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Artigo Completo

EVIDENCE OF CONTAMINATION IN THE MAIN BODY OF THE PUBLIC WATER SUPPLY OF CAMPO MOURÃO-PR: AN ANALYSIS FROM A BIOINDICATOR OF ENVIRONMENTAL QUALITY

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ABSTRACT
Nowadays, aquatic environments are affected by organic and synthetic pollutants that cause the destabilization of ecosystems, communities, and population. Besides their mutagenic and carcinogenic effects, pollutants can bioaccumulate and show a potential risk for all lives, including human. The detection and minimization of impacts and/or raising information about this issue can be of great relevance to society and to the regulation of laws that aim to control aquatic pollution. This study aimed to evaluate the environmental contamination of Rio do Campo, Brazil, with the fish lambari (Astyanax altiparanae) as a bioindicator of environmental quality. When a fish micronucleus test was done with the specimens collected at Campo River, higher frequency of nuclear abnormalities in their erythrocytes was observed when compared to a laboratory control. This observation indicates that those fish were under enough clastogenic agents to generate irreversible genetic damage in their blood cells. The results should warn the society and authorities about the danger of anthropogenic activities to ecosystems and public health, which will lead to actions of recuperation in the balance and sustainability of the local ecosystem.
Keywords: *environmental pollution; aquatic ecosystems; Astyanax altiparanae; piscine micronucleus test.*

INDÍCIOS DE CONTAMINAÇÃO NO PRINCIPAL CORPO HÍDRICO DE ABASTECIMENTO PÚBLICO DO MUNICÍPIO DE CAMPO MOURÃO – PR: UMA ANÁLISE A PARTIR DE UM BIOINDICADOR DE QUALIDADE AMBIENTAL

Resumo
Atualmente, milhares de poluentes de origem sintética e orgânica têm afetado o ambiente aquático, promovendo a desestabilização dos ecossistemas, das comunidades e das populações. Muitas destas substâncias, além de apresentarem efeitos mutagênicos e carcinogênicos a diferentes organismos, podem se bioacumular, representando um risco potencial, inclusive à saúde humana. Desta forma, detectar, minimizar e/ou subsidiar informações acerca deste problema, são ações de grande relevância para a sociedade e para regulamentação adequada de leis que promovam o controle da poluição aquática. Sabendo disso, o presente trabalho teve por objetivo avaliar a contaminação ambiental do rio do Campo, Campo Mourão-PR, a partir da utilização do lambari, Astyanax altiparanae, como bioindicador da qualidade ambiental. Através do teste do micronúcleo písceo (análise quali e quantitativa) foi possível verificar que os exemplares coletados no rio do Campo apresentaram taxa de danos nucleares em seus eritrócitos, significativamente maiores do que a situação controle realizada em laboratório. Esta verificação indica que estes indivíduos estiveram sujeitos a níveis de agentes clastogênicos suficientes para gerar danos genéticos irreversíveis em suas células sanguíneas. Os resultados obtidos servem para alertar a sociedade e as autoridades sobre os perigos que as atividades antrópicas podem trazer para os ecossistemas e para a saúde pública, viabilizando ações de recuperação do equilíbrio e de sustentabilidade do ecossistema local.
Palavras-chave: *poluição ambiental; ecossistemas aquáticos; Astyanax altiparanae; teste do micronúcleo písceo.*

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INTRODUCTION

Aquatic biodiversity and human health can be negatively affected by the inappropriate disposal of industrial, domestic or agricultural pollutants in water bodies (1). In Campo Mourão, the disposal of a variety of pesticides and industrial effluents in water bodies has been detected (2).

Campo Mourão has one of the most expressive agricultural production areas in Brazil, especially soy and corn due to its plain topography and fertile soil. Since 1970, monoculture areas have been expanding in the region, in detriment of small properties and diversity of agricultural production. Today, agriculture occupies up to 83.58% of the area, which not only made the local economy stronger, but has also increased population mass migrations from countryside to urban areas which results in 94.26% of the population living urban areas(3). In urban areas drenched by the water course, domestic waste disposals and sewage and industrial waste discharges worsen the degradation of aquatic environment, as occurs in Campo River (2).

Two important consequences to aquatic environments are: the large scale monocultures increases the use of agrochemicals in soil and superficial waters, and the concentration of population in urban areas increases the demand for sanitation, what intensify and concentrate the discharge of organic matter, heavy metals and pesticides in water courses.

Campo River is used for water abstraction, accounting for 80% of the drinking water supply of the city, and for discharges of effluents from the city's Water Treatment Station as well as from Sewage Treatment Station (4).

Although the river is used for the conservation of local wild fauna and human population nearby, the implementation of environmental monitoring programs becomes essential for the maintenance of environment quality and for the reduction of anthropic impacts. Biomonitoring programs in South and Southeast Brazil have been increasing in the last decade. These programs use fish and other aquatic organisms to evaluate the water quality, reflecting an attempt to regulate or establish rules regarding the safety of water supply in abstraction impoundments and rivers, and the possible risks to human exposure. These studies have enhanced with the increase of urbanization, industrialization, and agricultural activities around water bodies, what is usually associated with inadequate or insufficient water treatment (5).

In general, fish are selected as sentinels of water quality, because they live submerged in it, and occupy different trophic levels and are important means of contamination to human population (5).

When fish are exposed to degraded environments, they either die instantly or undergo permanently genetic alterations, depending on the quantity or structure of the genetic material of the affected cells (6). When the division of the genetic material happens, cellular anomalies such as micronucleus or nucleus with chromatin's projection (7) or with an invagination that alters its shape, results from errors in cellular division, specifically in anaphase.

The micronucleus test, technique that evaluate genetic damage rate applied in the present study, has been widely used as an environmental indicator, analyzing mutagenicity and estimating the damage level due to exposure to contaminants (8). This test has been applied successfully due to its simplicity, safety and sensibility, and its non-dependency on the study of animal's chromosomal characteristics (9). Besides, when fish erythrocytes are used, there is no excessive consumption of time or suffering for the animals.

In the same manner as micronucleus, nuclear morphological alterations may also happen, i.e., the nucleus does not show a regular oval shape, but has a projection or an invagination of chromatin. Ayllon and Garcia-Vazquez (10) showed that these alterations are induced by well known genotoxic compounds, even when the micronucleus has not been formed. It is believed that these nuclear anomalies are due to the problems with the nuclear lamina, that confers the regular oval shape and stability of the nucleus (11).

The bioindicator specie used, Astyanax altiparanae (Figure 1), is popularly known as tambiú or yellow tail lambari and is characterized by its silver body with a whitish ventral region and a grayish dorsal region (12). A. altiparanae was chosen due to its presence in large quantities in local rivers (13), its bioindicator potential and the possibility of being submitted to bioassays in laboratory.

Figure 1. *Astyanax altiparanae.* Fonte: Galvan (2011)(14).

The aim of this work was to evaluate the environmental quality of Campo River, in Campo Mourão/PR, through the rate of nuclear damage in erythrocytes of t Astyanax altiparanae fish (12).

MATERIALS AND METHODS

Study Area

Campo River (Fig. 2) belongs to Ivaí River watershed and it moves from the South to the North of Campo Mourão, Paraná. The stretch of Campo River where fish were sampled for this study is set near the Water Treatment Station of Campo Mourão (24°03'09"S and 52°23'33"W). This station still does not have an efficient method to dispose its final effluent that is rich in aluminum polychloride (heavy metal salt used in the water treatment process), which is discharged in Campo River (15). Biological material sampling and control group.

Figure 2. Localization of study area. Note= * Collection point in Campo River. Font: modified of Guerreiro and Parolin (16).

In the field, two samplings of biological material were made, with gill nets (5m X 2m, 1.5 cm between opposed knots) for 24 hours, from November to December 2010. The control group of A. altiparanae was acquired from a fish farming, supplied with water from a non-polluted source and were acclimatized in laboratory, for 30 days in a constantly aired 12L aquarium under regular temperature (22ºC) and photoperiod (12-h light/dark). The animals were fed 2-fold daily with commercial food standardized to 3% of their biomass (Alcon®). The water was from an urban supply, but it was ultra-filtrated before coming into contact with the animals and a semi-static control was performed (with removal of 1/3 water).

A. altiparanae sampled, as well as those acclimatized in the laboratory had their blood sampled for the piscine micronucleus test (PMT).

Piscine micronucleus test (PMT)

The technique to evaluate the erythrocytes was described by Heddle (17) and Schmid (18), with some modifications. The experiment was conducted in accordance with the national and institutional guide for the protection of animal well-being of the Brazilian College of Animal Experimentation (Colégio Brasileiro de Experimentação Animal – COBEA) (19). The individuals were anesthetized with alcoholic solution of benzocaine 5%. The blood of each individual was collected by cardiac puncture with a new and heparinized insulin syringe, and one drop was poured over the surface of a clean and identified slide.

The blood was spread over the slide surface using a cover slip. After air-drying, the slides were fixed in ethanol 96% for 30 minutes followed by staining with Giemsa 10% (diluted in phosphate buffer - pH 8.6) for 10 minutes, and then the slides were analyzed by random scanning until 1000 cells per individual were counted.

When counting, only nucleated erythrocytes with intact nuclear and cytoplasmic membranes were considered. The criteria for a particle to be considered a micronucleus are: it did not exceed 1/3 of the size of the main nucleus, be clearly separated from the main nucleus and had distinguishable boundaries, and has the same color and refringence as the nucleus. Alterations in the regular elliptic shape of erythrocyte's nuclei that did not fit in to the concept of micronuclei but could be described as nuclear morphological alterations according to Carrasco, Tilbury and Myers (20) were also analyzed.

Data analysis

The data of the fishes both from the control and the Campo River were put in a spreadsheet for statistical analysis. Before analysis, data were tested for: a) normality, through a Shapiro-Wilk test; b) variance homogeneity, through a Levene test followed by a t-test for groups by applying heterogeneous variance (21), with the aim of comparing the rate of nuclear damage found in fish from Campo River with the rate of nuclear damage found in the control group.

RESULTS AND DISCUSSION

In this study, 13 individuals of A. altiparanae were collected in Campo River and 14 were used as the control group. Consistent variations from the normally smooth, elliptical shape of the erythrocyte nucleus were apparent in the fish's blood analyzed during this research. Some of these nuclear morphological alterations are shown in Figure 3, including one micronucleus. Among current cytogenetic techniques, nuclear abnormalities and micronuclei are considered as well established indicators of cytotoxicity, and genetic toxicology, respectively (22).

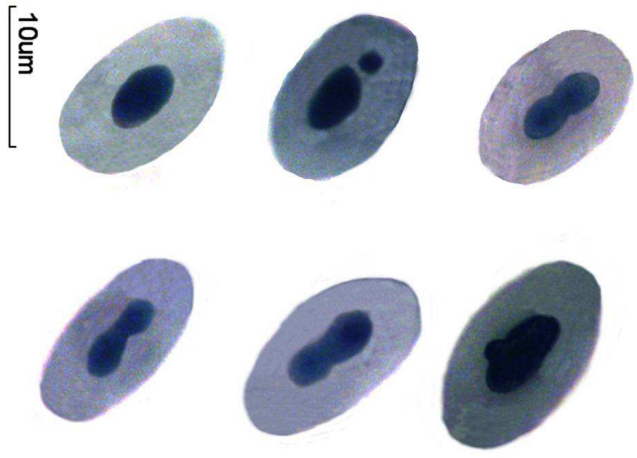

Figure 3. Photos of a micronucleus and nuclear morphological alterations found in erythrocytes of *Astyanax altiparanae*. The first cell represents a normal nucleus, the second cell has a micronucleus and all the other cells show nuclear morphological alterations.

Table 1 shows the results of the counts of erythrocytes with nuclear alteration. The control group had a nuclear damage mean rate of 3.57 with a standard deviation of 1.65, while Campo River individuals had a mean rate of 29.85 with a standard deviation of 8.44.

Table 1. Nuclear damage rate in erythrocytes of *A. altiparanae* in control and Campo River groups.

specimens	Control Group	Campo River
1	5	28
2	2	28
3	3	30
4	4	38
5	6	36
6	1	26
7	4	41
8	5	46
9	2	19
10	3	31
11	4	25
12	6	22
13	1	18
14	4	-

The nuclear damage rate found in erythrocytes of A. altiparanae from Campo River was significantly higher than the one found in the control group (t=-11.0248; df=12.852 and p=6.413 x 10-8, i. e., p<0.05) (Fig. 4). The nuclear morphology results agree with many others studies which have found elevated erythrocyte MNP frequencies in fish inhabiting contaminated site (7,23–26). Fish subjected to genotoxic exposure can suffer a variety on non-specific effects, such as enzyme impairment, changes in metabolism, degenerative organ changes and reduced growth, as well as reduced reproductive output (27). In extreme cases, there may be an increased mortality of the most sensitive individuals (7).

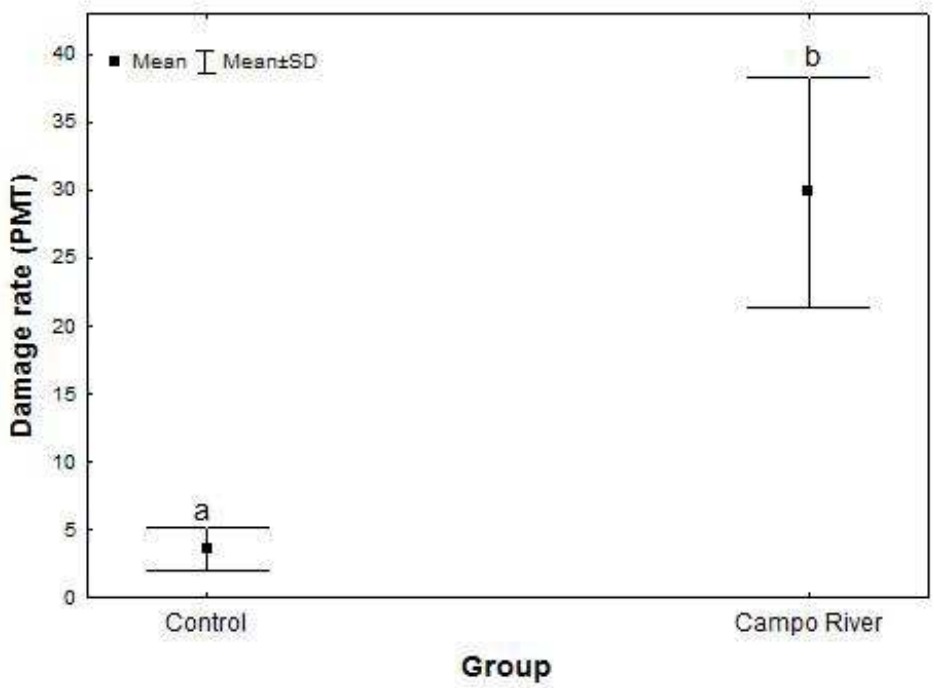

Figure 4. Mean and standard deviation of nuclear damage found in erythrocytes of *A. altiparanae* in control and Campo River. The letters 'a' and 'b' indicate statistical difference. SD= standard deviation; PMT=Piscine micronucleus Test.

Results indicate that some nuclear damage may occur spontaneously. Since they characterize mutation, individuals habiting Campo River are subjected to enough levels of clastogenic agents that cause irreversible genetic damage. Still, it is possible that these damages were sub-estimated, because species of genus Astyanax generally were not found as relatively answerable to pollution in studies (25,26). Grisolia et al. (26) verified that among six species living into a polluted river, Astyanax bimaculatus lacustres presented smaller nuclear damage rate. Therefore, it is fundamental that in future studies, species of other trophic guilds also be researched in regard to DNA alterations caused by pollution.

In environmental studies performed using native populations exposed in loco to the influence of polluting activities, it is important to establish baseline values for the biomarker in the sentinel organism (25). Thus, in our study, it was possible to verify that the specie A. altiparanae was a good bioindicator to evaluate the water quality in the Campo River.

It is worth emphasizing that the usage of bioindicators as sentinel of water quality has the advantage of providing information about long run environmental effects, which means that they are capable of reflecting states that no longer exist by the time of the investigation, but were led by processes that occurred previously in the community (28).

In the same manner, the capacity of many pollutants and their derivatives to modify their toxicity when in synergism makes the evaluation of environmental risk based only in abiotic parameter little trustworthy. Such panorama has been leading to the establishment of precocious warning signals, or biomarkers, which reflect alterations in the regular patterns expected in healthy individuals. Biomarkers can be measured in body fluids, cells, tissues, etc. (29).The results of this study indicate that the measurement of

biomarker PMT in A. altiparanae may be an appropriate means of detecting exposure to environmental genotoxins in Campo River. The Campo River, as a receptor of all kinds of waste in its course (mainly agrochemicals from agricultural activities and aluminum polychloride from the effluent of the Water Treatment Station), has increased its genotoxicity. The piscine micronucleus test reveals the alterations caused by substances that are not only genotoxic but also show mutagenic effects, which means that the alterations affect DNA in a permanent, irreversible manner and also may ultimately cause cancer (9).

As shown in the Lago Azul State Park Management Plan (Plano de Manejo do Parque Estadual Lago Azul) (30), it is important to emphasize that the invasion of the permanent preservation area of the riparian vegetation also contributes to the contamination of the river, especially in rainy periods, the occupation facilitates the entry of soil and organic matter particles in the river, which carry toxic substances from agricultural areas (pesticides).

All these actions have been transforming Campo River, a former nturally healthy habitat, into a degraded environment, which exposes the individuals, living and/or using its waters to potentially toxic compounds. Acknowledging the great use of the river for the population of Campo Mourão, the possible consequences of its contamination to the public health of the city has become really preoccupying.

CONSIDERAÇÕES FINAIS

The samples of A. altiparanae collected in Campo River are suffering genotoxic/mutagenic alterations in their blood cells. These alterations are probably related to the use of pesticides in the agricultural area of the region, and to the effluents discharged from the water treatment station (high concentration of heavy metals).---The results showed in this study, besides new data about the environmental quality of Campo River, also serves as a warning to the society and authorities about the danger of anthropic activities to ecosystems and public health.

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