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ARTICLE

Cytogenetic responses of *Allium cepa* L. after exposure to contaminated pond waters

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ABSTRACT: (Cytogenetic responses of *Allium cepa* L. after exposure to contaminated pond waters). Biomarkers may refer to certain changes in living cells induced by environmental contaminants. To assess these, plants act as excellent systems for genetic tests as they are sensitive to pollutants. Therefore, the present study aimed to evaluate a coastal pond (Brazil) used for fishing and recreation, but also affected by several environmental impacts. Three sampling stations were established around the pond to collect water during the rainy and a post-rainy period. The *A. cepa* test was conducted using seeds exposed to water samples and controls. Slides of root meristems were prepared using the Feulgen method and analyzed to calculate the mitotic index, the rate of chromosomal aberrations and the frequency of micronuclei. Statistical analysis was performed using ANOVA followed by the Tukey or Bonferroni test (P <0.05). The results showed significant changes in the mitotic index, chromosomal aberrations and frequency of micronuclei when compared to the negative control. Among the aberrations observed, C-metaphase, chromosomal breaks, nuclear buds and chromosomal losses showed significant values. In addition, it was observed that the rate of chromosomal aberrations during the rainy period was superior to that in the post-rainy period. These results may be associated with the presence of potentially toxic compounds such as aluminum and cadmium, although the pond presents a good water quality index. Therefore, we suggest a combination of more sensitive methods, such as the *Allium cepa* test, with traditional systems of surveillance and monitoring of aquatic environments.
Keywords: Mitotic index, chromosomal aberrations, micronucleus, metals.

RESUMO: (Respostas citogenéticas de *Allium cepa* L. após exposição às águas contaminada de uma lagoa). Biomarcadores podem se referir a certas mudanças em células vivas por contaminantes ambientais. Para a avaliação destes, as plantas atuam como ótimos sistemas testes genéticos sensíveis aos poluentes. Portanto, o presente estudo teve como objetivo avaliar uma lagoa utilizada para a pesca e recreação, mas também submetida a vários impactos ambientais. Foram definidas três estações amostrais ao longo da lagoa para a coleta de água durante períodos de chuva e pós-chuva. O teste do *A. cepa* foi realizado por meio de sementes expostas às amostras de água e controles. Lâminas dos meristemas das raízes foram montadas pelo método de Feulgen e analisadas para calcular o índice mitótico, a frequência de aberrações cromossômicas e a frequência de micro-núcleos. Análise estatística foi realizada utilizando ANOVA seguida de Tukey e teste de Bonferroni (P <0.05). Os resultados demonstraram alterações significativas nos índices mitótico, de aberrações cromossómicas e na frequência de micronúcleos quando comparado ao controle negativo. Entre as aberrações observadas, C-metáfase, quebras cromossômicas, brotos nucleares e perdas cromossômicas apresentaram valores significativos. Além disso, observou-se que a taxa de aberrações cromossómicas durante o período de chuva foi superior ao período pós-chuva. Estes resultados podem estar associados à presença de compostos potencialmente tóxicos, tais como o alumínio e cádmio que foram quantificados em estudo pretérito, embora a lagoa apresente índice de qualidade de água de boa / regular. Portanto, sugerimos a combinação de métodos mais sensíveis, tais como teste de *Allium cepa*, aos tradicionais métodos de fiscalização e monitoramento de ambientes aquáticos.
Palavra-chave: Índice mitótico, aberrações cromossômicas, micronúcleo, metais.

INTRODUCTION

Considering the contamination of biosphere components such as water with mutagenic and carcinogenic agents, as well as their interactions with humans, the monitoring of genotoxic compounds present in the environment should become an important aspect of public health with the intent to prevent or minimize human exposure to these substances (Feretti *et al.* 2007).

Jacuném Pond is a coastal pond environment that is undergoing an artificial eutrophication process resulting from effluents discharged directly or indirectly by its tributaries. Previous cytogenetic and molecular evaluations on *Oreochromis niloticus* obtained from this environment revealed high levels of genotoxicity and mutagenicity, as well as concentrations of Al and Cd higher than those recommended by the Brazilian legislation at three sampling stations in the pond during rainy and post-rainy periods (Duarte *et al.* 2012).

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Biomarkers may refer to certain changes in living cells induced by environmental contaminants, and also act as indicators of potential toxic input in organisms (Everaarts 1995). In this regard, plants act as excellent systems for genetic tests as they are sensitive to environmental pollutants; thus, they are commonly used in the monitoring of aquatic environments and industrial and domestic effluents (Matsumoto & Marin-Morales 2004, Grisolia *et al.* 2005, Egito *et al.* 2007, Nunes *et al.* 2011, Grippa *et al.* 2012, Gupta & Ahmad 2012).

Among the plants, *Allium cepa* L. is widely used as a test system for the evaluation of damage to chromosomes and the mitotic apparatus, due to the ease of analysis as well as its high sensitivity for detecting chemicals (Fiskesjö 1985, Leme & Marin-Morales 2009). Using the meristematic cells of *A. cepa* roots, the potential genotoxic and mutagenic effects of different compounds can be determined (Fernandes *et al.* 2007, Caritá & Marin-Morales 2008), and it is effective in the analysis of the pollution of water resources and for evaluating water quality (Radic *et al.* 2010).

Due to the degradation of water bodies, constant monitoring these environments is necessary using tests that are fast and sensitive. Therefore, this study aimed to assess the cytotoxicity, genotoxicity and mutagenicity, using the *A. cepa* test system, of water samples collected during rainy and post-rainy periods in Jacuném Pond, located in Serra, Espirito Santo, Brazil.

MATERIALS AND METHODS

Characterization of the study area

Jacuném Pond is a coastal pond environment, with a depth of less than 3 meters, located in Serra, Espírito Santo, Brazil (22°10'S and 40°13'W). According to the Resolution 357/2005 of the National Environment Council (CONAMA), it is classified as a Class 2 environment. Surrounded by industrial and residential neighborhoods, with an area of 1.46 km², the pond is undergoing an artificial eutrophication process due to the constant inflow of domestic and industrial effluents released directly or indirectly by its tributaries. The pond served as source of public water supply until the end of 1983, when the captation systems and conventional treatment by the Espírito Santo Sanitation Company (CESAN) were disabled. Today, it is used by locals for fishing, recreation and leisure. However, since the 1980s, it has experienced environmental impacts from the misuse and occupation of its drainage basin (32.06 km²), of which 48% is located in urban areas (Duarte *et al.* 2012).

Sampling stations

To characterize the conditions of Jacuném Pond, three sampling stations distributed along the pond and its drainage channel were defined. These stations are used by the State Institute for the Environment and Water Resources of Espírito Santo (IEMA) in the monitoring program of the water quality of watersheds of Espirito Santo State, Brazil. The station J1 is located in an urban area, latitude 20°10'10.80"S and longitude 40°14'21.80"W. The station J2 is located in the industrial perimeter in the old CESAN captation station, latitude 20°9'58.80"S and longitude 40°14'6.10"W. The station J3 is located in the urban and rural perimeter, corresponding to the effluent of Jacuném Pond, latitude 20°9'17.00"S and longitude 40°11'19.20"W.

The samples consisted of surface water samplings in triplicate, collected and stored in sterile polyethylene bottles, previously washed with 2% Extran solution. The samples were kept refrigerated until the tests. There were two sampling campaigns, the first at the end of August 2010, corresponding to a rainy period (20.7 mm of monthly total precipitation) and the second at the end of September of the same year, characterizing the post-rainy period (27.8 mm of monthly total precipitation).

Allium cepa test

Seeds of *A. cepa* from the Baia Periforme variety of the same batch were used in order to maintain physiological and genetic homogeneity, in addition of being available throughout the whole year, ensuring the reliability of the test as proposed by Leme *et al.* (2008). The *A. cepa* test, using meristematic root cells, was performed according to Grant (1982) with modifications. Seeds were germinated directly in the water from sampling stations J1, J2 and J3. The negative control (CON) was performed with seeds germinated in distilled water and the positive (CON+) control was a solution of methyl methanesulfonate (MMS) at a concentration of $4x10^{-4}$ mM. In each experimental group, 5 ml of water sample was added to a Petri plate, using three plates per treatment. Exposure was carried out for five days until the roots were roughly 2 cm in length.

The roots of the seeds submitted to the treatments were collected and fixed in Carnoy 3:1 (three parts of ethanol for one part of acetic acid) for 24 hours, after reaching approximately 2 cm in length. The meristems were subjected to acid hydrolysis in 1N HCl (60°C) for seven minutes, followed by washing in distilled water. Staining was performed using the traditional method of Feulgen and the slides were prepared by the commonly used method of gentle crushing with a drop of acetic carmine (2%). Samples were covered with coverslips that were subsequently removed with liquid nitrogen, and were finally mounted with Canada balsam for analysis.

Five slides were prepared in triplicate per treatment (CON, CON+, J1, J2 and J3). Approximately 15,000 cells, i.e. 1,000 per slide and 5,000 per replicate, were evaluated for each treatment under a light microscope. The cellular phases, as well as chromosomal abnormalities were assessed and the frequencies were used to calculate the mitotic index (MI), the rate of chromosomal aberrations (CA) and the frequency of micronuclei (MN).

The analysis of cytotoxicity measured by MI was obtained from the ratio of the total number of dividing cells with the total number of cells analyzed. For the

analysis of genotoxicity by the chromosomal aberrations index, different types of changes were considered in different stages of cell division (prophase, metaphase, anaphase and telophase), such as chromosomal bridges, chromosomal breaks, C-metaphase, multipolar anaphase, chromosomal retard, chromosomal adherence, chromosomal losses, fragmented nuclei, nuclear buds and binucleated cells. In these cases, the CA index was calculated by determining the ratio of the total number of cells with these aberrations and the total number of cells analyzed. For the assessment of mutagenicity, MN was used, and the number of cells carrying micronuclei in relation to the total number of cells was assessed (Leme & Morales-Marin 2008).

Statistical analyses

For the number of each cellular alteration, as well the MI, CA and MN indexes, analysis of variance (ANOVA) was performed followed by Tukey's Multiple Comparison Test at a significance level of 0.05, in order to compare the sampling stations and controls in each sampling campaign. For the comparison between first and second campaigns, Bonferroni's Multiple Comparison Test was used at a significance level of 0.05.

RESULTS AND DISCUSSION

The pluviometric data of 2010, collected by the National Institute of Meteorology, showed that the first sampling campaign was characterized as a period of rain, featuring a pond flood, given that the previous month was marked by severe rainfall, raising the water level of the environment; these effects extended to the following month. The months of May, June and July received 113.4, 68.6 and 94.9 mm of monthly total precipitation, respectively. In the second campaign, the previous month was marked by low rainfall that lasted until a month later, characterizing the post-rainy period, where the water level of the pond was low.

A study conducted by Duarte *et al.* (2012), with water samples from the same sampling stations during the same sampling periods of this study, revealed that the pond had a good water quality index (WQI), but also had aluminum and cadmium concentrations higher than those established by Brazilian resolution CONAMA 357/2005 for waters of Class 2 lacustrine environments. In the resolution, the allowed concentrations for Al and Cd are 0.1 mg/L and 0.001 mg/L, respectively. In the previous study, in J1 during the first campaign, the concentration of Al was 0.319 ± 0.005 mg/L, and in J2 during the second campaign, the Al concentration was 0.002 ± 0.015 mg/L. In J3, the Al concentration was 0.003 ± 0.015 mg/L in the first campaign and 0.002 ± 0.016 mg/L in the second. Cd was found at high levels in J3 during the first campaign (0.002 ± 0.0002 mg/L).

Besides the measurement of metals, Duarte *et al.* (2012) conducted ecotoxicological tests in *Oreochromis niloticus*. In this case, the evaluation of *O. niloticus* peripheral blood showed high genotoxicity through the DNA damage index measured by the comet assay, as well as high mutagenicity based on the frequency of micronuclei measured using the micronucleus test. These findings occurred in samples from J1, J2 and J3 during the rainy and post-rainy periods.

In a corresponding manner, the results of the analyses of *A. cepa* exposed to water samples from different sampling stations during the rainy and post-rainy periods revealed that all sampling stations in both campaigns had cytotoxic, genotoxic and mutagenic potentials. The MI values were significantly lower than those found in negative controls. The rate of CA and frequency of MN showed, in general, that all values were significantly higher than the negative control during both campaigns, suggesting that the water samples interfered with the cell division process (Table 1). In this context, J1 showed the lowest values of MI during both campaigns, i.e. the rainy and post-rainy periods. As for CA, during the first campaign, J2 and J3 showed the highest values, while in the second campaign, the three sampling stations showed similar values for this index. The frequency of MN was found to be higher in J1, J2 and J3 during the rainy period, while in the post-rainy period, J1 and J3 were significantly different from the negative control.

Utilizing the *Allium cepa* test system, Nunes *et al.* (2011) observed that water samples from the Sinos River, an environment under intense anthropic impact through effluent discharges, showed no significant effect on micronucleus frequency. However, a cytotoxic effect was observed through the MI values of some samples. In this study, it was found that the concentrations of some metals, including aluminum, were in contravention of CONAMA Resolution 357/2005 in some samples. A study conducted by Bianchi *et al.* (2011) on the water of the Monjolinho River, which receives untreated effluents, also showed that, during the summer (hot and humid), water samples interfered with cell division in *A. cepa*, reflected in the MI, MN and AC indexes. During the winter (cold and dry), there were no significant differences compared to the negative control. In this study, the water samples showed high concentrations of lead, nickel, copper, chromium and manganese in the different seasons and sampling campaigns.

The cell damage results analyzed individually (Table 2) showed significantly different numbers of chromosomal breaks during the rainy period at J2 and during the post-rainy period at J3. Regarding C-metaphases, it was observed that, at all the sampling stations in both campaigns, the number of C-metaphases was significantly higher than in the negative control. As for chromosomal retard, chromosomal adherence and chromosome losses, J3 showed significantly higher numbers during the first campaign. The number of nuclear buds, during the rainy period, was higher in J2 and J3, while in the post-rainy period only J2 showed significantly higher numbers.

Cytogenetic analyses performed by Fiskesjö (1983, 1988) using bulbs of *A. cepa* treated with aluminum salts, demonstrated that the genotoxicity of this element was

Table 1. Mitotic index (MI), the rate of chromosomal aberrations (CA) and the frequency of micronuclei (MN) evaluated using the *Allium cepa* assay after exposure to water samples from Jacuném Pond sampling stations (J1, J2, J3) as well as negative (CON) and positive (CON+) controls.

Index	Treatments	Campaign	Replicate			Mean±SEM	Difference Between Campaings
			I	II	III		
MI		Rainy period	0.223	0.228	0.228	0.226 ± 0.002^{A}	nc
	CON+		0.172	0.179	0.178	0.176 ± 0.002^{B}	nc
	J1		0.046	0.044	0.048	0.046 ± 0.001^{B}	-
	J2		0.077	0.080	0.078	0.078 ± 0.001^{B}	*
	J3		0.095	0.091	0.093	0.093 ± 0.001^{B}	*
	CON	Post-rainy period	0.142	0.144	0.147	0.144 ± 0.001^{A}	nc
	CON+		0.085	0.087	0.088	0.087 ± 0.001^{Ba}	nc
	J1		0.044	0.041	0.042	0.043 ± 0.001^{B}	-
	J2		0.089	0.085	0.086	0.087 ± 0.001^{Ba}	*
	J3		0.060	0.058	0.060	0.059 ± 0.001^{B}	*
AC	CON	Rainy period	0.001	0.003	0.002	0.002 ± 0.001^{A}	nc
	CON+		0.028	0.030	0.030	0.029 ± 0.001^{Ba}	nc
	J1		0.016	0.011	0.014	0.014 ± 0.001^{B}	*
	J2		0.028	0.025	0.028	0.027 ± 0.001^{Ba}	*
	J3		0.029	0.026	0.031	0.029 ± 0.001^{Ba}	*
	CON	Post-rainy period	0.001	0.001	0.001	0.001 ± 0.000^{A}	nc
	CON+		0.019	0.022	0.024	0.022 ± 0.001^{B}	nc
	J1		0.007	0.009	0.008	0.008 ± 0.001^{Ba}	*
	J2		0.013	0.015	0.013	0.014 ± 0.001^{Bb}	*
	J3		0.012	0.009	0.011	0.011 ± 0.001^{Bab}	*
MN	CON	Rainy period	0.000	0.000	0.000	0.000 ± 0.000^{A}	nc
	CON+		0.005	0.008	0.006	0.006 ± 0.001^{Ba}	nc
	J1		0.007	0.005	0.006	0.006 ± 0.001^{Ba}	-
	J2		0.008	0.007	0.008	0.008 ± 0.000^{Bab}	-
	J3		0.010	0.009	0.009	0.009 ± 0.000^{Bb}	-
	CON	Post-rainy period	0.001	0.001	0.002	0.001 ± 0.000^{A}	nc
	CON+		0.012	0.018	0.018	0.016 ± 0.002^{B}	nc
	J1		0.007	0.009	0.008	0.008 ± 0.001^{Ba}	-
	J2		0.005	0.007	0.006	0.006 ± 0.001^{Aa}	-
	J3		0.009	0.008	0.010	0.009 ± 0.001^{Ba}	-

Means followed by different capital letters differ significantly to the CON and means followed by the same lower case letter do not differ significantly ($P < 0.05$), according Tukey's Multiple Comparison Test. * Significant difference between the same sampling stations in different campaigns ($P < 0.05$), according Bonferroni's Multiple Comparison Test. nc: Not compared. SEM: Standard error of the mean.

probably due to nuclear dissolution and chromosomal adherence. According to Voutsinas *et al.* (1997), aluminum acts on the cytoskeleton, thereby inhibiting the polymerization of microtubules, interrupting the growth of roots and causing changes in its morphology.

Dovgalyuk *et al.* (2003) showed that aluminum nitrate leads to a dense packing of microtubules in the meristematic cells of *A. cepa*. Based on studies on the inhibition of phospholipase D into microtubules by aluminum ions, Pejchar *et al.* (2008) emphasized its cytotoxic effects. Tests performed by Achary & Panda (2009) with 200-800 μM concentrations of Al indicated the induction of DNA damage and cell death in *A. cepa* root cells.

Regarding Cd, Fiskesjö (1988), using bulbs of *A. cepa* treated with various concentrations of cadmium chloride, showed high frequencies of C-metaphase, as well as chromosome adherence. Studies by Dovgalyuk *et al.* (2003) on the phytotoxic action of cadmium chloride in meristematic cells of *A. cepa* revealed that, following exposure to 50 μM cadmium chloride, the roots showed disturbances and breakdowns in the organization of microtubules. Moreover, studies on *A. cepa* by Marcano *et al.* (2006) and Seth *et al.* (2008) demonstrated that Cd interferes with the mitotic spindle, inducing changes such as multipolar anaphases, chromosome bridges, chromosome losses, unequal distribution of chromosomes at the end of the cell division, the formation of micronuclei and inhibition of the mitotic index.

The comparison analyses of MI, MN and CA between the same sampling stations in different campaigns revealed that the mitotic index showed no significant differences between campaigns for J1, but was lower during the rainy season in J2 and lower during the post-rainy period in J3. In contrast, the values of chromosomal aberrations showed a more pronounced pattern; the values at all points were higher during the rain. The frequency of micronuclei showed no significant differences between campaigns (Table 1).

The interference of seasonality on the water level of lacustrine environments and its consequence on damage frequencies were observed in this environment by Duarte *et al.* (2012), where the intensification of cytogenetic

Table 2. Cellular damage evaluated using the *Allium cepa* assay after exposure to water samples from Jacuném Pond sampling stations (J1, J2, J3) as well as negative (CON) and positive (CON+) controls.

Damage	Rainy period					Post-rainy period				
	CON	CON+	J1	J2	J3	CON	CON+	J1	J2	J3
Chromosomal bridge	$0 \pm 0,000^{A}$	$5 \pm 2,082^{A}$	$3 \pm 1,582^{A}$	$6 \pm 1,528^{A}$	$2 \pm 0,577^{A}$	$0 \pm 0,000^{A}$	$6 \pm 1,155^{B}$	$2 \pm 0,577^{Aa}$	$2 \pm 0,577^{Aa}$	$0 \pm 0,000^{Aa}$
Chromosomal break	$2 \pm 1,155^{A}$	$16 \pm 3,512^{Ba}$	$1 \pm 0,577^{A}$	$12 \pm 1,732^{Ba}$	$11 \pm 1,528^{Aa}$	$0 \pm 0,000^{A}$	$20 \pm 2,082^{B}$	$5 \pm 0,577^{Aab}$	$2 \pm 1,00^{Aa}$	$9 \pm 1,155^{Bb}$
C-Metaphase	$4 \pm 1,155^{A}$	$90 \pm 5,132^{B}$	$51 \pm 3,215^{Ba}$	$67 \pm 2,309^{Bb}$	$63 \pm 3,055^{Bab}$	$4 \pm 0,577^{A}$	$56 \pm 0,000^{B}$	$23 \pm 2,082^{B}$	$32 \pm 1,155^{Ba}$	$36 \pm 2,082^{Ba}$
Multipolar anaphase	$0 \pm 0,000^{A}$	$1 \pm 0,577^{A}$	$0 \pm 0,000^{A}$	$0 \pm 0,000^{A}$	$0 \pm 0,000^{A}$	-	-	-	-	-
Chromosomal retard	$0 \pm 0,000^{A}$	$3 \pm 1,155^{Aab}$	$0 \pm 0,000^{Aa}$	$3 \pm 0,577^{Aab}$	$7 \pm 2,517^{Bb}$	$0 \pm 0,000^{A}$	$2 \pm 0,577^{Aa}$	$0 \pm 0,000^{Aa}$	$1 \pm 0,577^{Aa}$	$2 \pm 0,577^{Aa}$
Chromosomal adherence	$0 \pm 0,000^{A}$	$4 \pm 1,528^{Aa}$	$2 \pm 0,577^{Aa}$	$4 \pm 0,577^{Aa}$	$6 \pm 1,528^{Ba}$	$0 \pm 0,000^{A}$	$4 \pm 1,528^{Ba}$	$2 \pm 0,000^{Aab}$	$1 \pm 0,577^{Aab}$	$0 \pm 0,000^{Ab}$
Chromosomal losses	$0 \pm 0,000^{A}$	$15 \pm 1,55^{Aa}$	$0 \pm 0,000^{Ab}$	$2 \pm 1,000^{Ab}$	$15 \pm 3,055^{Ba}$	$0 \pm 0,000^{A}$	$1 \pm 0,577^{Aa}$	$1 \pm 0,577^{Aa}$	$1 \pm 0,000^{Aa}$	$0 \pm 0,000^{Aa}$
Fragmented nucleus	$1 \pm 0,577^{A}$	$2 \pm 0,000^{A}$	$0 \pm 0,000^{Aa}$	$0 \pm 0,000^{Aa}$	$0 \pm 0,000^{Aa}$	-	-	-	-	-
Nuclear bud	$2 \pm 1,55^{A}$	$9 \pm 2,517^{Aa}$	$11 \pm 2,082^{Aa}$	$40 \pm 2,082^{Bb}$	$39 \pm 3,606^{Bb}$	$0 \pm 0,000^{A}$	$20 \pm 4,359^{Ba}$	$7 \pm 1,155^{Ab}$	$30 \pm 1,528^{Ba}$	$5 \pm 1,000^{Ab}$
Binucleated cell	$0 \pm 0,000^{A}$	$1 \pm 0,577^{Aab}$	$0 \pm 0,000^{Aa}$	$2 \pm 0,000^{Bb}$	$0 \pm 0,000^{Aa}$	-	-	-	-	-

Means (means ± SEM) followed by different capital letters differ significantly to the CON and means followed by the same lower case letter do not differ significantly ($P < 0.05$), according Tukey's Multiple Comparison Test. SEM: Standard error of the mean.

damage was noted in test organisms used during the rainy period in which the water level is usually high. In this regard, Ergene *et al.* (2007) described that the concentration of pollutants generally depends on the phenomenon of enrichment or dilution caused, for example, by rain. In such cases, dilution can promote the precipitation of pollutants or exacerbate the effects caused by the greater leaching of pollutants from the soil and sewage.

In the present study, only the index of chromosomal aberrations showed an intensification pattern during the rainy period, showing that the greatest genotoxic potential occurs during flood periods in Jacuném Pond. However, it should be emphasized that, independently of the rainy and post-rainy periods, the computed endpoints (MI, MN and AC) exhibited high values, suggesting that this environment contains large amounts of substances that are potentially cytotoxic, genotoxic and mutagenic, such as Al and Cd, detectable by the *Allium cepa* test.

Bioindicators capable of responding in a more reliable way to the potential risks of pollutants present in aquatic environments complement the physical and chemical analyses usually performed to monitor the water quality of aquatic environments. Therefore, according to Buss *et al.* (2008), in order to achieve more efficient evaluations in aquatic environments, it is essential to combine traditional methods to classify waters with more sensitive biotic methods, as presented in this study. This study and the one by Duarte *et al.* (2012) show that biotic test systems are valuable for assessing the quality of aquatic environments. Other studies concomitantly using assays with *O. niloticus* and *A. cepa* also point to the necessity of these tests for the evaluation of environmental quality, considering the sensitivity of both species (Matsumoto *et al.* 2006, Barbosa *et al.* 2010).

Based on these results, it is concluded that the water samples analyzed from the sampling stations established in Jacuném Pond, located in the municipality of Serra, showed cytotoxic, genotoxic and mutagenic potential in at least one of the campaigns. On that account, it is emphasized that the inspection and monitoring of the pond and its drainage basin be intensified, along with the use of ecotoxicological methods, such as cytogenetic analysis, to assess water quality.

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