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Micronucleus test for monitoring the genotoxic potential of the surface water of Luján River (Argentina) using erythrocytes of *Lithobates catesbeianus* tadpoles

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Abstract

Luján river is a peri-urban ecosystem dominated by urban, industrial and agricultural discharges, receiving contributions from about one hundred streams along its path. The toxicological quality of the surface water in the middle course of the river was monitored by means of the micronucleus (MN) test, using peripheral erythrocytes of *Lithobates catesbeianus* premetamorphic tadpoles. This cytogenetic assay was performed to evaluate the effects of a particular domestic/industrial effluent poured continuously into the river, by incubating the larvae for 6 days to water samples from two sites: upstream and downstream the point where the effluent is dumped. The positive control was a cyclophosphamide solution. Five seasonal samplings were conducted between 2007 and 2008. The physicochemical analyses and the values of three water quality indices showed a highly polluted condition of the river. The MN frequency in the erythrocytes of the larvae exposed to the river samples was significantly higher than that of negative controls in all the cases and, in most samples, without differences with respect to the positive controls or even higher. There were no significant differences in the frequency of MN between the two environmental samples. The results allowed showing the genotoxic potential of the Lujan river water.

Key words: genotoxicity; erythrocytes; micronucleus test; *Lithobates catesbeianus* tadpoles; water pollution; river monitoring; Lujan river (Argentina) .

El test de micronúcleos en eritrocitos de *Lithobates catesbeianus* para el monitoreo del potencial genotóxico del agua superficial del Río Luján (Argentina)

Resumen

El río Luján es un ecosistema periurbano afectado por descargas urbanas, industriales y agrícolas; y de vertidos de aproximadamente un centenar de arroyos. La calidad toxicológica del agua superficial en el curso medio del río fue monitoreada por medio del ensayo de micronucleos (MN), utilizando eritrocitos periféricos de larvas premetamórficas de *Lithobates catesbeianus.* El ensayo citogenético fue realizado para evaluar los efectos de un efluente doméstico/industrial, vertido en forma continua al río, incubando las larvas por 6 días en las muestras de agua provenientes de dos sitios: aguas arriba y aguas abajo del sitio donde el efluente es volcado. El control positivo fue una solución de ciclofosfamida. Entre 2007 y 2008 se realizaron cinco muestreos estacionales. Los análisis fisicoquímicos y los valores de tres índices de calidad del agua del río mostraron una condición de alta contaminación. En todas las muestras ambientales la frecuencia de MN en los eritrocitos de las larvas fue significativamente mayor que en los controles negativos; en la mayoría de los casos no se registraron diferencias con respecto a los controles positivos; en algunos casos la frecuencia fue aún mayor. No se registraron diferencias significativas entre los valores registrados de MN en los dos sitios muestreados. Los resultados mostraron el potencial genotóxico del agua del río Luján.

Palabras-claves: genotoxicidad; eritrocitos; *test* de micronúcleos; larvas de *Lithobates catesbeianus;* contaminación acuática; monitoreo del río; río Luján (Argentina).

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INTRODUCTION

Luján river is peri-urban watercourse of the Metropolitan Area of Buenos Aires, Argentina that flows into the Río de la Plata basin. It is 128 km-long and its basin covers 2690 km^2 (Busso, 2010). In the upper basin, the area surrounding the river is characterized by low population density, predominantly agricultural and ranching activities. Then, the river crosses through the city of Luján, an urban center with 107,000 inhabitants, and later continues its journey through an area of low population density and production activity. The main contributions are urban and industrial liquid discharges that begin upstream and increase downstream (Giorgi, 2001). In the lower basin, the river receives discharges of a large industrial park and of a municipal effluent treatment plant (Sánchez Caro, 2010).

Several authors have conducted studies aimed to understanding the environmental quality of the surface water of this river (Di Marzio *et al*., 2005), by focusing mainly on the physicochemical analysis of the samples. It is accepted that this information can be expressed quantitatively in the form of integrated indices of several parameters, and that it is useful to warn about environmental and health risks on the basis of maximum permissible concentrations of toxic substances according to the uses of the ecosystem (aquatic life protection, drinking water, recreation, etc.). However, such indexes provide incomplete knowledge since they do not consider the variations and interactions of the biotic and abiotic factors and influence the characteristics of each particular ecosystem and the relation with its users.

An important breakthrough in the ecotoxicological assessment of aquatic environments has been the incorporation of bioassays. They allow complementing the physicochemical information with biological information in order to know the consequences of exposing an organism to an environment under stress conditions, in a more realistic ecotoxicological approach. The bioassays that can be used to evaluate genotoxic and cytotoxic damage involve scoring chromosomal aberrations, sister chromatic exchanges, and comet and/or micronuclei in proliferating cell populations (Mudry & Carballo, 2006). The micronucleus (MN) test in bone-marrow and peripheral blood erythrocytes is one of the best established *in vivo* cytogenetic assays in the field of genetic toxicology, providing a convenient and reliable index of both chromosome breakage and chromosome loss (Fenech, 2000). During micronuclei analyses, some authors have observed the occurrence of other nuclear abnormalities, suggesting that they must be taken into consideration along conventional micronuclei analysis. Such abnormalities are related to cell division failures, cell death processes, as well as to genotoxicity and/or mutagenicity (da Silva Souza & Fontanetti, 2006).

The aim of the present study was to assess, for the first time, the genotoxic potential of surface waters from a particular area of Luján river by means of the MN test, using peripheral erythrocytes of *Lithobates catesbeianus* premetamorphic tadpoles.

MATERIALS AND METHODS

Description of the study area

Sampling sites (S1 and S2, Fig. 1) were located on the right bank of the middle course of the river, separated by approximately 60 m; the sites were located 70 km away from headwaters (S1: 34°31’15.20’’S and 50°02’15.50’’W; S2: 34°31’13.49’’S and 59°02’12.67’’W), 12 km downstream from the city of Luján, the main urban conglomerate crossed by the river. The flow direction of the river is from S1 to S2. The area of the sampling locations is characterized by a low population density. Between the two sites there is an important permanent effluent discharge point of a mixture of domestic and industrial (beer and cardboard industries) effluents. The chemical profile of the S1 site was considered as representative of the river quality before the effluent discharge. The margin of the site where the effluent is dumped is characterized by the presence of an abundant and permanent amount of white foam.

Tadpoles

Bullfrog *(Lithobates catesbeianus,* syn *Rana catesbeiana)* (Shaw, 1802) tadpoles were selected to carry out the present study. This species has an extensive distribution and it is relatively easy to handle and acclimate to laboratory conditions. It is exotic for the local herpetofauna but its distribution has expanded due to its great colonizing capacity and ability to adapt to any kind of freshwater body. It was introduced to be used in aquaculture ventures, as an ornamental species and/or for biological control (Akmentis & Cardoso, 2009).

Premetamorphic larvae (stages 25-31) (Gosner, 1960), without previous contact with contaminants, were obtained from a commercial supplier. The length (cm) of all tadpoles used in this study (N = 185) was (mean ± SEM) 6.1 ± 0.1 whereas their body weight (g) was 2.5 ± 0.1.

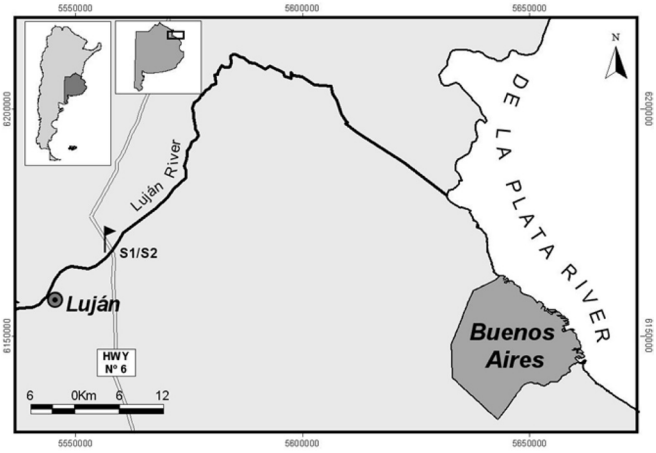


Figure 1 - Geographic location of Luján City and sampling sites. S1 upstream and S2 downstream, after an urban-industrial discharge.

Sampling of river water. Environmental conditions

Five sampling surveys of the near-surface water (15-20 cm depth) of each site of the river were performed in December 2007 (summer) and March (summer), June (autumn), August (winter) and December (spring) during 2008. The mean temperature of the sampled area ranged between 10.2 and 21.7 ºC. The annual mean rainfall rate in the watershed during the sampling period was 661.8 mm, whereas during the week before each sampling, it was 23.8, 6.0, 34, 1.5 and 31.5 mm, respectively.

Water samples for physicochemical analyses were conditioned in clean plastic bottles. Aliquots of samples for determination of heavy metals were kept acidified with HNO_3 (pH $\leq$ 2). An additional sample of 100 L for bioassays was simultaneously collected in plastic containers in each site. Samples were immediately transported to the laboratory and stored at 4-8º C until analyzed (3-5 days).

Experimental design – Test organisms - In vivo exposure experiments

The design of the bioassays is shown in Figure 2.

Stock animals were kept in glass aquaria with aerated Luján tap water (TW). Prior to the experiments, tadpoles were acclimated for 7 days in the laboratory at constant temperature (21 $\pm$ 1 ºC); the photoperiod was adjusted according to the sampling season. During acclimation, animals were fed *ad libitum* once a day with commercial fish pellets of the following composition (in g 100 g^{-1}): lipids, 12.7; protein, 39.7; carbohydrates, 28.6; humidity, 7.8, and ash, 11.2. Then, they were transferred to 24 L-glass aquaria with aerated TW, under a constant circuited flow regime (Cole Palmer peristaltic pump, model N 7553-85) at a rate of 17-22 mL min^{-1}.

After the acclimation period, two groups of tadpoles were exposed to the river water samples (S1, S2) for 6 days. A third group of animals were exposed to TW (Negative Controls; NC); TW was used as negative control because at present no part of the river can be considered devoid of pollutants. Another group of tadpoles, Positive Control (PC), were exposed to TW containing 40 mg L^{-1} cyclophosphamide monohydrate (Sigma-Aldrich, CAS No. 6055-19-2).

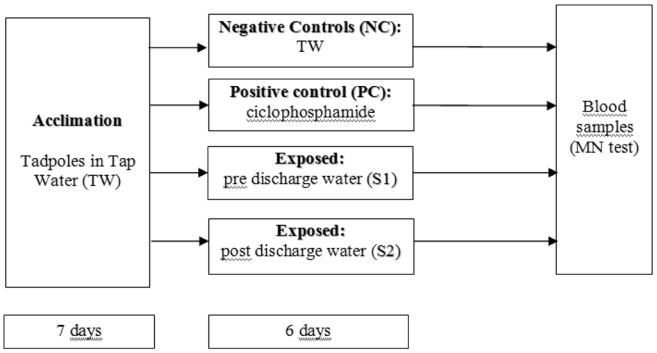


Figure 2 - Scheme of the experimental design.

Each group consisted of 10 tadpoles. During the assays, the media were renewed every 48 h. Two hours before the renewal, animals were fed, with an amount equivalent to 2 % body weight; the remaining food was removed and the medium of each aquarium was replaced by fresh medium.

Chemical analyses

The parameters measured and the analytical procedures used are shown in Table 1. The heavy metal total concentrations of Mn, Zn, Cu, Cr, Ni, Pb and Cd in all samples were measured by atomic absorption spectrophotometry, with a Shimadzu 6700 equipment with a graphite furnace GFA 6000 and an ASC 6000 autosampler. Results were expressed as µg L^{-1}; the detection limit was in the range of 0.5-1.0 µg L^{-1}.

In samples collected in Summer 07 and Summer 08, a screening of pesticides was conducted by high resolution capillary gas chromatography (Hewlett Packard; 61530 Plus A6890) equipped with appropriate capture detectors (ECD, FPD and NPD). The screening included the following pesticides: *Organochlorines*: Aldrin, α-, β- and γ-Chlordane, DDT and metabolites, Dieldrin, α- and β-Endosulfan, Endrin, Heptachlor, Heptachlor epoxide, Hexachlorobenzene, α- and β-Hexachlorocyclohexane, Methoxychlor and Mirex; *Organophosphates*: Bromophos, Chlorfenviphos, Chlorpyriphos, Coumaphos, Diazinon, Ethylbromophos, Ethion, Fentrothion, Malathion and Methylparathion. The detection limit was 0.03 µg L^{-1} for organochlorines and 0.02 µg L^{-1} for organophosphates.

Water Quality Indices

Three Indices (WQI) were calculated: 1) an index of domestic pollution based on DO, Cl^-, BOD_5 and NH_4^+ (Berón, 1984); 2) the Index of Lacoste & Collasius (1995), which quantifies industrial contamination; the parameters used for the calculations were DO, COD, and the total concentration of Zn, Cu and Cr, and 3) a modification of the fresh water pollution index proposed by Pesce & Wunderlin (2000), based on hardness, Cl^-, DO, NH_4^+, NO_2^-, NO_3^-, soluble reactive phosphorus, BOD_5 and COD. These WQIs are unitless and ascribe a quality value in a scale inversely proportional to the pollution ranging from zero (highly polluted-sewage quality) to ten (high-purity water quality).

Blood sampling – Micronucleus test

Blood cells were obtained by cardiac puncture with heparinized syringes from the heart of animals previously anesthetized in ice cold water. Two peripheral blood smears for each larva were prepared on clean slides and fixed with ethanol 96 % for 10 min, air-dried and stained with Acridine Orange (Sigma-Aldrich, CAS No. 158550) following the method described by Schmid (1975) and Ueda *et al.* (1992). For MN frequency analysis, erythrocytes were examined using a Zeiss HBO50 epifluorescence microscope at 400x magnification. For each tadpole, 1,000 erythrocytes were analyzed in each

Table 1 - Methods used for the determination of the physicochemical parameters of the water samples.

Parameters	Units	Methods
pH		pHmeter Orion EA 940
Conductivity	µS cm^{-1}	Field sensor, Hanna HI 98240
Hardness	mg $CaCO_3$ L^{-1}	Kit Merck 108039
Total alkalinity	mg $CaCO_3$ L^{-1}	Tritiation with H_2SO_4 [a]
Chlorides	mg Cl^- L^{-1}	Tritiation with $AgNO_3$ [a]
DO	mg O_2 L^{-1}	Winkler [a]
Ammonium	mg N-NH_4^+ L^{-1}	Kit Merck 14752
Nitrites	mg N-NO_2^- L^{-1}	Colorimetric [a]
Nitrates	mg N-NO_3^- L^{-1}	Kit Merck 14773
Soluble reactive phosphorous	mg P-PO_4^- L^{-1}	Colorimetric [a]
Biochemical oxygen demand (DBO_5)	mg O_2 L^{-1}	Winkler [a]
Chemical oxygen demand (COD)	mg O_2 L^{-1}	Kit Merck 14540
Heavy metals	µg L^{-1}	Atomic absorption spectrophotometry
Pesticides	µg L^{-1}	Gas chromatography

[a] APHA-AWWA, 2005

replicated slides and the frequency of micronucleated cells was recorded. Coded and randomized slides were scored blind by a single observer; the results are expressed as the number of micronucleated cells over 2,000 erythrocytes. Several criteria were adopted for the identification of MN; in relation to the main nuclei, they had: a) to be smaller than one-third, b) to be clearly separated, c) exhibit the same color and intensity and d) exhibit well preserved cytoplasm (Fenech, 2000; Grisolia, 2002). It is worth mentioning that Polard *et al.* (2011) showed that the assay was more sensitive when Acridine Orange staining of slides was applied, avoiding false positives as when other stains were used.

Statistical analysis

Mean values ± SEM of MN/2000 cells were calculated. The results were tested with non-parametric one-way ANOVAs, Kruskal-Wallis and post-hoc Dunn´s test. In addition, a correlation between the WQIs and the frequency of MN was carried out, coupled with a statistical analysis using the *t* test.

Statistical analyses were performed using GraphPad InStat (3.01) and Statistica 7.0 softwares for Windows. A probability value of less than 0.05 was regarded as significant (Zar, 2010).

RESULTS

The physicochemical parameters analyzed in the river water samples and in tap water (Negative Controls) as well as the WQIs are summarized in Table 2.

Table 2 - Physico-chemical parameters and Water Quality Indices of Luján river and Tap Water (NC)

		Summer 07		Summer 08		Autumn 08		Winter 08		Spring 08		NC
Parameter	Units	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	
pH		7.8	7.7	7.8	7.9	8.5	8.6	8.3	8.0	7.8	8	8.1-8.5
Conductivity	µS cm^{-1}	2940	2890	1090	1500	1850	1667	1350	2300	913	1570	920-1000
Hardness	mg $CaCO_3$ L^{-1}	250	240	130	120	170	150	190	150	120	90	60-95
Total alkalinity	mg $CaCO_3$ L^{-1}	837	728	484	499	600	772	655	946	437	946	380-450
Chlorides	mg Cl^- L^{-1}	344	328	160	190	205	135	238	167	62	35	13-31
Dissolved oxygen	mg O_2 L^{-1}	4	3	1.5	1.7	3.3	5.3	1.63	0.8	2.8	0	6-11
Ammonium	mg N-NH_4^+ L^{-1}	2.6	1.5	5.8	5.8	4.6	3.1	4.3	2.4	2.3	1.9	0.4-1.6
Nitrites	mg N-NO_2^- L^{-1}	0.7	0.6	0.8	0.8	0.2	0.2	0.8	0.5	0.5	0.7	0.02-0.1
Nitrates	mg N-NO_3^- L^{-1}	0.7	0.2	0.7	0.7	1.5	3.7	0.8	2.8	0.3	0.1	3.6-3.9
Soluble reactive phosphorus	mg P-PO_4^{-3} L^{-1}	0.6	0.5	0.7	1.1	1.1	0.7	1.3	1.4	1.2	2.7	0.1-0.3
Biochemical oxygen demand (BOD_5)	mg O_2 L^{-1}	11.1	5.3	2.1	1.8	6.9	8.4	5.6	10.3	14.5	3.6	0.7-1.2
Chemical oxygen demand (COD)	mg O_2 L^{-1}	120	28	33	81	59	67	65	96	36	>150	0
COD/BOD_5		10.8	5.2	15.8	45.7	8.5	8.0	11.5	9.3	2.5	≥41.7	0
Heavy metals:	µg L^{-1}											
Mn		**1900**	700	101	96	20	< 5	31	13	44	< 5	ND
Zn		170	**730**	**88**	35	26	15	26	17	**65**	18	33
Cu		< 10 D	< 10 D	**22**	< 10 D	< 5	< 5	3	4	**180**	**32**	< 10 D
Cr		< 10 D	**11**	< 10 D	< 10 D	**11**	**6**	**5**	**3**	**3**	**3**	< 10 D
Ni		< 10 ND	< 10 ND	< 10 ND	< 10 ND	NM	NM	NM	NM	NM	NM	ND
Pb		< 20 ND	< 20 ND	**41**	< 20 D	< 5	< 5	< 2	< 2	< 2	< 2	**23**
Cd		< 6 ND	< 6 ND	< 10 D	< 10 D	< 1	< 1	< 1	< 1	< 1	< 1	< 10
WQI:												
WQI (Berón, 1984)		3	5	4.6	5	4	4	5	3	3	5	9
WQI (Lacoste & Collasius, 1995)		5	7	6.5	5	6	6	6	4.5	6	4	9
WQI (Pesce & Wunderlin, 2000)		5	6	5	5	5	4	4	3	4	3	8

NC: Negative Controls; NM: not measured; ND: not detected; D: Detected. In bold heavy metals over de guidelines of Water Resources Secretary; WQI: Water Quality Indices.

The pH of the river water was fairly stable, always slightly alkaline, whereas the conductivity, hardness and alkalinity were higher than the controls in all cases; the DO levels were very low in all samples. The COD:BOD ratio was indicative of an important contribution of non-biodegradable organic matter. Other indicators of domestic, municipal and industrial discharges (Cl^-, PO_4^{3-}, $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$) were always very high. The concentration of heavy metals exhibited temporal variability without a uniform pattern of changes, although in several cases their levels were above those provided by local legislation for aquatic life protection.

The concentrations of organochlorine and organophosphorus pesticides determined in samples of Summer 07 and Summer 08 were below the detection limit; for that reason were not included in Table 2.

The three river water quality indices were consistently in the range between 3 and 6 throughout the monitoring; the values calculated for the Negative Controls (TW) oscillated between 8-9.

During the bioassays (with river water and TW controls), possible changes in a limited number of physical-chemical parameters (DO, pH, hardness, conductivity) of the media were regularly monitored; the ranges of the measured parameters are shown in Table 3.

Figure 3 shows the number of MN found in peripheral erythrocytes of tadpoles exposed to Luján river water samples, before (S1) and after (S2) the effluent discharge, as well as in Negative and Positive Controls.

In the sampling carried out in summer 2007, there were no significant differences in the frequency of MN relative to the negative control. In this regard, it is interesting to remember that that metal toxicity is influenced by the ambient chemistry which affects their speciation and its impact on their bioavailability (Gandhi *et al.* 2011); it is known that the ecotoxicity of metals in particular is inversely related to hardness: as it increases, metal toxicity decreases. In our case, hardness in summer 2007 was higher than in other samples. In contrast, statistically significant differences in the frequency of MN were found in most of the remaining S1 and S2 samples compared to NC.

The number of MN in the blood samples of larvae exposed to cyclophosphamide showed a significant increase (250 %) as compared to the negative control values. Except in S1 samples of summer 2007, the frequency of MN was close to or higher than that found in the Positive Controls; in addition the recorded MN frequencies were always slightly higher in S1 than in S2.

Figure 4 shows an erythrocyte of *L. catesbeianus* tadpole exposed to Luján river water with one MN; it should be noted that in a few cases the blood smears of larvae exposed to environmental samples and cyclophosphamide solutions exhibited erythrocytes with two MN. It is important to note that there was no mortality in the test organisms during the bioassays.

The correlation between the three WQIs calculated for samples from each site (see Table 2) and the frequency of MN in erythrocytes was found to be always negative: high values of the WQIs (indicating a low polluted water) were accompanied by a decrease in frequency MN. The best correlation obtained corresponded to WQI calculated as Pesce & Wunderlin (2000) (Figure 5); in this case, the Pearson coefficient (r) was - 0.7034 and statistically significant ($p = 0.003$). For the other indices, the coefficients obtained and their significance were: $r = - 0.6950$, $p = 0.004$ (Lacoste & Collasius, 1995) and $r = - 0.5841$, $p = 0.022$ (Berón, 1984).

DISCUSSION

Surface aquatic environments are the sinks for complex chemical mixtures of diverse origin, including a variety of toxics from industrial effluents and urban wastes. This is the case of the water of Lujan river, as its physicochemical profile showed signs of important degradation of its quality.

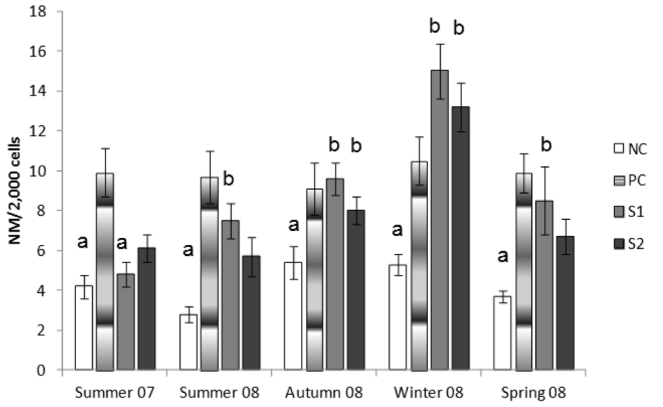
The DO levels showed that the river water could be characterized as under permanent hypoxia. Besides the conditions of hypoxia, the high levels of nitrogen compounds and heavy metals present in the samples make the river environmentally adverse for the aquatic biota. This conclusion is reinforced by the values found in the quality indices of the water samples, whose values ranged from 3-5 (Berón), 4-7 (Lacoste & Collasius) and 3-6 (Pesce & Wunderlin) showing an important degree of deterioration, while the indices of the Controls remained stable along the assays oscillating between 8-9 (Table 2).

The *in vivo* micronuclei frequency assay has been widely used as a technique for genotoxicity monitoring of polluted aquatic media and in the screening for the presence of toxic compounds suspected to be genotoxic (Ossana *et al.*, 2010; Wirz *et al.*, 2005). This test is recommended to be conducted

Table 3 - Physicochemical parameters of the bioassay media during the exposure in the laboratory.

		Summer 07		Summer 08		Autumn 08		Winter 08		Spring 08		NC	PC
Parameter	Units	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2		
pH		7.8-8.1 (4)	7.7-8.0 (4)	7.8-8.0 (3)	7.8-7.9 (3)	8.3-8.5 (3)	8.6-8.7 (3)	8.0-8.6 (3)	8.0-8.7 (3)	7.7-8.3 (4)	8.0-8.6 (4)	7.8-8.6 (14)	8.5-8.7 (6)
Conductivity	$\mu S\ cm^{-1}$	2940-3060 (4)	2890-2960 (4)	1442-1500 (3)	1090-1478 (3)	1839-1914 (5)	1667-1963 (3)	2210-2350 (3)	2300-2400 (3)	842-970 (4)	1340-1570 (4)	843-1021 (13)	870-1055 (4)
Hardness	$mg\ CaCO_3\ L^{-1}$	170-240 (4)	200-250 (4)	120-140 (3)	130-135 (3)	110-170 (3)	105-150 (3)	170-190 (3)	150-180 (3)	100-120 (4)	90 (4)	60-90 (12)	70-95 (6)
Dissolved oxygen	$mg\ O_2\ L^{-1}$	6.5-7.6 (4)	5.2-7.5 (4)	4.2-4.7 (3)	4.7-5.9 (3)	3.6-4.1 (3)	7-13 (3)	6.6-8.5 (3)	7.0-7.5 (3)	5.3-5.7 (3)	5.7-6.7 (3)	6.1-8.0 (12)	6.4-8.2 (3)

NC: Negative controls; PC: Positive controls; Data as ranges. Number of measurements between parentheses.



NC: negative controls; PC: positive control; S1: pre discharge Luján river; S2: post discharge Luján river. a, significant differences from positive controls (PC); b, significant differences from negative controls (NC).

Figure 3 - Frequency of micronuclei (MN) in peripheral erythrocytes from *Lithobates catesbeianus* tadpoles exposed to negative and positive controls and water from pre (S1) and post (S2) discharge sites of Luján river. Data as means ± SEM; N= 7-10.

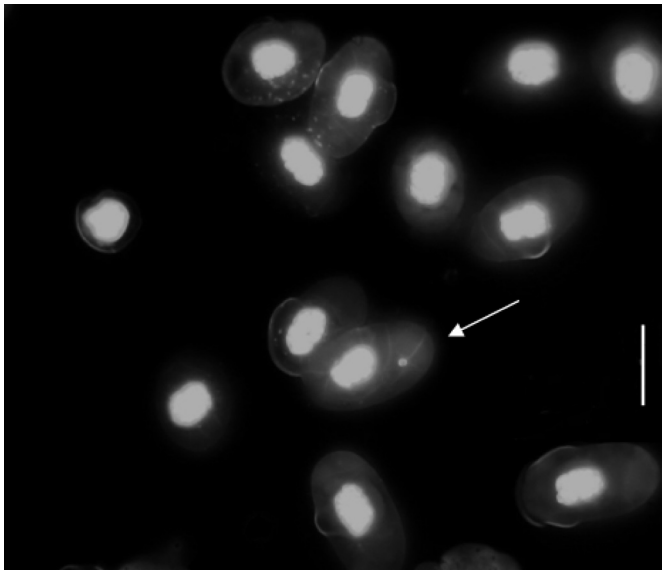


Figure 4 - Micronucleated erythrocyte (arrow) in peripheral blood smear from premetamorphic *Lithobates catesbeianus* exposed during 6 days to a Lujan river water (S1 sample) (bar: 20 µm)

as part of the monitoring protocols in aquatic toxicological assessment programs (Ohe *et al*., 2004; Udroiu, 2006); it has been proved to be a reliable index of both chromosome breakage and chromosome loss (Schmid, 1975).

Amphibians at early premetamorphic stages of their development showed to be an appropriate model as bioindicator organisms to explore the toxicant effects of environmentally relevant mutagens, under both laboratory and field conditions (Cabagna *et al.*, 2006; Campana *et al.*, 2003; Gauthier, 1996; Gauthier *et al.*, 2004; Krauter, 1993, Lajmanovich *et al.*, 2005). It is important to note that the red blood cells in amphibians are nucleated and undergo cell divisions in the circulation (Gauthier, 1996), which makes them a suitable material for genotoxicity assays.

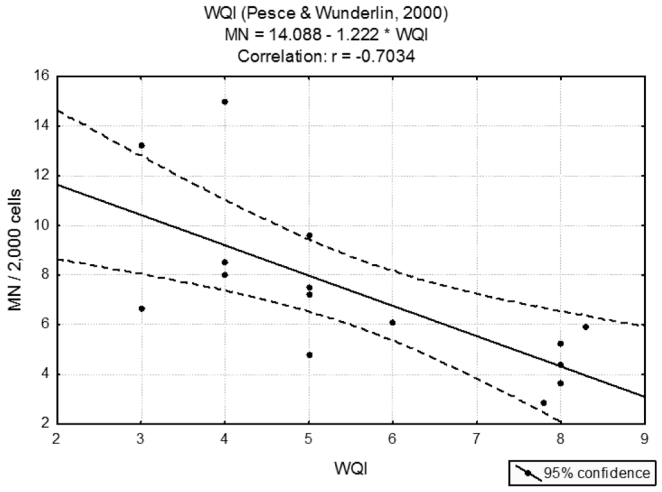


Figure 5 - Correlation between WQI (Pesce & Wunderlin, 2000) and MN frequency.

This test allows detecting the effects of mutagenic agents present in the analyzed samples on chromosomes by the identification of lagging chromosomes and/or acentric fragments that remain separate from the nucleus. From the ecotoxicological perspective, the exposure of aquatic fauna to mutagens may enhance, among other adverse effects, the frequency of recessive mutations, thus promoting the decline of particular sensitive animal populations. Some authors have demonstrated a direct relationship between genomic instability and some nuclear abnormalities, such as nuclear buds, broken-eggs and micronuclei as genotoxic responses (da Silva Souza & Fontanetti, 2006; Gravato & Santos, 2002; Serrano-García & Montero-Montoya, 2001).

It is also important to relate the frequency of MN with the chemical profile of the samples, which showed the existence of certain heavy metals that are highly toxic to aquatic life. The concentrations of some heavy metals (Zn, Mn and Cr) remained at high levels while the values of the other metals (Cd) were generally low. Although the concentrations of several metals in most samples were above the limits allowed by local environmental legislation, the concentrations of other organic or inorganic contaminants may have increased incidentally, thus causing a toxic response, which may have contribute to increases in the frequency of MN in some samples.

Correlations between physicochemical parameters considered for the calculation of WQIs were statistically significant in relation to the frequency of MN registered in erythrocytes of larvae exposed to environmental samples. In this regard, it is important to point out that the erythrocytes of tadpoles exposed to the samples of the river showed increases in the frequency of MN in animals exposed to S1 and S2 related to their baseline levels of MN in NC (tap water) which oscillated between 3 and 5 MN/2000 cells.

Partial results referred to the concentrations of organochlorine and organophosphorus pesticides in the environmental sampling suggest that genotoxicity could not be attributed to those pesticides.

It is worth mentioning that during the two weeks before winter 08 sampling, the total rainfall was 0 and 1.5 mm, in contrast to what happened in the other samplings in which values were much higher; this circumstance could explain the significant increase of the MN frequency at both S1 and S2 sites, possible due to a concentration of the media. Similar results were found by da Silva Souza & Fontanetti (2006) in Paraíba do Sul river.

The frequency of MN in the erythrocytes of the exposed tadpoles was in most cases higher in S1 than in S2, although the differences were not significant. The effluent dumped in the river did not contribute to increase the previous genotoxicity of the river water.

The negative controls allowed determining the basal frequency of MN, which indicated a spontaneous micronuclei formation. In addition, the assays with cyclophosphamide, an indirect alkylating agent well known as a genotoxic substance, indicated a highly significant increase in the frequency of micronuclei as compared to the Negative Controls. It is important to point out that, except in summer 07 samples, the MN frequency found in the animals exposed to cyclophosphamide (PC) was not statistically different from that of the animals exposed to environmental samples; moreover, the values of the samples taken during the winter 2008 were higher than those of the PC.

In this study, the first report on the genotoxicity of the water of Luján river, we showed evidences that the increasing frequency of MN in the blood of premetamorphic *L. catesbeianus* tadpoles exposed to the river samples is an early biological marker of chromosomal damage to assess the genotoxicity of this peri-urban polluted watercourse and that the protocol applied was apt to describe the toxicological quality of water samples.

CONCLUSIONS

The results presented in this work revealed an important deterioration in the water quality of the Lujan river. In addition, this is the first report of genotoxicity of surface water of the river, which was high and comparable to that found by exposure of test organisms to a reference toxicant (cyclophosphamide). The correlation between WQIs and the frequency of MN were negative and statistically significant. It is also important to note that these effects were early as they were recorded after a brief exposure period (6 days) and sublethal.

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