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Environmental Degradation at a Public Park in Southern Brazil as Revealed Through a Genotoxicity Test (MN) on Peripheral Blood Cells from *Poecilia vivipara* (Teleostei)

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Abstract The effects of anthropogenic activities on water, environment, and consequently quality of life can be evaluated using genetic, biochemical, and microbiological parameters. Regarding genetic parameters, the micronucleus test is a fast, efficient, inexpensive method for detecting alterations in genetic material induced by a variety of genotoxic agents. In the present study, blood cells from *Poecilia vivipara* from the Belém River in the city of Curitiba, Paraná, Brazil were evaluated for genotoxic effects stemming from human-produced pollution, as expressed by the micronucleus. The water in the river was evaluated with regard to physiochemical and microbiological parameters as well as for heavy metals. The analysis revealed the presence of copper, zinc, and nickel, with high concentrations of copper. The micronucleus analysis revealed significant differences in relation to the groups (study and control), suggesting a positive relation between the water quality of the Belém River and micronucleus expression as a result of the pollution to which this river is subjected.

Keywords Micronuclei · Genotoxicity · Public parks · Environmental degradation · *Poecilia vivipara*

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1 Introduction

Human activities are among the most important causes of the release of toxic elements into water, sediment, and the biota. Such elements can have harmful effects on organisms, as they are biomagnified in the food chain (Curtius et al. 2003). Moreover,

toxic elements can persist and become biotransformed in the environment (Papagiannis et al. 2004).

The biomonitoring of aquatic environments is of extreme importance. These environments are the final destination for nearly all urban, industrial, and agricultural waste (Ferraro et al. 2004). Traditionally, methods based on the control of waste water flow and other pollution sources have been used in the monitoring of the effects of anthropogenic activities on aquatic systems (Blum and Speece 1990). However, due to the harm caused by their toxic effects, a number of pollutants require greater effectiveness with regard to their control and monitoring (Blum and Speece 1990). In terms of methodological evolution, approaches that measure the biological effects of pollutants have begun to be used (Abessa et al. 2008). The effects of such pollutants on water, the environment, and quality of life can currently be evaluated using genetic, biochemical, and microbiological parameters (Vargas et al. 1993, 2001; Kaiser 1998; Begum et al. 2005; Prá et al. 2005; Principi et al. 2006; Manier et al. 2009). The cytogenetic monitoring of environmental pollution is an important component of an integrated system of ecological monitoring. Such methods are based on the analyses of cells and chromosomes, as these components are the most vulnerable to pollutants (Butorina et al. 2002).

A number of chemical substances released into the environment are made up of agents that cause genetic mutations and chromosome alterations (Ferraro et al. 2004). Such substances are called genotoxic components and are considered aneugenic when inducing alterations in chromosome distribution during the cell division process, thereby giving rise to aneuploidies. Other genotoxic substances are called clastogenic, which induce breaks and produce alterations in the chromosome structure. These breaks and alterations can be detected by cytogenetics, such as chromosome analysis, the analysis of micronucleus (MN) frequency, and comet assays. Therefore, by analyzing the clastogenic and/or aneugenic effects, it is possible to evaluate the genotoxic effects of a given agent on any organism through an analysis of its cells (Rabello-Gay et al. 1991; Padrangi et al. 1995; Alberts et al. 1997; Norppa and Falck 2003; Ferraro et al. 2004; Manier et al. 2009).

A number of studies have used the analysis of micronucleus frequency in the detection of the genotoxicity of many substances in the aquatic environment (Grisolia and Starling 2001; Llorente et al. 2002; Barsiené et al. 2002; Viganó et al. 2002; Swartz et al. 2003; Lemos et al. 2007; Galindo and Moreira 2009). This method has proven to be a very effective, simple, inexpensive procedure with considerable sensitivity (Norppa and Falck 2003). It is based on the observation of micronuclei as a genotoxic effect caused by chromosome breaks, acentric and/or dicentric chromosomes, chromosome rearrangements, and entire chromosomes that were not incorporated into the nucleus of dividing cells (Lindholm et al. 1991; Ford and Corell 1992; Catalán et al. 1995; Saunders et al. 2000; Falck et al. 2002; Norppa and Falck 2003). Studies on the genotoxicity of potentially toxic substances allow determining the responses of a certain organism to a given contaminant. Moreover, such an approach can focus on evaluating possible population disturbances that could compromise the genetic variability and conservation of species (Padrangi et al. 1995; Galindo and Moreira 2009).

The aim of the present study was to analyze genotoxicity in blood cells from *Poecilia vivipara* resulting from an increasing degree of urban development around the Belém River, taking samples from a public park in the city of Curitiba (Southern Brazil).

2 Materials and Methods

2.1 Sampling and Study Area

Two sampling groups of *P. vivipara* were determined in the present study. In November 2005, seven specimens were collected from the Belém River (Curitiba, Southern Brazil) to make up the study group. Another seven specimens of *P. vivipara* were sampled from the Piraquara River (Piraquara, Southern Brazil) to serve as the control group.

The Belém River runs through industrial and limestone mining areas, located in metropolitan Curitiba. The river also runs through a large portion of urban areas, including public parks, such as São Lourenço Park (the study area). The Belém River also receives considerable load from domestic sewers along its urban course, which contributes toward its apparent state of degradation. São Lourenço Park is an area of 203.918 m^2, with an artificial lake

throughout its length, which is supplied by water from the Belém River (Fig. 1).

The Piraquara River (control group) has no similar conditions of human impact and free of chemical and/or biological pollution (IAP 2005). The river is located in a conservation region that contains springs used for the human water supply.

2.2 Genotoxicity Analyses

Blood cells were obtained from a peripheral blood smear on a smooth slide after sacrificing the fish, fixed in absolute methanol and stained with Giemsa solution (7.5%). About 3,000 cells per specimen were analyzed under an optical microscope for the counts of normal and micronucleated cells. The statistical evaluation of the number of micronuclei found in both samples was performed using one-way analysis of variance (ANOVA) with a 95% confidence interval.

2.3 Physiochemical and Microbiological Analysis of the Water

The physiochemical (determination of total hardness, alkalinity, nitrite concentration, nitrate concentration, pH, and temperature) and qualitative microbiological (test for total coliform bacteria) tests were performed on two samples of 1,000 mL of water. Samples were collected from three points in the study location, with specific analyses performed for each sample. These analyses were performed on the samples from November 2005. The water analysis methods followed the determinations established by Resolution 357/3 of CONAMA (2005) [Brazilian National Environmental Council].

2.4 Analysis of Heavy Metals

The samplings of the heavy metals copper, nickel, and zinc were performed using decontaminated glass bottles at the same sampling sites as the physiochemical and microbiological analyses. Samplings were performed in two different seasons (November 2005 and July 2006). The heavy metal analyses were performed on 100 mL of each sample by adding 20 mL of nitric acid p.a. This solution was heated on a hot plate until 60 mL of the original solution had evaporated. After reaching room temperature, 40 mL of ultra-pure water were added to obtain a final solution of 100 mL for a proper analysis. The solution remained stored in a volumetric balloon. The readings

Fig. 1 Satellite image of São Lourenço Park (Curitba, Southern Brazil). The sampling site is in the center of the image (lake from the damming of the Belém River in the region). Note the remarkable degree of urban occupation around the lake. Source: Google Earth, 2006

Table 1 Summary of the results observed in *Poecilia vivipara* cells from the Piraquara and Belém rivers regarding the presence of micronuclei (MN)

Specimen	Piraquara River group				Belém River group			
	MN cells		Normal cells	Frequency	MN cells		Normal cells	Frequency
	1 MN	2MN			1MN	2MN		
01	02		2,998	0.00066				
02	02		2,998	0.00066				
03	01		2,999	0.00033				
04	06		3,029	0.00197				
05	07		3,023	0.00231				
06	05		3,097	0.00161				
07	09		3,131	0.00286				
08					46	02	2,952	0.01598
09					41	04	2,955	0.015
10					34	03	2,960	0.01333
11					60	06	2,959	0.02181
12					38	01	2,963	0.01299
13					50		2,958	0.01662
14					48		2,969	0.01590
total	32		21,275	0.0015	317	16	20,716	0.0158
Mean±standard deviation	4.57±2.99				47.42±9.51			

of the heavy metals were performed in an atomic absorption spectrophotometer (AA-6800 Shimadzu), using acetylene and compressed air in a flame analysis with hollow cathode lamps, as recommended by the manufacturer.

3 Results

The cell analysis revealed a micronucleus frequency (total number of micronuclei/total number of cells analyzed) of 0.01394 in the sample from the Belém River and 0.00172 in the sample from the Piraquara River (Table 1). The mean number of micronuclei observed for each group was 4.57±2.99 and 47.42± 9.51 in the fish from the Piraquara and Belém rivers, respectively, with a significant difference in the number of micronuclei between groups (ANOVA, $p < 0.05$; Fig. 2). The physiochemical and microbiological analyses revealed no altered results based on the Brazilian National Environmental Council (CONAMA) classification of class III freshwater environments. However, the microbiological analysis revealed the presence of coliforms (Table 2). The results of the heavy metal analyses for the first sampling campaign revealed no differences in nickel and zinc concentrations in comparison to reference values. However, there was a quite high concentration of copper in comparison to its reference value

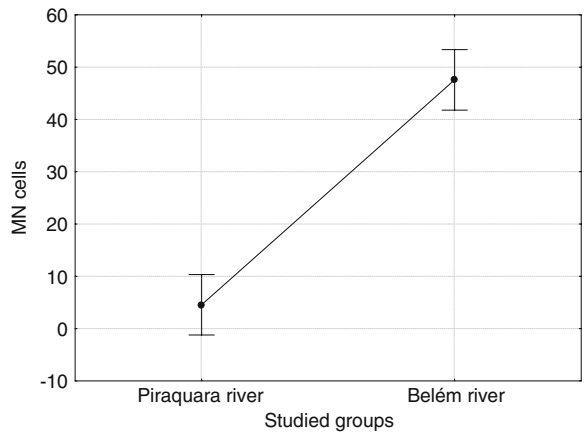

Fig. 2 Result of one-way ANOVA regarding the number of micronuclei found in the groups studied. *MN cells* micronucleated cells

Table 2 Physiochemical and microbiological analyses of the water from the Belém River (Curitiba, Southern Brazil) performed in November 2005

Sample	Hardness (mg of $CaCO_3$/L)	Alkalinity	pH	Temperature (°C)	Nitrite (mg/L)	Nitrate (mg/L)	Total coliforms
1	79.5	132.48	7.0	18.9	0.064	0.18	Positive
2	80	120.06	7.1	19.1	0.046	0.16	Positive
3	81	103.50	7.2	19.9	0.089	0.33	Positive

Reference values: pH=6–9, nitrite=1 mg/l, nitrate=10 mg/l. The obtained results classify the samples as Class III Water based on the classification of the Brazilian National Environmental Council (CONAMA) for freshwater environments. Resolution No. 357, March 17, 2005

(Table 3). In the second sampling campaign, no altered values were found for nickel, zinc, or copper (Table 4).

4 Discussion

The evaluation of genotoxicity of polluted waters has frequently addressed the phenomena of contamination from industrial and urban waste, as genome damage may lead to reductions in aquatic fauna (Lemos and Erdtmann 2000; Vargas et al. 2001; Viganó et al. 2002; Tagliari et al. 2004; Horn et al. 2004; Chen and White 2004; Ohe et al. 2004). Environmental degradation was evidenced in the present study by the MN frequency in the fish inhabiting the Belém River in the vicinity of São Lourenço Park (Curitiba, PR).

Although the evaluation of water quality parameters (Table 2) revealed no discrepancies in relation to the limits established by Resolution CONAMA-357 (class III freshwater environments), aspects such as odor, presence of oils, solid waste, microorganisms, and heavy metals suggest an effect from the urban pollution on the genetic material of *P. vivipara.* This hypothesis is be supported by the high frequency of micronuclei in the blood cells of specimens from the Belém River (Table 1).

While microbiological analyses suggest possible risks (presence of coliforms) to human and fauna health (Table 2), the toxicity tests underscore such risks. Sewage and chemical toxicity are two distinct variants aging in water. The occurrence of sewage can suggest toxicity, but certain toxic agents can cause a reduction in the amount of coliforms, acting as a bactericide (Zagatto and Goldstein 1991). However, the presence of coliforms and heavy metals in the Belém River suggests a possible association of these factors as causes of the remarkable genotoxicity observed. The heavy metal concentrations found in the sampling region in both periods require prompt, conclusive efforts at recuperating the ecosystem from the threats imposed by these chemicals. The presence of heavy metals is a significant ecological and public health problem due to their toxicity and ability to accumulate in living beings (Alloway and Ayres 1993; Langston 1998).

The features of the Belém River in the vicinity of São Lourenço Park, especially its lake-like physiog-

Table 3 Results of the first sampling campaign for heavy metals in the Belem River performed in November 2005

Sample	Cu		Ni		Zn	
	Conc	Abs	Conc	Abs	Conc	Abs
1	0.0471	0.0061	0.0208	0.0033	0.0540	0.0413
2	0.0398	0.0055	0.0291	0.0038	0.4999	0.1734

Reference values (CONAMA 357/3): Cu: 0.013 mg/l, Ni: 0.02 mg/l, Zn: 5 mg/l

Cu copper, *Ni* nickel, *Zn* zinc, *Conc* concentration (mg/l), *Abs* absorbancy

Table 4 Results of the second sampling campaign for heavy metals in the Belem River performed in July 2006

Sample	Cu		Ni		Zn	
	Conc	Abs	Conc	Abs	Conc	Abs
1	−0.0559	0.0006	0.0068	0.0044	0.0124	0.0127
2	−0.0547	0.0007	0.0187	0.0051	0.0860	0.0340
3	−0.0559	0.0006	0.0203	0.0052	0.0228	0.0157

Reference values (CONAMA 357/3): Cu: 0.013 mg/l, Ni: 0.02 mg/l, Zn: 5 mg/l

Cu Copper, *Ni* nickel, *Zn* zinc, *Conc* concentration (mg/l), *Abs* absorbancy

raphy, favor the scenario of degradation (Papagiannis et al. 2004; Begum et al. 2005). According to the authors cited, lakes are more vulnerable to heavy metal pollution than lotic ecosystems. Yet it should be considered that the concentrations of copper, nickel, and zinc found are an underestimate of the real in loco concentrations, as the quantity of metal in dissolved ionic form is often much lower than the total content (Curtius et al. 2003). Moreover, suspended sediment is the main vehicle for the transportation of metals in water. Metals can be also deposited on the bottom, where they build up, further contributing toward the contamination of the water (Amado Filho et al. 1999; Rodrigues and Formoso 2006).

Among the heavy metals evaluated, different concentrations of copper were found in the two sampling periods, whereas nickel and zinc concentrations remained practically constant and close to their reference values (0.02 mg/l–CONAMA). The findings on nickel contamination should be considered with caution, as its concentration may have been underestimated. This notion is supported by high concentrations of nickel in waste waters from chemical, metal, and mining industries (Fresenius et al. 1988), as the upper course of the Belém River (near the sampling region) receives waste water from all these industries. Moreover, the recognized genotoxicity of nickel further stresses the need for caution in interpreting the results of the present study (Ohe et al. 2004).

Zinc concentrations in the water samples are quite below the reference value (5 ml/l–CONAMA), which suggests no relationship with the results on MN frequency. Contrarily, copper concentrations (Tables 2, 3) were higher than the reference values. The impact of copper on the environment, even at low concentrations, is recognized (Jannaschk et al. 1999; Chassagnole et al. 2003; Carattino et al. 2004; Strydom et al. 2006). Copper ions are also known to exhibit high affinity with the catalysis of free radicals, with severe consequences in both DNA repair and duplication, thereby inducing genome damage (Hartwig 1995). Thus, the presence of copper at the concentrations observed has a direct association with the high frequency of micronuclei found in fish from the Belém River.

A number of studies have demonstrated copper genotoxicity in aquatic organisms (Guecheva and Henriques 2001; Arkhipchuk and Garanko 2005), but the mechanisms of this genotoxicity are poorly discussed (Bagdonas and Vosyliené 2006). One possible mechanism of copper genotoxicity is the induction of oxidative stress and the production of reactive oxygen species (ROS), which damage the DNA (Gabbianelli et al. 2003). (Guecheva and Henriques 2001) suggest that copper genotoxicity occurs via the action of ROS, while the inhibition of DNA repair enzymes could be due to the non-specific binding Cu^{2+} to essential sites in the enzyme molecule. Some heavy metals, such as lead and mercury, are known to interact with the motor protein system. Tubulins (components of the mitotic spindle) are possibly responsible for genotoxic effects (MN) due to the negative interference of heavy metals on motor proteins (Their et al. 2003). Zinc, copper, and cobalt reduce the numbers of sulfhydryl groups in the tubulins, which may be responsible for their effects on tubulin polymerization (Webster and Oxford 1996). Taking into consideration all of these possibilities of genotoxic intervention, the high frequency of micronuclei demonstrated in the present study suggests that copper could be acting as an aneugenic and/or clastogenic agent.

Another relevant factor observed in the present analysis was the considerable difference in micronucleus frequency between the study and control groups. This difference (tenfold more micronuclei in the Belém River) indicates severe genotoxic effects on these fish, even during periods of milder copper concentrations. Such high genotoxicity has important implications for the ecological stability of the area, such as fish mortality or at least compromised reproduction. The high genotoxicity may also reduce genetic diversity, with population declining due to the reduction in the evolutionary potential stemming from agents of environmental stress (Swartz et al. 2003). From the results of the present study, *P. vivipara,* which is an endemic fish species in the region (Martins and Barrella 2008), is endangered.

Based on the entire set of results, we would like to highlight a possible similar impact occurring throughout both the terrestrial fauna and humans, given that São Lourenço Park is a place of considerable public visitation and is inhabited by several native plant and animal species. Thus, the results of the present study reinforce the idea that even simple methods, such as genotoxicity as measured by micronuclei, can contribute toward biomonitoring and provide precise

information on the risks to human and animal health as well as the quality of the environment.

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