

Table 3. Micronucleus test (MN) frequency and micronucleated cells (MNC) in erythrocytes from *R. quelen* and *G. brasiliensis* collected in the Paranaíba River basin, Uberlândia, MG, Brazil.

Treatments	Species	Nº of individuals	X(‰) ± SD	
			MN	CMN
Positive Control	*Rhamdia quelen*	12	0.764 ± 0.123[a]	0.764 ± 0.123[a]
Positive Control	*Geophagus brasiliensis*	12	0.704 ± 0.117[a]	0.704 ± 0.117[a]
Reference Site (S1)	*Rhamdia quelen*	12	0.053 ± 0.037[b]	0.053 ± 0.037[b]
Reference Site (S1)	*Geophagus brasiliensis*	12	0.037 ± 0.021[b]	0.037 ± 0.021[b]
Site 2 (Óleo stream)	*Rhamdia quelen*	12	0.194 ± 0.102[a,b]	0.186 ± 0.113[a,b]
Site 2 (Óleo stream)	*Geophagus brasiliensis*	12	0.181 ± 0.073[a,b]	0.163 ± 0.070[a,b]
Site 3 (Liso stream)	*Rhamdia quelen*	12	0.701 ± 0.318[a]	0.693 ± 0.278[a]
Site 3 (Liso stream)	*Geophagus brasiliensis*	12	0.637 ± 0.223[a]	0.627 ± 0.213[a]

[a] Significant difference when compared to the S1, according to the Tukey test (α=0.05)
[b] Significant difference compared to the positive control (α=0.05)

Table 4. Frequency of mutants spots observed in the marked trans-heterozygotes descendants (MH) of *Drosophila melanogaster* derived from the standard cross (ST) treated with surface water samples from the Paranaíba River basin sites.

Genotypes and treatment	N. of flies	Spots per fly (number of spots); statistical diagnosis[a]				Total spots	Mean Clone	Frequency of clone formation (10^5 cells per cell division)[f]	
		small single	large single	twin spots	Total spots				
	(N)	(1-2 céls)[b]	(>2 céls)[b]			mwh[c]	and class[c,d]	Observed[d,e]	Control corrected[d,e]
		m = 2	*m* = 5	*m* = 5	*m* = 2	(*n*)	($\hat{i}$)	*n/NC*	($2^{\hat{i}-2}$) X (*n/NC*)
mwh/flr³									
S1	60	0.08 (5)	0.05 (3)	0.00 (0)	0.13 (8)	8	2.13	0.27	0.30
S1+DXR	60	0.68 (41) +	0.30 (18) +	0.18 (11) +	1.17 (70) +	69	2.52 {2.57}	2.36 {2.08}	3.38 {3.10}
S2	60	0.18 (11) i	0.12 (7) i	0.05 (3) i	0.35 (21) +	21	2.52 {2.77}	0.72 {0.44}	1.03 {0.76}
S3	60	0.27 (16) +	0.17 (10) +	0.08 (5) +	0.52 (31) +	31	2.65 {2.83}	1.06 {0.79}	1.66 {1.39}
mwh/TM3									
S1	30	0.03 (1)	0.00 (0)		0.03 (1)	1	1.00	0.07	0.03
S1+DXR	30	0.20 (6) +	0.07 (2) i	[g]	0.27 (8) +	8	1.88 {2.00}	0.55 {0.48}	0.50 {0.48}
S2	30	0.07 (2) i	0.00 (0) i		0.07 (2) i	2	1.50 {2.00}	0.14 {0.07}	0.10 {0.07}
S3	30	0.13 (4) i	0.07 (2) i		0.20 (6) +	6	1.83 {2.00}	0.41 {0.34}	0.37 {0.34}

[a]Statistical diagnostic according to Frei and Wurgler (1988): (+) positive (compared to the negative control); (−) negative; (i) inconclusive; m, minimal risk multiplication factor for the assessment of negative results; probability levels α = β = 0,05.

[b]Including rare single *flr³* spots.

[c]Considering the mwh clones for the single spots and mwh for the twin spots.

[d]Numbers in square brackets are induction frequencies corrected for spontaneous incidence estimated from negative controls

[e]Frequency of clone formation: clones/flies/48,800 cells (without size correction).

[f]Calculated according to Frei et al. (1992).

[g]Only mwh single spot can be observed in BH individuals; Balancer chromosome TM3 does not carry the flr3 mutation.

Table 5. Frequency of mutants spots observed in the marked trans-heterozygotes descendants (MH) of *Drosophila melanogaster* derived from the bioactivation cross (HB) treated with surface water samples from the Paranaíba River basin sites.

Genotypes and treatment	N. of flies	Spots per fly (number of spots); statistical diagnosis[a]										Total spots	Mean Clone		Frequency of clone formation (10^5 cells per cell division)[f]			
		small single		large single		twin spots		Total spots										
	(N)	(1-2 céls)[b]		(>2 céls)[b]								mwh[c]	and class[c,d]		Observed[d,e]		Control corrected[d,e]	
		m = 2		*m* = 5		*m* = 5		*m* = 2				(*n*)	(*î*)		*n/NC*		($2^{\hat{i}-2}$) X (*n/NC*)	
mwh/flr³																		
S1	60	0.10 (6)		0.02 (1)		0.02 (1)		0.13 (8)				8	1.75		0.27		0.23	
S1+DXR	60	0.83 (50)	+	0.52 (31)	+	0.43 (26)	+	1.78 (107)	+			104	3.04	{3.15}	3.55	{3.28}	7.30	{7.25}
S2	60	0.22 (13)	i	0.13 (8)	+	0.05 (3)	i	0.40 (24)	+			24	2.63	{3.06}	0.82	{0.55}	1.26	{1.14}
S3	60	0.45 (27)	+	0.28 (17)	+	0.13 (8)	+	0.87 (52)	+			50	2.50	{2.64}	1.71	{1.43}	2.41	{2.24}
mwh/TM3																		
S1	30	0.03 (1)		0.00 (0)				0.03 (1)				1	1.00		0.07		0.03	
S1+DXR	30	0.30 (9)	+	0.13 (4)	i			0.43 (13)	+			13	2.08	{2.17}	0.89	{0.82}	0.94	{0.92}
S2	30	0.07 (2)	i	0.03 (1)	i	[g]		0.10 (3)	i			3	2.00	{2.50}	0.20	{0.14}	0.20	{0.19}
S3	30	0.20 (6)	i	0.10 (3)	i			0.30 (9)	+			9	2.00	{2.13}	0.61	{0.55}	0.61	{0.60}

[a]Statistical diagnostic according to Frei and Wurgler (1988): (+) positive (compared to the negative control); (−) negative; (i) inconclusive; m, minimal risk multiplication factor for the assessment of negative results; probability levels α = β = 0,05.

[b]Including rare single *flr³* spots.

[c]Considering the mwh clones for the single spots and mwh for the twin spots.

[d]Numbers in square brackets are induction frequencies corrected for spontaneous incidence estimated from negative controls

[e]Frequency of clone formation: clones/flies/48,800 cells (without size correction).

[f]Calculated according to Frei et al. (1992).

[g]Only mwh single spot can be observed in BH individuals; Balancer chromosome TM3 does not carry the flr3 mutation.

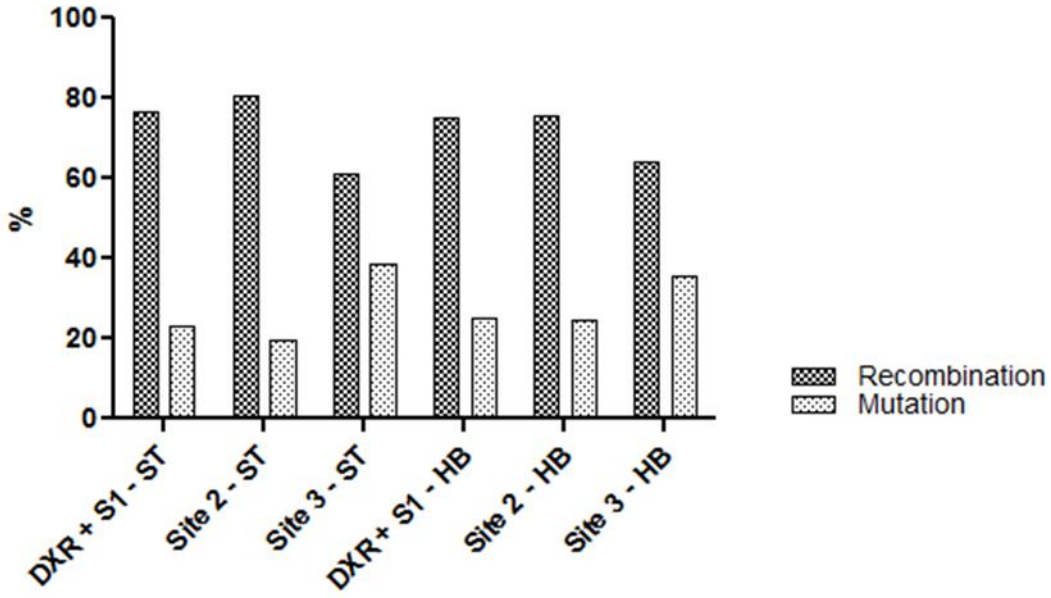

Fig. 2. Comparison of recombinogenic and mutagenic events obtained of clone-induction frequencies in standard (ST) and high bioactivation (HB) crosses.

Capítulo III

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Monitoring genotoxicity potential in the Mumbuca stream, Minas Gerais, Brazil

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Running title: Genotoxicity in Mumbuca stream

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Abstract

Rivers are sites for water catchment to supply metropolitan areas but also serve as receptors for discharge of urban sewage, wastewater and agri-industrial effluents. Bioindicators, or sentinel organisms, are widely used as markers of pollution in various environments. The objective of the present study was to evaluate the genotoxic potential and consequent quality of the water from the Mumbuca stream, which supplies the city of Monte Carmelo, located in the Minas Triangle region, Minas Gerais, Brazil. This was achieved using two variable response bioindicators (*Rhamdia quelen* and *Geophagus brasiliensis*), the micronucleus (MN) test and determining the presence of metals by flame atomic absorption spectrometry. Results showed that site 3 (region of residential flow and intense industrial pottery activity), from the chemical and biological aspects, presented a greater possibility for induction of genotoxic activity, as confirmed by the increase in the micronucleus frequency in *Rhamdia quelen* and *Geophagus brasiliensis* in comparison with the reference site. The water of the Mumbuca stream was influenced by genotoxic agents, especially lead and chrome, assessed by the *in vivo* micronucleus test. Data suggest that discharge of industrial effluents in a specific stretch of the stream interfered with biota.

Keywords: Micronucleus; *Geophagus*; *Rhamdia;* Bioindicator

1. INTRODUCTION

Water quality in Brazil, according to Brasil (2005), has been regulated since March 17, 2005 (Resolution no.357 of the National Council for the Environment - CONAMA). This regulation classifies waters and determines the acceptable physical and chemical levels that cannot offer risks to organisms that use this resource (Barbério et al., 2009). However, concern has arisen about the accuracy of the established tolerance levels because potential genotoxicity indices have been observed and presented with significant effects, even when the parameters were in agreement with those determined by law (Matsumoto et al., 2006).

Anthropomorphic activity significantly increases oscillations in the chemical makeup of aquatic environments, and pollution with compounds with deleterious effects is important (Araújo et al., 2001). Contamination of the water of large cities throughout the world is of concern for the water resource conservation authorities. Rivers are both water catchment points to supply metropolitan areas and receptors for the discharge of urban sewage, wastewater and agro-industrial effluents. In some regions, the environment has been disturbed such that degradation of these contaminants and restoration of the natural balance is not possible (Wengrat and Bicudo, 2011).

The presence of chemical products in the environment should be detected so that a prognosis can be made of the probable effects on the organisms and the impacts that they may have on animal, plant and human populations (Gadzała-Kopciuch, 2004).

Regarding components with potential genotoxic action, lead (Pb) is emphasized as a chemical that can interact directly with genetic material via

covalent chemical linking (Hong et al., 2007). The lead concentrations in aquatic environments are higher in the proximities of industries that process various materials when compared to environments without human interference (Atsdr, 2007). In addition to lead, other heavy metals are also labeled as toxic or even genotoxic, as is the case of Mercury (Hg), which is widely used in mining operations and present in sewage and industrial discharges. Copper (Cu) comes from mining, industrial and agricultural activities, is widely distributed in water and has a high capacity to form complex chemicals. Chrome (Cr) is used on a large scale in industrial activities, is present in domestic effluent, and is known for its genotoxic characteristic that causes damage by its ions when in the hexavalent oxidation state. Other metals with known genotoxic potential include cadmium (Cd), nickel (Ni) and manganese (Mn) (Templeton et al., 2000).

In Brazil, several studies have reported the toxic environmental effects of lead (Costa et al., 2007). Such effects, according to Suiçmez et al. (2006), are found in various concentrations in the different tissues of exposed animals.

Bioindicators, or sentinel organisms, that act as environmental signalers of polluted areas are widely used. Biomarkers are equally useful because they indicate systems that generally include a subsystem of a complete organism, used to identify a specific target (Reddy and Baghel, 2012). Fish are used as test organisms because of their importance in the study of water genotoxicity, considering that they are susceptible to the presence of environmental xenobiotics and their cytogenetic type is easy to handle in the laboratory. Fish can act as bioindicators of polluted water bodies because some species are highly sensitive to various contaminants through exposure to anthropological

activities, or even directly by feeding from other organisms that have also been exposed (Lakra and Nagpure, 2009).

According to Callisto et al. (2001), benthic water aquatic organisms are more tolerant to organic pollution because they are adapted to lower oxygen levels, a factor reflected in the diverse sensitivity among different species. Benthic water fish are also in greater contact with the benthic layer, which harbors higher concentrations of chemical compounds than other parts of a water body (Ahlf et al., 2002; Fracácio et al., 2003; Hollert et al., 2002; Rocha et al., 2009).

Various species are considered efficacious bioindicators because of their capacity to indicate environmental deterioration caused by the interaction of contaminants present in the environment (Feretti et al., 2008). The “acará” or “cará” (*Geophagus brasiliensis*) belongs to the Cichlidae family, most of the species of which are freshwater fish with a few found in saline environments, and is considered a surface organism. These fish are very sensitive to the environment and therefore present bioindicator capacity. The “acará” are omnivorous and can feed on fish, micro-crustaceans and gastropods, among others. They are not significant in the market but are important in subsistence fishing (Beatty, 2013). Another species with bioindicator characteristics is the *Rhamdia quelen* catfish*,* adapted to the lentic environment that is typically found in holes on river and lake bottoms. These fish are nocturnal and prefer environments with sand, mud and old tree trunks, where they hide during the day (Gomiero and Braga, 2007).

Cytogenetic trials are used for biomonitoring because they can assess damaged species at variable concentrations and exposure times (Pimenta et

al., 2013). This damage results from the action of toxic and genotoxic substances on sensitive organisms. During the cell division processes, more specifically in telophase, whole or partial chromosomes are not incorporated in the main nucleus and form smaller nuclei known as micronuclei. The micronucleus was defined by Winter et al. (2007) as a chromatin fragment, identified as detached from the main nucleus, and originates by chromosome breaks or mitotic dysfunctions.

In addition to the micronuclei, other variables of the principal test can be considered that can also indicate the quality state of the environment assessed, such as micronucleated cell counting and the presence of cell abnormalities of the lobed, blebbed and notched types (Ayllón et al., 2000; Carrasco et al., 1990; Çavas and Ergene-Gozukara, 2007; Ergene et al., 2007; Lemos et al., 2005).

The region of the Minas Triangle is located in the southeast region of the state of Minas Gerais, Brazil, and has a hydrographic network of great economic and environmental importance. The city of Monte Carmelo, situated in this region, has many pottery industries that discharge effluents with toxic residues from washing the varnish tanks directly into the Mumbuca stream, which harms the water quality in this region. The objective of the present study was to evaluate the genotoxic potential and consequent water quality of the Mumbuca stream that supplies the city of Monte Carmelo, Minas Triangle region in the state of Minas Gerais, Brazil, with two bioindicator fish (*Rhamdia quelen* and *Geophagus brasiliensis*) using the micronucleus test and genotoxic metal quantification by flame atomic absorption spectrometry.

2. MATERIALS AND METHODS

2.1 Study area

The Mumbuca Stream (fig. 1) is an affluent of the Perdizes River in the Paranaíba River hydrographic basin and is an area used for water resource extraction. The fish used in the present study were collected from the following areas:

Site 1 (Reference site): located at the main site of water catchment for residential supply. It has well preserved riverbank vegetation and natural resources. Geographic coordinates: 18º44'39.9"S and 47º29'93.6"W.

Site 2: Located in an area of residential flow and little industrial activity. Geographic coordinates: 18º43'63.3"S and 47º29'56.4"W.

Site 3: The last site sampled, it is also located in a region of residential flow and intense industrial pottery activity. Geographic coordinates: 18º41'93.7"S and 47º29'41.7"W.

2.2 Sample collection and physical-chemical analysis

The physical-chemical parameters of the water from the sampled sites were assessed according to standard methods (Awwa, Apha and Wpcf, 2005).

A total of eight samplings were made during the dry season (low water) and the wet season (high water), between February and October 2013. One hundred and twenty biological samples were used, including fish of the *Geophagus brasiliensis* (mean weight*:* 35 ± 6 g and mean length: 15 ± 4 cm) and *Rhamdia quelen* species (mean weight*:* 700 ± 150 g and mean length: 23 ± 4 cm). To standardize the samples, 183 individuals were collected at each collection site and 43 were discarded because they did not meet the suggested biometric characteristics. Fifteen specimens of each species were sampled per

site, including the positive control. Bait and a fishhook were used to capture the fish. The fish were kept in polystyrene boxes with water from the location and proper aeration until transport. The fish samples collected (bioindicators) contained individuals of both sexes. They were transported live to the laboratory for later analysis and kept in a 90 x 50 x 45 cm aquarium.

2.3 Water Quality Indices - WQI

Water quality indices were determined as proposed by the Water Management Institute of the State of Minas Gerais, Brazil, the Instituto Mineiro de Gestão das Águas – IGAM (2005). The WQI was determined by weighting some of the analyzed chemical parameters (dissolved oxygen, fecal coliforms, biochemical oxygen demand, total phosphate, temperature, turgidity and total solids) using the IQADATA software. The indices obtained were classified as follows: $90<IQA\leq100$ (excellent); $70<IQA\leq90$ (Good); $50<IQA\leq70$ (Regular), $25<IQA\leq50$ (poor); $0<IQA\leq25$ (very poor).

2.4 Micronucleus Test

The micronucleus test was carried out according to the criteria validated by Countryman; Heddle (1976) and Fenech (1993). After collection and acclimatizing for 12 hours, the fish were anaesthetized with 25 ml of 1.5% benzocaine alcohol solution in an aquarium with 2 L of water. Shortly after a 30-min sedation, a blood sample was extracted from each fish by puncturing the tail vein with a heparinized syringe to smear on slides (two per fish). Approximately 40 µl of blood was applied to each slide. The slides were dried at ambient temperature and fixed after 24 hours in 100% ethanol for 15 min and

then stained with 4% Giemsa solution diluted in phosphate buffer (60 mM KH2 PO4 and 60 mM Na2HPO; pH 6.8) for 15 min. The slides were washed with distilled water and dried at ambient temperature and later prepared for use. The cytological analysis was carried out as proposed by Schmidt (1975) in an optical microscope with 1000x magnification. A total of 4000 mononucleated erythrocytes were examined per fish on each slide. Some criteria previously proposed to identify the micronucleus were used to guarantee the count accuracy (Fenech et al., 2003). The number of micronucleated cells (CMN) was also quantified because some of the cells analyzed presented more than one micronucleus.

2.5 Nuclear abnormalities

To analyze the erythrocytic nuclear abnormalities (ENA), alterations were ranked according to Carrasco (1990) using three classifications: blebbed (nuclei with small evaginations and that have chromatin), lobed (nuclei with larger evaginations, including alterations that determine a deformed nucleus), and notched (membrane invaginations, do not contain genetic material in the invaginated location). All of the records of alterations were placed in a single category so that the ENA could be assessed as a single endsite. The MN, CMN and ENA indices were calculated according to Costa et al. (2009) as the number of alterations per 1000 mononucleated erythrocytes.

2.6 Positive control

For the positive controls, 15 fish of each species were collected from the reference site because in this location, there was a higher demand for fish. They were placed in three aquariums and acclimatized for one week. The fish were then weighed and anesthetized with 0.1 g/L benzocaine to administer cyclophosphamide (0.04 mg/g fish). They were killed after 96 hours of exposure.

2.7 Preparation of biological material for the Flame Atomic Absorption Spectrophotometer (FAAS) assay

Ten fish of each species from sites 1, 2 and 3 were used for the heavy metal analysis, assessed by the physico-chemical analyses of the water. The fish were dissected, and the organs (gills and liver) were removed from the test groups (sites 2 and 3) and compared with the specimens from the reference site (site 1). The tissues were washed in distilled water and preserved in a formaldehyde solution, which was later removed. The procedure was carried out following the recommendations of the FAO (1975) and Cyrille et al. (2012) using 500 mg of each tissue placed separately in Teflon test tubes for digestion with nitric acid (HNO_3) and perchloric acid ($HClO_4$) for 24 hours at ambient temperature, followed by 4 hours at 100ºC. The samples were filtered and analyzed for heavy metal (lead, chrome and manganese) content using a Perkin Elmer Flame Atomic Absorption Spectrometer (FAAS) model AAnalyst 100. To calibrate the spectrophotometer, standard solutions were used at different concentrations (0.2, 0.5, 1 and 2.5 µg/g) of the metals assessed. The detection limits (DL) were 0.01 µg/ml, 0.01 µg/ml and 0.005 µg/ml for lead,

chrome and manganese, respectively. To guarantee the quality of the method, the analyses were carried out in duplicate, applying the protocol by Boadi et al. (2011).

2.8 Statistical analysis

The data were analyzed using the AnalysSOft BioStat statistical program, Professional version 2009. The results were compared using the parametric-type analysis of variance test (one-way ANOVA) followed by the Tukey test. All of the tests were carried out using a significance threshold of 0.05. For correlations, the calculation was made according to Pearson and the level of significance was adjusted to 95%.

3. RESULTS AND DISCUSSION

Interference in water bodies by any polluting agent is widely known and has already been discussed in various studies (Hoshina et al., 2009; Ohe et al., 2003; Rocha et al., 2009; Vargas et al., 2001).

The Mumbuca stream is considered Class 2 by CETESB (2009). This classification includes waters intended for human supply in addition to other activities, such as fishing (Brasil, 2005). The water collected from the Mumbuca stream was submitted to chemical analysis and, as suggested by the resolutions of the National Council of the Environment (CONAMA), the parameters obtained were classified into three categories for water quality: class 1 (excellent level), class 2 (regular level) and class 3 (poor level).

Effluents of agricultural origin were not observed in the area of influence of the collection sites of the research. All of the parameters (Table 1) in the

study were ranked as class 1 at the reference site according to the chemical data. In this stretch, translucent inodorous water can be seen, which is ideal for consumption after conventional treatment. At site 2, which mainly has residential characteristics, many parameters were found within the ideal conditions, but some parameters, such as biochemical oxygen demand (BOD), dissolved oxygen (DO), fecal coliform and total solids levels, were ranked as class 3. The residues assessed at site 2 reflected the presence of organic effluents, mainly related to the depletion of dissolved oxygen in the environment, indicating a high organic matter load (Ballester et al., 1999). At site 3, many chemical parameters were observed to be outside of the limits proposed by the Minas Gerais agency for water management, such as those of class 3 (BOD, DO, fecal coliform, total solids, total phosphorus, turbidity, total lead, chrome and manganese).

The reference area (site 1) was chosen after pre-analysis, and its standard of excellent water quality was confirmed after grouping some of its categories according to the data of the water quality index (WQI) presented in Table 1. The water at site 2, because of its influence from domestic discharges and, in some cases, intervention from industrial activity, presented medium WQI values but was still considered a stretch with regular quality water. At site 3, many actions of interference from human activity were found, mainly of the industrial type, and after weighting some parameters, values were observed to be much lower than expected for the stretch according to the limits proposed by IGAM (2005). It was therefore considered a very poor quality environment. Thus, site 3 showed the greatest potential for damage to organisms present in the Mumbuca stream.

It is important to consider that the water quality and the respective integrity of the biota assessed are mostly involved with industrial effluents that typically have a high genotoxic capacity and that the untreated residential effluents that can contain loads of toxins that induce water degradation, or even cause cell damage to the biological material assessed, should also be considered.

Many chemical compounds, even when free in the environment, may not interact directly with the present water organisms. To better understand the actions of the irregular compounds, especially heavy metals, flame atomic absorption spectrometry (FAAS) methodology was applied so that the presence of any heavy metal, which would normally have genotoxic properties, could be quantified. According to Evans et al. (1993), the gill epithelium is directly related to the heavy metal concentrations found in water resources because the gills, responsible for gas exchange, are in direct contact with the pollutants in the environment. Other tissues can also accumulate heavy metals, such as the liver, which, according to Romeo et al. (1999), represents the main organ associated with detoxification and other metabolic activities associated with toxic compounds.

Among the metals analyzed according to the FAAS methodology, only the tissue levels of lead, chrome and manganese were determined in the fish, due to the heavy metal presence indicated by the physical-chemical analyses and in consideration of the industrial activities in the proximities of the sites assessed. Table 2 shows that the lead, chrome and manganese levels were in accordance with the norm (above detection limit) in the individuals sampled from the reference site, a fact that was used as a parameter for statistical

comparison. At site 2, low lead and chrome indices were quantified, but they were not as significant as those observed at site 3. These results indicated toxic action of the lead and chrome, detected by the two bioindicators *Rhamdia quelen* and *Geophagus brasiliensis*, but their means did not differ statistically. The presence of low manganese levels was also observed at site S3, but these indices were not above legal limits for hazardous substances in fish.

The presence of heavy metals in this water body may have been due to the production of effluents from pottery activities that contain varnishes and paints with derivatives of lead compounds (Kasuba et al., 2012). Although site 2 had the prevalent characteristic of a residential area and its respective effluents, it still has some relatively small industrial activities because the municipality in question does not regulate its activities in sectors, as proposed by the public administration of other cities. Thus, the low lead and chrome indices found in these individuals from site 2 and free in the water as shown in the chemical analysis may have come from these activities at this intermediate stretch of the Mumbuca stream.

Using the same methodology, a study by Cyrille et al. (2012) also detected indices of heavy metal presence in water organisms. This assessment can indicate the contribution of any heavy metal in the induction of cell abnormalities in bioindicator species. The differences found regarding the presence of the metals in fish tissues can be explained by the use of these trace elements at different concentrations by the industrial activities in the area of influence. Thus, lead-derived compounds are used at higher concentrations than the others, while chrome presented intermediate indices and manganese presented low incidence, both in the tissue and the physical-chemical

assessments and was close to the legal limits pre-determined by the legislation. The indices obtained in the physical-chemical assessment of the Mumbuca stream in stretch 3 were above the limits established by the Brazilian legislation. This finding suggests that the effects found at this site were the result of the interactions of mixtures causing varied effects on the environmental resources and for the biota that use this environment (Ohe et al., 2004).

For each site, 15 viable individuals were assessed for the principal test (MN test), in addition to the complimentary assessments (CMN and total ENA) shown in Table 3. The micronucleus test frequency at site 2 can be treated as intermediary because it presents a significant difference compared to the reference site, but it was not significant when compared to the indices from the positive control. Therefore, it cannot be foreseen as a stretch of genotoxic potential. The site 3 assessment presented high indices of MN, CMN and ENA for both the biological species with statistical similarity ($p<0.05$) to the positive control (genotoxicity induction with cyclophosphamide) following the Tukey test, establishing evidence that site 3 suffers deleterious action from genotoxic products.

The complimentary assessments were in agreement with the variations observed in the main test. Sites S2, S3 and the positive control (treated samples) were characterized by the presence of more than one micronucleus per cell. Based on this assessment, the micronucleus cell ratio, which also differed significantly at the treated sites from the reference site, was described.

According to Serrano-Garcia and Montero-Montoya (2001), the ENA and micronucleus originate by similar processes and both indicate interference from genotoxic factors. This fact is in line with the present study because in the three

abnormality assessments carried out, the results were shown to be similar across sites. In the assessments by Ayllón and Garcia-Vasquez (2000), Bolognesi et al. (2006) and Ergene et al. (2007), a positive correlation was also shown among the nuclear abnormalities and the micronucleus rates. In agreement with these authors, it can be inferred from the results obtained in the present study that the total cell abnormalities and the micronucleus rates indicated the genotoxicity potential at the sites sampled. Similarly, Ferraro (2004) stated that the relationship between the number of nuclear abnormalities and an index of genotoxicity effects suggested that each sign of morphological alteration could represent a manifestation of the effects of chemicals present in the resources assessed.

The micronucleus test was used because it is a simple methodological procedure that is accessible to any small municipality that does not have many resources or advanced research centers. The test can be applied by agents from the environmental area, and the results obtained can be complemented with more specific biological or chemical assessments to reach the expected objectives. Regardless of using the complimentary assessments, the micronucleus induction assay is known for its validity as a monitor of genotoxic effects in fish. Thus, the viability of the test was recognized (Udroiu, 2006).

It is important to note that there were no significant differences in any of the tests carried out regarding the bioindicators because both were performed with the same statistical relevance compared to the controls (S1 and the positive control), supporting the use of both species for eco-toxicological tests. According to Pimenta et al. (2013), different species react differently to genotoxic agents. In the present study, however, no significant differences were

shown to demonstrate variation in the indicators used. Thus, species with variable habitats, performance and location (surface or benthic) were shown to be similar by the tests applied.

Site 3, from the chemical and biological aspects, presented greater possibilities for inducing genotoxic action, as confirmed by the increase in the indices at this stretch in *Rhamdia quelen* and *Geophagus brasiliensis* compared to the reference site. This increase in the micronucleus frequency at site 3 was directly linked to the relation between the lead rates (Fig. 2) found in the fish assessed according to Pearson correlations ($\alpha = 0.05$). The comparison between MN frequency and mean lead and chrome concentrations in tissues of *Rhamdia quelen* and *Geophagus brasiliensis* shows a positive correlation. This relationship was determined by Omar et al. (2012), who stated that metals have genotoxic potential, a fact shown in previous studies (Wierzbicka, 1989), and demonstrated the capacity of lead and chrome to induce formation of diverse nuclear alterations by interfering in cell division mechanisms. A correlation between Pb and Cr levels may simply indicate that there are toxic substances in the environment because pollutants generally occur in mixtures. Thus, factors such as chemical compound interactions, which cannot be predicted, can also contribute to the increase in damaging effects to the cells.

The city of Monte Carmelo depends on the income from pottery activities, but the environmental damage involved in this process is immeasurable and affects both the quality of public health related to water supply and the development of aquatic organisms sensitive to the chemical products, which are irregularly present in the water in some stretches.

4. CONCLUSIONS

It was concluded that the water from the Mumbuca stream is influenced by genotoxic agents, especially lead. This was determined by assessment with an *in vivo* micronucleus test, the results of which suggested that discharges of industrial effluents in a specific stretch of the stream (S3) interfered with the biota. This fact should be considered regarding the present concern of industrial effluent into the environment, as well as long term to maintain the viability of the stream in question for urban supply after processing by municipal treatment stations. Regarding the bioindicator used, the benthic fish (*Rhamdia quelen*) presented a higher rate of abnormalities (MN, CMN and ENA) than the surface fish (*Geophagus brasiliensis*). This increase was not statistically significant, so both types of fish, when placed in different locations in the body of the stream, had sensitivity potential for viable bioindication to carry out the genotoxicity tests.

5. CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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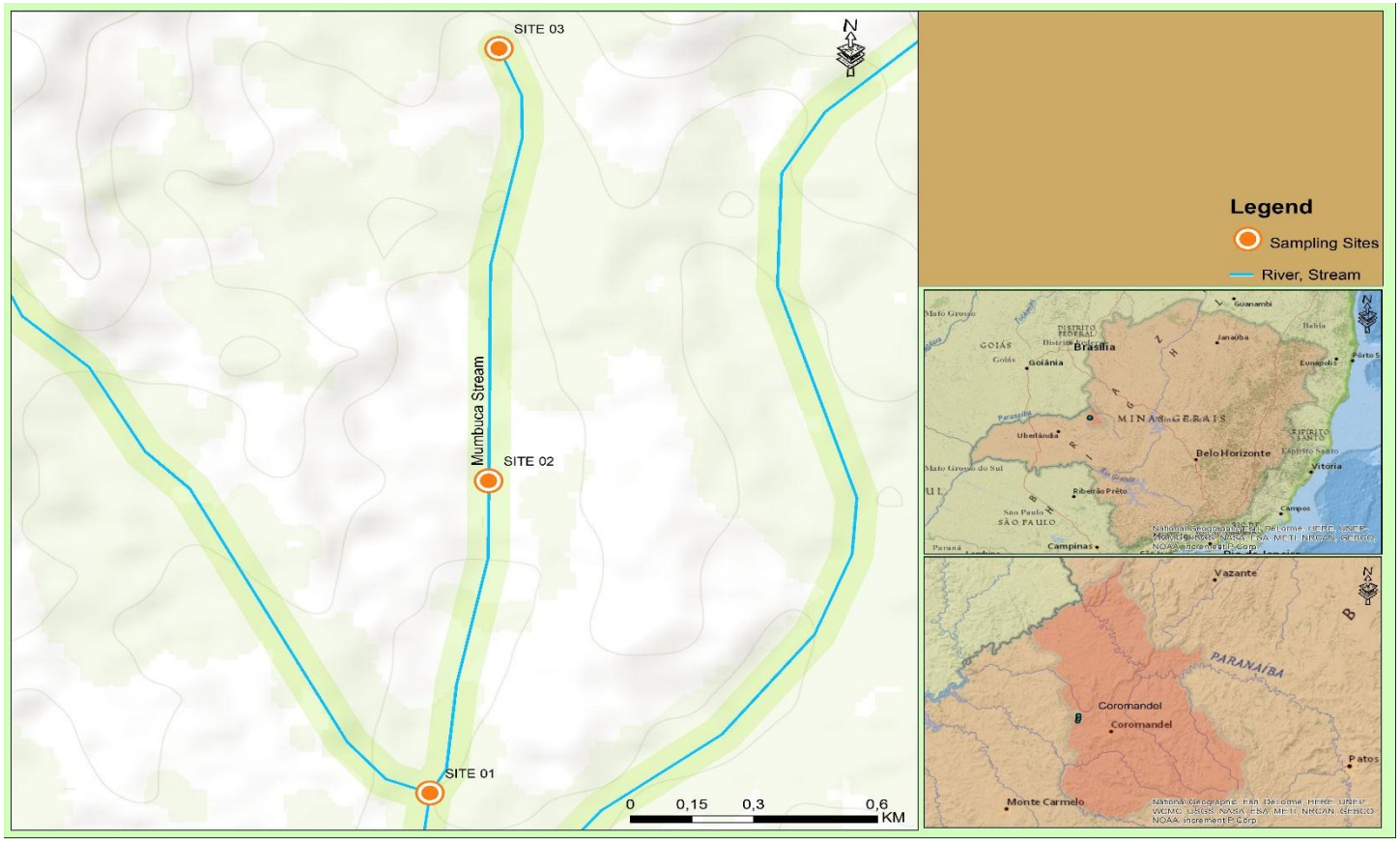

Fig. 1 Location of the sites assessed (Site 1, Site 2 and Site 3) in the Mumbuca stream, Monte Carmelo, MG.

Table 1: Physical-chemical parameters and water quality index (WQI) of stretches in the Mumbuca Stream.

Parameters	**Site 1** (Mean and SD)	**Site 2** (Mean and SD)	**Site 3** (Mean and SD)
Biochemical Oxygen Demand (mg L^{-1})	0.68 ±1.05	5.67 ±3.14*	8.88 ±2.56*
Dissolved Oxygen (mg L^{-1})	7.33 ±1.16	5.89 ±2.67*	4.34 ±1.07*
Fecal Coliform (NMP/100 ml)	19.33 ±8.67	305 ±121*	467 ±344*
Nitrates (mg L^{-1})	1.08 ± 2.14	6.34 ±10.45	5.36 ± 5.67
pH (UpH)	6.66 ±2.34	6.09 ±1.14	6.13 ±2.21
Total phosphorus (mg L^{-1})	0.002 ±0	0.014 ±0.08	0.068 ±0.14*
Total Solids (mg L^{-1})	39 ±28	580 ±173*	1030 ±84*
Turbidity (UNT)	12 ±6	56 ±34	132 ±12*
Temperature (°C)	23.12 ±2.34	23.47 ±1.69	23.41 ±1.52
Total lead (mg L^{-1})	0.0001 ±0.0022	0.013 ±0.08	0.081 ±0.58*
Chrome (mg L^{-1})	0.003 ±0.009	0.01 ±0.07	0.08 ±0.07*
Manganese (mg L^{-1})	0.012 ±0.021	0.27 ±0.005	0.36 ±0.014*
Cadmium (mg L^{-1})	0.0001 ±0.0003	0.0001 ±0.0008	0.0002 ±0.0007
Water Quality Index (WQI)	**91.34**	**68.51**	**37.45**
Classification	**Excellent**	**Regular**	**Very poor**

*Values above the allowed level as determined by CONAMA Resolution no. 357 (2005).

Table 2: Lead (Pb), Chrome (Cr) and Manganese (Mn) concentration means (µg/g) in samples of *Rhamdia quelen* and *Geophagus brasiliensis* at sites S1, S2 and S3.

Sites (Species sampled)	Pb		Cr		Mn	
	Liver	Gills	Liver	Gills	Liver	Gills
Site 1 (*Rhamdia quelen*)	<DL	<DL	<DL	<DL	<DL	<DL
Site 1 (*Geophagus brasiliensis*)	<DL	<DL	<DL	<DL	<DL	<DL
Site 2 (*Rhamdia quelen*)	0.12± 0.22	0.52± 0.36	0.13± 0.08	0.17± 0.07	<DL	<DL
Site 2 (*Geophagus brasiliensis*)	0.03± 0.06	0.44± 0.27	0.08± 0.01	0.09± 0.03	<DL	<DL
Site 3 (*Rhamdia quelen*)	2.26± 0.65*	5.32± 0.74*	1.17± 0.78*	1.25± 0.44*	0.11± 0.07	0.25± 0.18
Site 3 (*Geophagus brasiliensis*)	1.87± 0.98*	2.36± 0.66*	0.68± 0.55*	0.77± 0.19*	0.07± 0.03	0.15± 0.09

*****Levels above FAO (1983) and FEPA (2003) recommended limits.

Table 3: Micronucleus test (MN) frequency, micronucleated cells (MNC) and total erythrocytic nuclear abnormalities (ENA) in erythrocytes from *Rhamdia quelen* and *Geophagus brasiliensis* collected in the Mumbuca stream, Monte Carmelo, MG, Brazil, from February to September 2013.

Collection Sites	Biological Material	Nº of Individuals	Cell Totals	X(‰) ± SD		
				MN	CMN	TOTAL ENA
Reference Site (S1)	*Rhamdia quelen*	15	60000	0.078±0.014	0.078±0.014	0.097±0.043
Reference Site (S1)	*Geophagus brasiliensis*	15	60000	0.067±0.018	0.067±0.018	0.088±0.057
Positive Control (CP)	*Rhamdia quelen*	15	60000	0.745±0.116[a]	0.713±0.104[a]	5.367±3.314[a]
Positive Control (CP)	*Geophagus brasiliensis*	15	60000	0.685±0.096[a]	0.661±0.086[a]	4.362±1.104[a]
Site 2	*Rhamdia quelen*	15	60000	0.245±0.067[a,b]	0.225±0.056[a,b]	2.122±0.056[a,b]
Site 2	*Geophagus brasiliensis*	15	60000	0.284±0.089[a,b]	0.264±0.077[a,b]	1.767±0.056[a,b]
Site 3	*Rhamdia quelen*	15	60000	0.667±0.223[a]	0.633±0.204[a]	5.158±4.433[a]
Site 3	*Geophagus brasiliensis*	15	60000	0.597±0.125[a]	0.589±0.108[a]	3.965±2.895[a]

[a] Significant difference when compared to the reference site (S1) according to the Tukey test with a $p<0.05$ level of significance.
[b] Significant difference compared to the positive control ($p<0.05$).

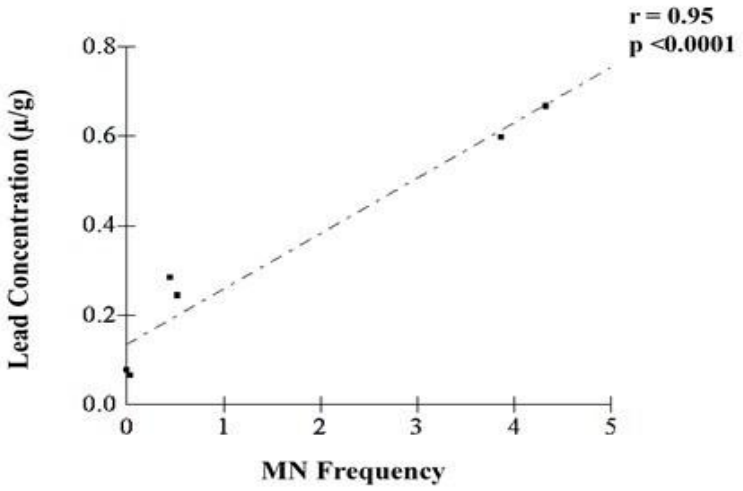

Fig. 2: Correlation between MN frequency and mean lead concentrations in *Rhamdia quelen* and *Geophagus brasiliensis* exposed in the locations tested according to Pearson correlation (α = 0.05).

CONCLUSÃO GERAL

A avaliação dos sítios de coleta (Fig.1), indicam que a bacia do Rio Paranaíba, na região do Triangulo Mineiro- MG, apresenta diversos que são alvo de impacto ambiental (S1- MU e S2-MU do córrego Mumbuca; S2- Córrego do Óleo e S3 – Córrego Liso). Os sítios foram descritos com potencial genotóxico, devido a presença de metais pesados (Cr, Cd e Pb), oriundos de efluentes industriais. Além disso, foi avaliado nesses locais a presença de efluentes domésticos, que interferem negativamente na determinação do índice de qualidade de água. O Teste de Micronúcleo foi utilizado como avaliação principal, e para sua realização, os indicadores, *Rhamdia quelen* e *Geophagus brasiliensis*, foram coletados em todos os trechos, e comparados com grupos controle. O Micronúcleo é um teste de aplicação técnica simples, e que pode ser realizado por Municípios que precisam monitorar seus reservatórios hídricos, prezando assim, pela manutenção da qualidade de água. Essa metodologia (MN), indicou que os sítios que continham alta taxa de degradação do meio em decorrência da presença de metais pesados, apresentaram maiores frequência de Micronúcleos. Demais testes como, a Espectrometria de Absorção Atômica de Chama e o Teste de Mutação e Recombinação Somática, podem ser utilizados afim de aumentar a confiança dos dados e estabelecer a classificação dos agentes genotóxicos e sua real capacidade acumulativa nos indivíduos testados. De maneira geral, os indicadores e testes utilizados se mostraram eficientes para a aplicação de metodologias integradas de monitoramento biológico em reservatórios hídricos.

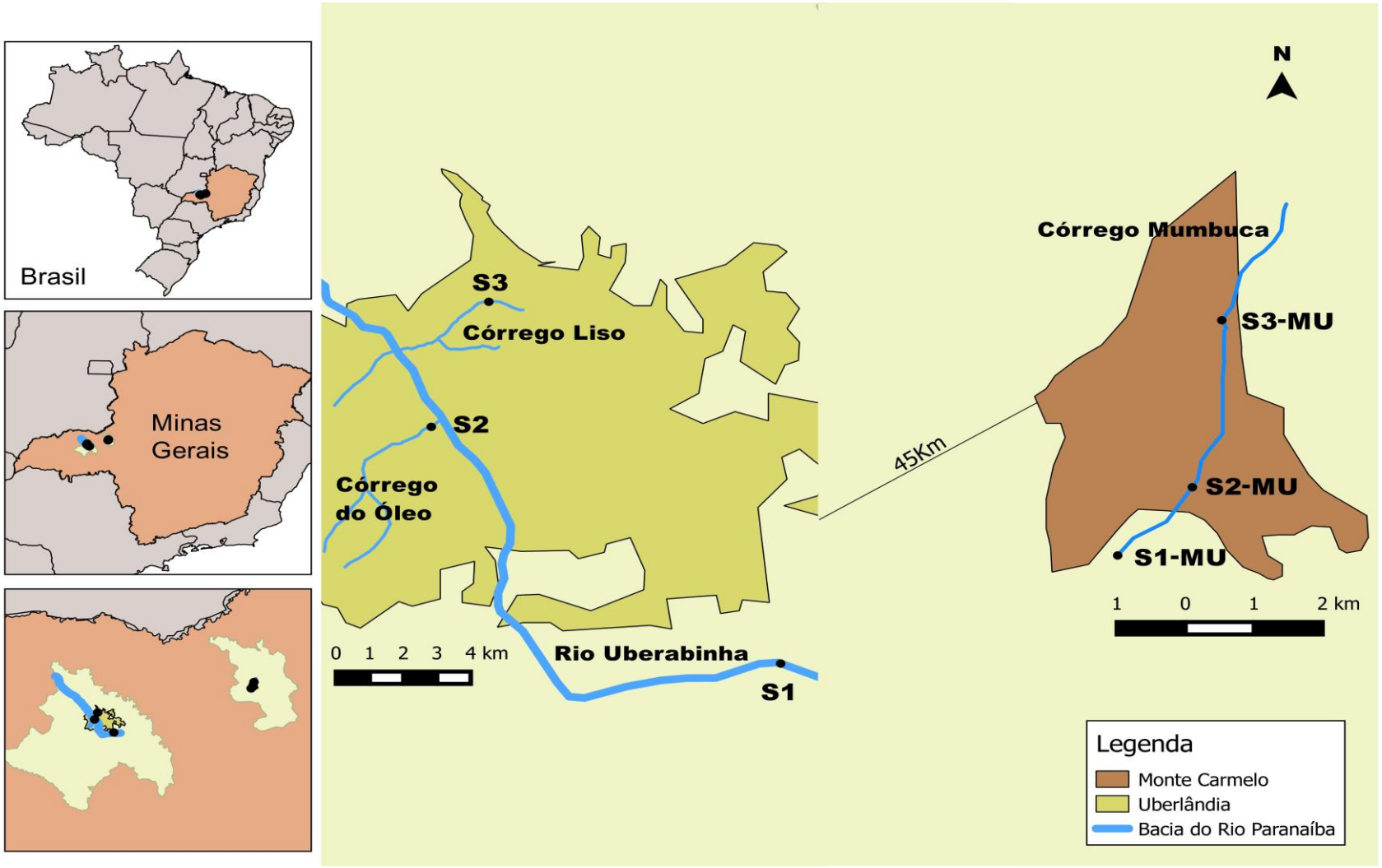

Fig. 1 Sítios de coleta na Bacia do Rio Paranaíba, região do Triângulo Mineiro, MG de Fevereiro de 2013 à Junho de 2015