Ecotoxicological evaluation of sediment and water samples from Sinos River, Rio Grande do Sul, Brazil, using *Daphnia magna* and V79 cells

Avaliação ecotoxicológica de sedimento e amostras de água do Rio dos Sinos, Rio Grande do Sul, Brasil, utilizando *Daphnia magna* e células V79

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Abstract: Biological tests are essential to evaluate the quality of environmental compartments in order to classify their capacity to preserve aquatic life and human health. The Sinos River is important in the state of Rio Grande do Sul, for socioeconomic and environmental reasons, since it is part of the Guaíba Lake Hydrographic Basin, the source of the public water supply for the state capital. Four river sites were monitored for sediment quality (Mar./01 to Mar./05), using (chronic) assays with *Daphnia magna*. Genotoxicity by micronuclei analysis (MN) and cytotoxicity in the V79 cell line (Chinese hamster lung) were evaluated in surface water at five sites in May/05 and Jun./05 to characterize the area. In assays with *D. magna*, the ANOVA test and MDS (multiple comparisons) were used for statistical analysis. Analysis of variance showed interaction between month and site with significant differences between the reproduction means per site, depending on the month. The results showed that the negative response for mortality (absence of acute toxicity) was often accompanied by a positive response to the reproductive deficiency (presence of chronic toxicity). In survival, the negative responses predominated at the sites sampled, while in reproduction the situation was the opposite, with frequent positive responses. In sediment, the most critical sites were Si008 with 54% of the samples presenting a low reproductive level, followed by Si056 with 42% of samples with these characteristics. The data on this compartment suggest a good recovery of river water. In surface waters Si008 and Si188 (June/2005) and Si048 presented cytotoxicity in both samplings analyzed. No positive responses were detected for micronuclei induction provoked by samples from the area studied, but signs of genotoxicity were observed at Si028 (May/05 and June/05) and Si008 (May/05). These results agree with the highest level of anthropic influence found at these sites.

Keywords: ecotoxicology, pollution, Cladocera, *Daphnia magna*, micronuclei.

Resumo: Testes biológicos são fundamentais para avaliar a qualidade dos compartimentos ambientais visando classificá-los quanto à capacidade de conservação da vida aquática e da saúde humana. O rio dos Sinos possui importância sócio-econômica e ambiental para o Rio Grande do Sul, por ser um dos formadores da Bacia Hidrográfica do Lago Guaíba, responsável pelo abastecimento público de água da capital. Foram monitorados quatro locais para a qualidade do sedimento (mar./01 a mar./05) através de ensaios (crônicos) com *Daphnia magna*. Em amostras de água superficial de cinco locais foram avaliadas a genotoxicidade pela análise de micronúcleos (MN) e a citotoxicidade na linhagem celular V79 (pulmão de hamster chinês), nos meses de maio/05 e jun/05, visando uma caracterização da área. Nos ensaios com *D. magna*, para análise estatística, utilizou-se o teste ANOVA e MDS (comparações múltiplas). A análise de variância mostrou interação entre mês e ponto amostral com diferenças significativas entre as médias de reprodução entre os pontos, dependendo do mês. Os dados mostraram que a resposta negativa para a mortalidade (ausência de toxicidade aguda) foi acompanhada muitas vezes por resposta positiva para a deficiência reprodutiva (presença de toxicidade crônica). Na sobrevivência predominaram as respostas negativas enquanto que na reprodução a situação foi inversa sendo as respostas positivas, freqüentes. Em sedimento, os pontos mais críticos foram Si08 com 54% das amostras apresentando baixo nível reprodutivo, seguido por Si056 com 42% de amostras com essa característica. Os dados desse compartimento sugerem sensível recuperação na qualidade do rio. Em águas superficiais, apresentaram citotoxicidade, Si008 e Si188 (june/05) e Si048 em ambas as amostragens. Não foram detectadas respostas positivas para indução de micronúcleos provocadas pelas amostras da área estudada, porém, observou-se indícios de genotoxicidade em Si028 (maio/05 e june/05) e Si008 (maio/05). Estes resultados são concordantes com uma maior influência antrópica verificada nestes locais.

Palavras-chave: ecotoxicologia, poluição, Cladocera, *Daphnia magna*, micronúcleo.

1. Introduction

The Sinos River is 200 km long from the source to the mouth. Along its course it is influenced by several sources of pollution, including sanitary and industrial sewage, garbage, oil refinery, sand dredging and navigation.

This is one of the rivers forming the Guaíba Lake, at Porto Alegre, the state capital of Rio Grande do Sul, Brazil. The lake is used as a primary contact recreation area for the population of the capital and surroundings, besides being the main source of water supply, after conventional treatment.

The Sinos River is classified from Class 1 at its source to Class 3 at the site furthest downstream. According to Brazilian law (Brasil, 2005), Class 1 consists of water for primary contact recreation, protection of aquatic communities and human consumption after simplified treatment. Class 2 are the rivers that provide water supply for human consumption after conventional treatment, and protection of the aquatic community and primary contact recreation, and Class 3 includes the rivers that are appropriate for domestic supply, after conventional or advanced treatment, irrigation, fishing, watering animals and secondary contact recreation.

The condition of having toxic compounds in the sediment that could be absorbed by living beings is bioavailability, since the adsorption of chemicals to the organic particles may result in riverbed deposits, which may return to the water column and thus enter in the trophic chain. Laboratory experiments detect adverse effects resulting from exposure to sediment according to time of exposure and feeding habits of the species. *Daphnia magna*, a cladoceran species was chosen based on the success achieved in the laboratory such as reproducibility of responses, its sensitivity to aquatic contaminants, and because this species has been widely used in many studies (Olmstead and LeBlanc, 2000, Terra et al., 2001; 2003; 2004; 2007; Terra and Feiden, 2003, Gillis et al., 2005), it is possible to compare the answers for different countries. Furthermore, the results are comparable over time because individuals with the same range of sensitivity were used.

The microcrustacean *D. magna* has shown good responses in sediment exposure tests (Nebecker et al., 1988; Suedel et al., 1996; Terra et al., 2004; 2006; Gillis et al., 2005). Because of these factors it is important to perform chronic tests to evaluate the real impact of samples on organism development specially when organisms such as the cladocera *D. magna.* According Suedel et al. (1996) *D. magna* with ≥48 hours old are used, which actively graze on sediment surfaces mobilizing these substances.

Observations on living beings are important because substances that are not detected by chemical analyses may interfere in the organisms, triggering biological responses.

Sediment, like water, is a spatially and temporarily variable source, but it has the capacity of accumulating persistent substances, and it has a higger probability of detecting a positive response. The two compartments, therefore, reflect different moments of environmental contamination.

A progressive system to evaluate aquatic environments is being adopted by FEPAM-Fundação Estadual de Proteção Ambiental, the environmental protection agency of the southernmost state of Brazil to obtain ecotoxicological information at different levels. This scheme requires the genotoxic characterization of the area to continue evaluating chronic toxicity. Based on Sinos river characteristics and the different anthropic pollution released in its water course, this study aims to propose an integrated approach to evaluate chronic and genotoxic effects. Biological tests are essential to evaluate the quality of the ecosystem for its capacity to maintain aquatic life. In this study the Sinos river sediment quality was evaluated by observing reproductive changes and survival of *D. magna* exposed to sediment samples from the selected sites and micronuclei induction by water samples in V79 cells.

2. Material and Methods

Between March 2001 and March 2005, samplings were performed at eight Sinos River sites in order to preserve the biota.

Four sites in the Sinos river were defined for sediment sampling and, according to water use, the most upstream site is Class 2 (Si056), while the others are class 3 (Si044, Si038 and Si008) (Figure 1).

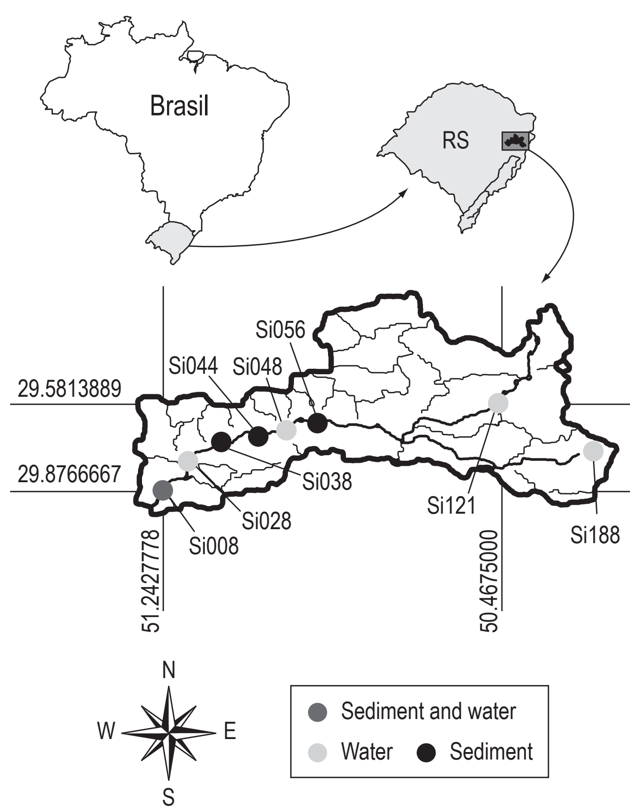

Figure 1. Location of the sampling sites at Sinos River Basin, Rio Grande do Sul, Brazil: Si008, Si028, Si038, Si044, Si048, Si056, Si121 and Si188.

Five sites in the Sinos River were defined to evaluate water quality. According to water uses, the first site is Class 1 (Si188), the next Class 2 (Si121) and the others Class 3 (Si048, Si028 and Si008). (Figure1).

In this study, sometimes only water samples from Si188, Si121, Si48, Si28, were evaluated or, in some periods, only sediment samples from Si056, Si044, Si038 and the two compartments, water and sediment samples from Si008, were performed. *D. magna* bioassays were used for sediment samples evaluation and V79 micronuclei induction for water genotoxicity analysis.

In this study, the sampling sites are denoted by the initials of the river name (Si) followed by the number of kilometers from the mouth.

Si188 (29° 43' 26" S and 50° 16' 46" W) – is 5 m wide, the reach is irregular, with gallery forests and a rocky bed with pebbles and cascades. No sources of pollution have been described;

Si121 (29° 34' 53" S and 50° 28' 03" W) – is 30 m wide, straight, with gallery forests and a rocky bed with pebbles. Sources of pollution are small farms and small pig and cattle breeding operations;

Si056 (29° 43' 50" S and 51° 05' 00" W) – is 50 m wide, with gallery forests and a sandy bed. It is used for the municipal water supply in Novo Hamburgo. Sources of pollution are garbage, sanitary and industrial sewage;

Si048 (29° 44' 21" S and 51° 07' 22" W) – is 50 m wide, with curves, gallery forests and a sandy bed. In this region sanitary and industrial sewage and garbage are discharged into the river. There are sand dredging and navigation activities. It is located at the mouth of the Luiz Rau stream, which is contaminated by tanneries;

Si044 (29° 45' 24" S and 51° 08' 16" W) – is 40 m long, with curves, gallery forests and a sandy bed. It is used as a water intake for the city of São Leopoldo. Sanitary and industrial sewage and garbage are discharged into it. There are sand dredging and navigation activities;

Si038 (29° 45' 50" S and 51° 10' 36" W) – is located downstream from the João Correa canal, 60 m wide, straight reach, with gallery forest and a sandy bed. Sources of pollution are sanitary and industrial sewage, garbage, navigation, paper recycling and sand dredging;

Si028 (29° 47' 53" S and 51° 11' 24" W) – is 60 m wide, straight, with grassed embankments, gallery forests, and a sandy bed. The sources of pollution are agricultural pesticides, tanneries, and sand dredging;

Si008 (29° 52' 36" S and 51° 14' 34" W) – is 100 m wide, straight, with grassed embankments and a sandy bed. Sources of pollution are sanitary and industrial sewage, garbage, refinery, sand mining and navigation.

2.1. Sediment (Chronic bioassays with Daphnia magna)

Sediment was collected at a depth of 1.20 m by a Petersen grab sampler, transported on ice to the laboratory, and stored in the dark at 4 °C until use (Burton, 1995; Ingersol et al., 1995; Terra et al., 2001).

All tests were performed with bulk sediment samples (Ankley, 1991; Burton, 1992; Schuytema et al., 1996; Cardozo et al., 2006), without sieving the sample, but removing the large organisms with pincers (Ingersoll et al., 1995), because the presence of indigenous organisms may greatly affect the chronic end point in sediment toxicity tests (Reynoldson et al., 1994).

The glassware was carefully washed to in order to use material that would be free of any organic or metallic toxic waste. For this neutral liquid soap, nitric acid p.a. diluted at 40% and acetone p.a. was used.

Daphnia magna (clone A) used in all our experiments were brought from the Institut für Wasserboden und Lufthygiene des Bundesgesundheitsamtes, Germany and have been successfully cultured under controlled laboratory conditions for more than 10 years. Cultures and tests were kept in separate germinators to minimize contamination.

The daphnids utilized in the assays were obtained through cultures maintained at a density of 25 adult individuals per 1000 mL in M4 medium (Elendt and Bias, 1990). These culture conditions kept the microcrustaceans in the parthenogenetic reproductive stage. All daphnids used in this study were from stock maintained for at least three generations and additionally were from the third or later brood.

Since these are long duration assays, before the exposure began the lots of animals were submitted to tests for sensitivity to $K_2Cr_2O_7$, and those that presented LC50-24 hours in 0.97 ± 0.24 mg$K_2Cr_2O_7$ were accepted, ensuring the use of lots with similar sensitivities. The Trimmed Spearman-Karber Method, Toxstat (Version 1.5) was used to calculate LC50-24 hours.

Adding ten newborn water fleas, each of them kept in its own glass beaker, made up the number of replicates for each sample at the beginning of experiments. The beakers were covered with laboratory film to prevent medium evaporation or contamination and the end of the bioassays, 960 microcrustaceans had been exposed to sediment samples, 240 per site. At the same time as these treatments, individuals exposed only to M4 were observed, thus constituting the assay control g roup.

Twenty-four 21-d semi-static tests were conducted randomly exposing the microcrustacean newborns, 2-26 hours old, to a Sinos River sediment sample, in a beaker, using M4 medium fresh (pH 7.8 and hardness of 230 mg $CaCO_3$/L), and sediment (v:v), at a proportion of 4:1, as already used successfully by other researchers (Burton, 1992; Suedel et al., 1996; Terra et al., 2001; Cardozo et al., 2006). All experiments were conducted in incubators at 20 ± 2 °C under cool white fluorescent light with a 16 hours light/8 hours dark photoperiod (Pieters and Liess, 2006; Smolders et al., 2005; Nebecker et al., 1988; Gersich and Mayes, 1986).

The cladocera were monitored on Mondays, Wednesdays and Fridays for mortality (total lack of movement by the animal) and reproductive status, at which time neonates were counted and removed. After this procedure, the culture medium was removed with a microcrustacean collector and M4 medium fresh was introduced through the inner wall of the beaker, avoiding breaking down the stability of the sediment surface layer. The daphnids were fed three times a week with a culture of green algae, *Scenedesmus subspicatus* at a concentration of 10^7cells-cm^{-3}, in a sufficient amount (1 mL) to guarantee food until the next handling.

For statistical analysis the ANOVA test with two factors was used and the response variable was the level of reproduction. In this analysis, the "month" and "site" factors were fixed and the beakers were the repetitions (n = 10). The SLICED option of the SAS software was used distributed by month. The assumption of homogeneity (constant variance) for the ANOVA test was not attained, then the data were weighted by the inverse of the variance of each group, where the group is formed by the combination of each point (4 points) with each month (24 months), totaling 96 groups, with ten repetitions each (beakers). The MDS (minimum difference significant) test of multiple comparisons was used. The level of significance used was 5% for all tests.

The level of change of the ecosystem was defined using the reproductive mean per brood (chronic), where the minimum number of 20 individuals per offspring was expected, and the percentage of survival (acute) when the expected value was to be equal to or higher than 80% (Terra et al., 2006). Complementing the analysis of these data, indexes were conferred ranging from zero to three in each parameter (reproduction and survival) (Terra et al., 2006). The lowest index identified the highest quality and zero indicates the ideal condition. The survival indexes were defined as follows: 0 = 8 to 10 survivors, 1 = 5 to 7 survivors, 2 = 2 to 4 survivors and 3 = 0 to 1 survivor. For reproduction, the indexes were based on the mean births per brood, according to the following schedule: 0 = ≥ 20 neonates, 1 = 15 to 19 neonates, 2 = 10 to 14 neonates and 3 = ≤ 9 neonates (Terra et al., 2007).

2.2. Water (Cytotoxicity and Micronucleus in V79 cells)

The cytotoxicity of water samples to V79 cells was determined by measuring the effect on cloning efficiency. Cultures containing 200 cells were incubated with 5 mL of minimum essential medium (MEM) for 24 hours at 37 °C and 5% of CO_2. River water samples (200 µL) were added for 3 and 24 hours before the cells being washed with PBS and incubated with fresh medium for 7 days. The colonies were fixed with methanol: acetic acid (3:1) and stained with violet crystal 0.1%. Any sample that presented a number of colonies equal to or less than 70% of the number of colonies in the negative control (sterile distilled water) was considered cytotoxic (Cardozo et al., 2006).

To analyze micronuclei (MN), V79 cells were inoculated in 25 cm^2 culture flasks at a density of 5×10^4/flask with 5 mL of MEM medium and 200 µL of samples, for 24 hours at 37 °C and 5% of CO_2. The negative control was sterile distilled water (200 µL). The positive control was bleomycin (Blenoxane from Bristol, 2 µL). The cultures were performed in duplicate and in parallel. At the end of the treatment, the cultures were washed twice with PBS and trypsinized. The cultures were centrifuged, treated with hypotonic solution (1% sodium citrate). The slides were stained with Giemsa and the MN analysis followed the criteria used by Countryman and Heddle (1976) and Fenech (1993). Two thousand cells were analyzed for each sample and controls. In order to perform statistical comparison of micronuclei frequency in V79 induced by samples and controls, the Z-test was used comparing two Poisson distributions. The responses are considered positive for micronuclei induction when it differs from controls by at least $P \leq 0.05$. The response obtained in the cultures exposed to the samples in which there was a double frequency of micronuclei as compared to the parallel negative control, without reaching statistical significance was considered a sign of genotoxicity.

3. Results

Figure 2 shows the percentage of survival observed per site, considering the 24 samplings performed. The sites where survival was less than expected (80%) are indicated below the interrupted line.

The sites Si008 and Si056 are those that caused most deaths among the cladocerans. The final period of sampling (September 2004 to March 2005) shows a lower number of deaths, characterizing improved river quality.

The most critical situations according the survival indexes were at the sampling sites: Si056, in the months of February 2002 (90% deaths by the 12th day of exposure), April 2002 (100% deaths by the 3rd day of exposure), November 2003 (100% deaths by the 5th day of exposure), February 2004 (40% deaths by the 9th day of exposure). For Si008, survival was critical in april 2002 (50% deaths by the 7th day of exposure), March 2003 (50% deaths by the 7th day of exposure), September 2003 (70% deaths by the 8th day of exposure), November 2003 (100% deaths by the 2nd day of exposure), April 2004 (90% deaths by the 9th day of exposure) June 2004 (90% deaths by the 16th day of exposure) and August 2004 (100% deaths by the 5th day of exposure). At Si044 the only critical moment for survival occurred in March 2001 (90% deaths by the 8th day of exposure). At Si038 the critical moment occurred in June 2003 (100% deaths by the 4th day of exposure). At Si008, 37.5% of the samples presented mortality above 20%, while Si056 presented 21% and the other sites, 4% each.

Table 1 show the percentage of survival and reproduction indexes found, per site sampled, during the period from March 2001 to March 2005. Zero index, i.e., absence of mortality was a frequent observation.

Figure 3 shows the mean of the number of births per offspring at each time in sampling. The interrupted line delimits the mean number of births (20) per offspring appropriate to maintain the population balance (Cowgill et al.,

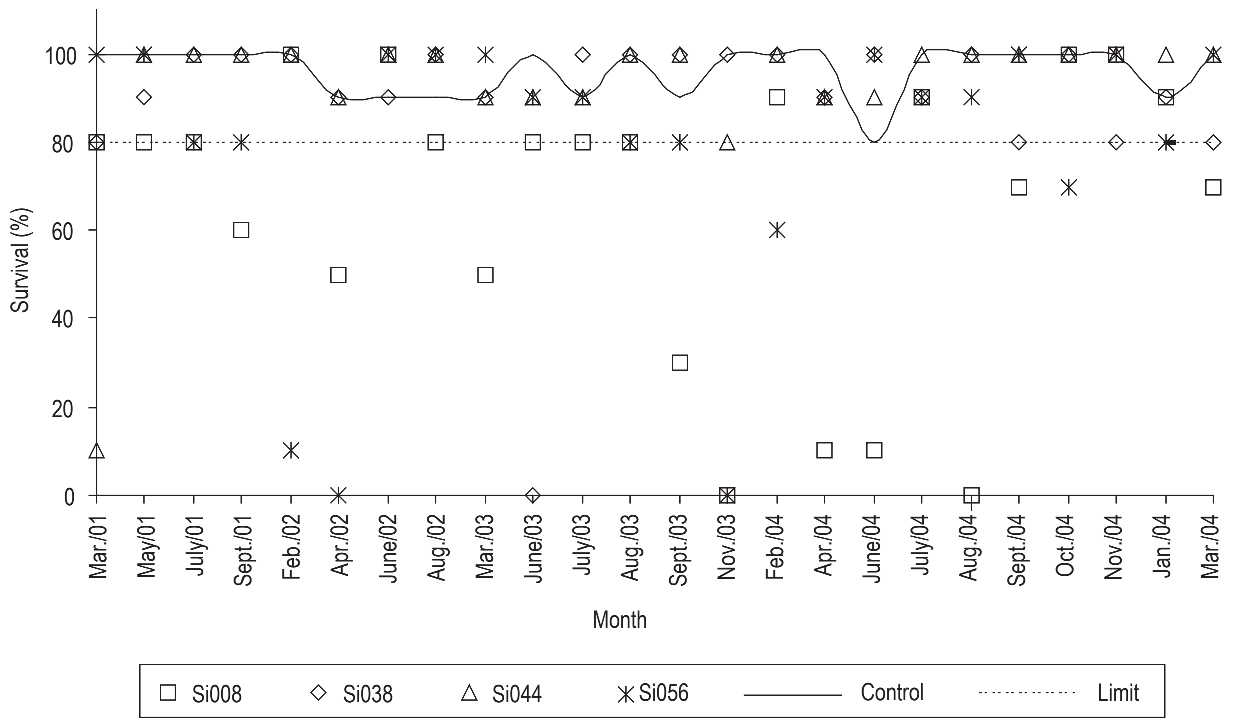

Figure 2. Survival percentage of *Daphnia magna* exposed to Sinos river sediment samples, control group and expected survival from Mar./01 to Mar./05.

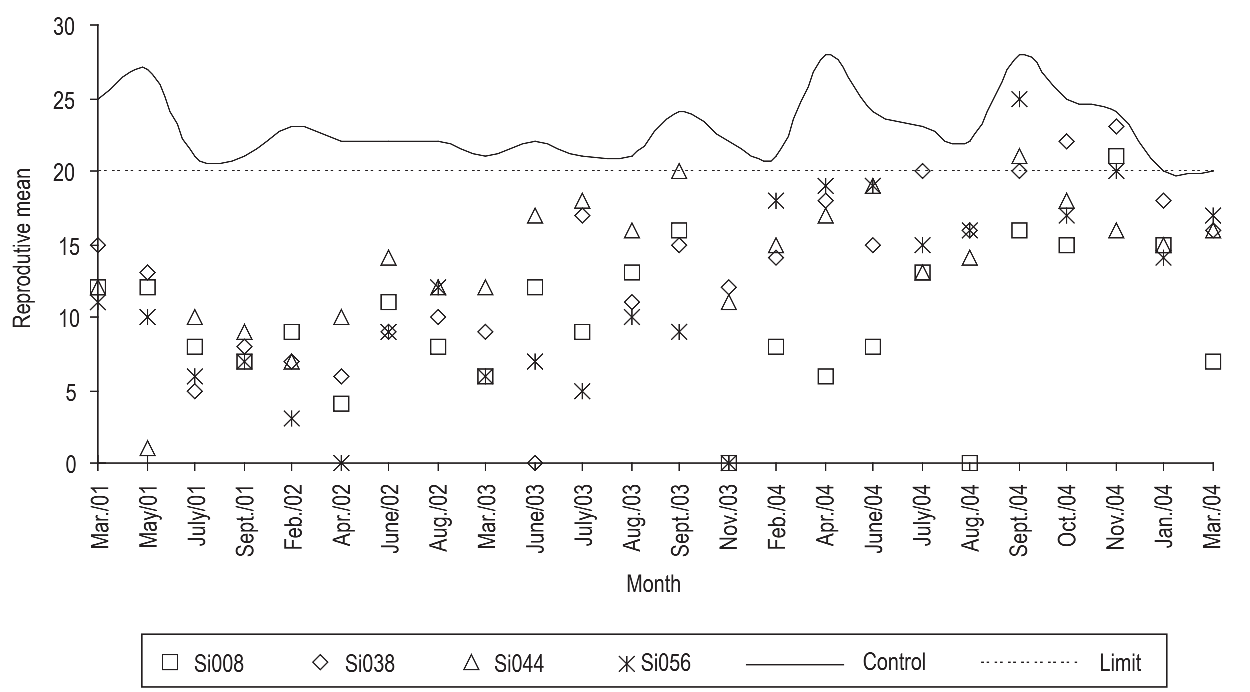

Figure 3. Reproduction mean of *Daphnia magna* exposed to Sinos river sediment samples, control group and expected mean, from Mar./01 to Mar./05.

Table 1. Percentage of *Daphnia magna* survival and reproduction indexes per site sampled from Mar./01 to Mar./05.

Indexes	Survival				Reproduction			
	0	1	2	3	0	1	2	3
Si008	62	21	4	12	4	17	25	54
Si038	96	0	0	4	12	29	29	29
Si044	96	0	0	4	8	37	42	12
Si056	75	12	0	12	8	25	25	42

Survival indexes: 0 = 8 to 10 survivors; 1 = 5 to 7 survivors; 2 = 2 to 4 survivors; 3 = 0 to 1 survivor
Reproduction indexes: 0 = ≥20 neonates; 1 = 15 to 19 neonates; 2 = 10 to 14 neonates; 3 = ≤9 neonates

Table 2. Total number, means and standard deviations of the *Daphnia magna* births per sampling site, from Mar./01 to Mar./05.

Sites	Number of neonates	Mean	SD
Si008	9520	397	260
Si038	14964	623	265
Si044	16188	674	223
Si056	12427	518	321
Control	26577	1107	133

1985), favoring environmental homeostasis. According Figure 3 a slow, but progressive increase in the mean of the number of births per offspring, indicate an improvement of the environmental quality in the reach studied, beginning in July 2003, except for Si008 and Si056. The same pattern is followed by the reproduction indexes (Table 1), although the reproduction indexes shows a bigger number of critical situations, since usually this parameter responds to lower and cumulative doses of pollutants.

Figura 4 shows that the total sum of reproduction indexes is higher than that of survival. Analysis of reproductive activity indicates that preservation of the species was often threatened.

Table 2 presents the absolute number of individuals generated, as well the corresponding mean and standard deviation, including the control group values, thus enabling the comparison of these data.

Considering the total number of neonates, we observe the diminished births at Si008, which lies closest to the mouth, while at Si044 the greatest number of births occurred. The standard deviation that occurred between the births is normal among cladocerans, but we observe that the deviation is smaller in the control, supporting the idea that environmental pollution interferes in reproduction (Table 2).

Table 3 supply multiple comparisons using the MDS (minimum difference significant) test. Analysis of variance showed that the main factors (month and site) and the interaction between them, presented significant differences between the reproduction means ($p < 0.001$). In other words, the differences between the reproduction means observed at each site depend on the month in which they were observed. Analyzing interaction between factors (site and month), distributed by month, the months with $p < 0.05$ presented significant differences in the reproduction means at each point (Table 3).

Figure 5 shows the cytotoxic analysis using the assay of plating efficiency in V79 cells exposed to surface water samples collected at five study sites along the Sinos river. A

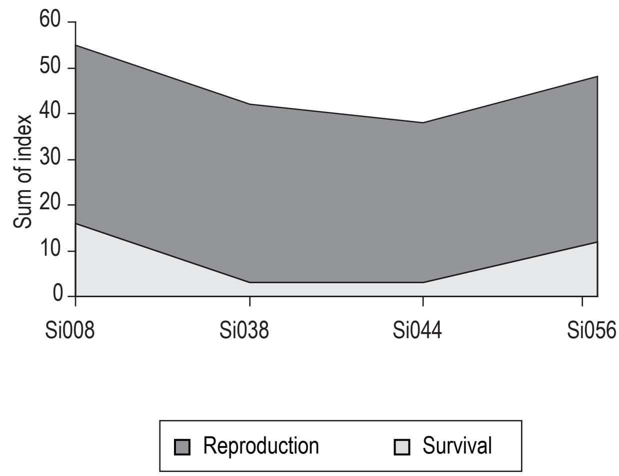

Figure 4. Sum of reproduction and survival indexes in *Daphnia magna*, per site sampled from Mar./01 to Mar./05.

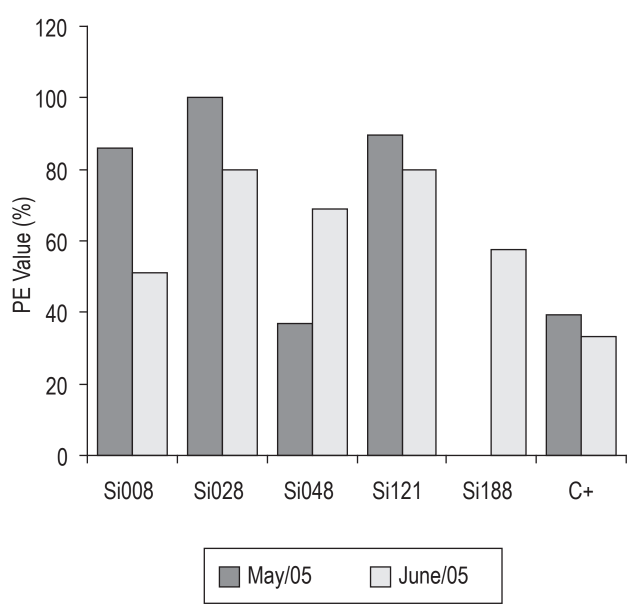

Figure 5. Cytotoxicity of Sinos river samples evaluated in V79 cultures by plating efficiency method. C+ is positive control, Bleomycin.

Table 3. Comparisons of the means of births (N = 10) at the sites sampled from Mar./01 to Mar./05. Means followed by the same letter are not significantly different according to the DMS test.

Mar./01		June/02		Sept./03		Aug./04	
Site	Mean	Site	Mean	Site	Mean	Site	Mean
Si038	69.7^{a}	Si044	64.9^{a}	Si044	100.5^{a}	Si056	82.8^{a}
Si044	57.9^{a}	Si008	52.8^{a}	Si038	74.1^{b}	Si038	80.5^{a}
Si056	57.7^{a}	Si056	45.7^{a}	Si056	37.8^{c}	Si044	68.5^{b}
Si008	52.0^{a}	Si038	43.4^{a}	Si008	23.9^{c}	Si008	0$^{\text{non-est}}$
May/01		Aug./02		Nov./03		Sept./04	
Site	Mean	Site	Mean	Site	Mean	Site	Mean
Si038	53.8^{a}	Si044	61.4^{a}	Si038	68.6^{a}	Si056	123.9^{a}
Si056	49.8^{a}	Si056	59.4^{a}	Si044	48.6^{b}	Si044	103.6$^{a,\ b}$
Si008	47.3^{a}	Si038	50.3$^{a,\ b}$	Si008	0$^{\text{non-est}}$	Si038	85.0$^{b,\ c}$
Si044	4.8^{b}	Si008	36.3^{b}	Si056	0$^{\text{non-est}}$	Si008	74.5^{c}
July/01		Mar./03		Feb./04		Oct./04	
Site	Mean	Site	Mean	Site	Mean	Site	Mean
Si044	47.2^{a}	Si044	56.4^{a}	Si044	81.6^{a}	Si038	111.7^{a}
Si056	25.8^{a}	Si038	42.4^{b}	Si038	68.0^{b}	Si044	91.2$^{a\ b}$
Si008	25.4^{a}	Si056	30.5^{c}	Si056	54.6$^{b,\ c}$	Si008	75.1^{b}
Si038	23.4^{a}	Si008	14.5^{d}	Si008	37.8^{c}	Si056	61^{b}
Sept./01		June/03		Apr./04		Nov./04	
Site	Mean	Site	Mean	Site	Mean	Site	Mean
Si038	41.7^{a}	Si044	75.6^{a}	Si056	83.6^{a}	Si008	103.0^{a}
Si044	39.3^{a}	Si008	52.0^{b}	Si038	83.1^{a}	Si038	101.3^{a}
Si056	33.0^{a}	Si056	29.2^{c}	Si044	76.0^{a}	Si056	98.7^{a}
Si008	25.4^{a}	Si038	0$^{\text{non-est}}$	Si008	2.8^{b}	Si044	80.3^{a}
Feb./02		July/03		June/04		Jan./05	
Site	Mean	Site	Mean	Site	Mean	Site	Mean
Si008	44.6^{a}	Si044	86.2^{a}	Si056	93.9^{a}	Si038	80.5^{a}
Si044	39.6^{a,b}	Si038	83.6^{a}	Si044	85.4^{a}	Si044	75.2^{a}
Si038	34.8^{b}	Si008	39.6^{b}	Si038	77.6^{a}	Si008	65.6^{a}
Si056	3.2^{c}	Si056	21.1^{c}	Si008	18.7^{b}	Si056	60.6^{a}
Apr./02		Aug./03		July/04		Mar./05	
Site	Mean	Site	Mean	Site	Mean	Site	Mean
Si044	52.2^{a}	Si044	79.6^{a}	Si038	88.5^{a}	Si056	82.9^{a}
Si038	27.0^{b}	Si038	53.5^{b}	Si056	67.3^{a}	Si044	79.3^{a}
Si008	11.0^{b}	Si008	53.1^{b}	Si044	64.2^{a}	Si038	65.5^{a}
Si056	0$^{\text{non-est}}$	Si056	40.2^{b}	Si008	63^{a}	Si008	24.8^{b}

cytotoxic effect was detected by samples from sites Si008, Si188 (June/05) and Si048 (May/05 and June/05). The results of the genotoxic analysis of the area, evaluated in V79 cells, can be seen in Figure 6. No statistically significant response was found for MN induction but there were signs of genotoxicity at Si008 (May/05 and June/05).

The evaluation of metals in Sinos River revealed that, in some sites and years, water presented concentrations above the permitted values of Brazilian water quality law as for exemple: Si056 (for Cd, Fe, Pb and Zn), Si044 (for Cu), in 2001; Si056 (for Cu, Fe), Si044 (for Fe), Si038 (for Cu) in 2002; Si056 (for Cu, Fe), Si044 (for Cu), Si038 (for Cu) in 2003; Si056 (for Cu, Fe), Si044 (for Cu, Fe), Si038 (for Cu) in 2004 and Si056 (for Fe), Si044 (for Fe) in 2005.

4. Discussion

Previous analyses of the area, between the months of May/98 and Oct./02, the same methodology was applied to this river at sites close to those currently evaluated (Terra et al., 2003), and it was observed that the critical moments for survival occurred during the dry season (summer). In the present study Si008 presented critical results in various seasons, probabily due to the anthropic influence characteristic of this site. Site Si056, located upstream in

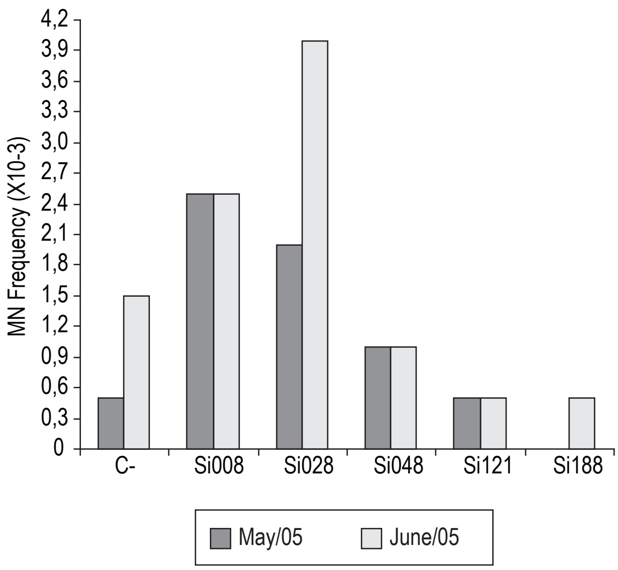

Figure 6. Frequencies of micronuclei observed in V79 cultures exposed to water samples from different sites in the Sinos river on two sampling occasions. C- is negative control, distilled water.

the river, also presented critical data, mostly in February 2002, April 2002, November 2003 and February 2004. This site receives sources of pollution like garbage, sanitary and industrial sewage;

The same study mentions that Si028 and Si036 (sites between Si008 and Si038) presented only 7% of offspring within the expected amount. Si008 presented critical results for aquatic life survival mostly in Apr./02, Mar./03, Sept./03, Nov./03, Apr./04, Jun./04 and Aug./04, probably due to its location close to the river mouth, receiving all contaminants released upstream, besides the local influence of pontual garbage, sanitary and industrial contribution. When sites Si028 and Si036 were evaluated for a genotoxic response (Ames Test), this was positive in both (Vargas et al., 2001).

Since the total sum of reproduction indexes is higher than that of survival (Figure 4), this shows that preservation of the species was often threatened. Figure 4 highligths the differences of biological action between survival and reproduction. The graphic representation shows that the reproduction area is bigger than the survival one.

The effect of sediments on the test-organisms demonstrates that persistent substances deposited may be reused by the trophic system, damaging one or more links in the food chain due to the mortality of the organisms, or else due to the reproductive reduction observed at many times. This process may trigger a lack of agreement or break in the ecosystem balance.

The presence of a few metals throughout the samplings in different months is observed in water samples. Although usually some values are below the standards allowed by Brazilian law (Brasil, 2005), they have been recorded because they are cumulatively polluting metals. They may influence the organisms when they are constant, even at low concentrations. These substances can be adsorbed to the sediment and released into the environment by biological and chemical actions or by the effect of weathering on organisms. At Si056, we often observe values above that allowed by law. This occurs because this site belongs to Class 2 (more noble use), and not because metal peaks occur, and the values at this site are similar to those found at the others.

Cytotoxic analysis performed in V79 cultures showed cytotoxicity induced by samples from sites Si008 and Si188 (June 2005) and Si048 (in both samplings analyzed). In the present study, the results of survival of *D. magna* exposed in sediment samples from Si008 indicate this site as critical in relation to quality with acute toxicity effects. Previous studies on toxicity in this river (Terra et al., 2003) showed that Si048 samples had critical effects for the survival of *D. magna*, with acute toxicity during the summer months. In the same study, sediment samples from site Si188 were toxic in 57% of the analyses, and Si028 presented the best survival indexes among the others, 79% of the responses being satisfactory for this parameter. In the present study Si008 showed bad results in 37% of the samples while in Si056 it was 21%. These data are consistent with the toxicity results obtained in V79, showing that the cytotoxic characteristics remained similar at these sites, although the period of this study was only autumn and winter.

As to genotoxic evaluation, the signs of a positive response, found at Si028 and Si008 are compatible with the strong anthropic influence found at these places. Sites Si188 and Si121, upstream from these areas, presented negative responses in the assays with V79 performed in this study.

In a genotoxicity study performed in this river, using the *Salmonella*/microsome assay with water samples, the authors found negative responses for the presence of frameshift and base-pair substitution mutagens (Vargas et al., 2001). However, in the same evaluation using a microscreen phage induction assay performed with *E. coli*, the sites located near Si008, Si028 and Si188 presented positive responses in some samplings. The latter data agree with the results obtained in this paper only for Si008, Si028, since few samplings of these sites have produced signs of a positive response for the induction of micronuclei in V79. This response is compatible with the events of chromosome loss or break provoked by substances with direct effect on the DNA, since the tests were performed without metabolic activation. These effects may be produced by compounds that are characteristic of areas around cities with complex mixtures of pollutants (such as PAHs and metals) that can be found in the study area, especially at sites Si008 and Si028 which are under the influence of public and industrial waste disposal and have proximity to agricultural areas. The influence of tanneries on sites Si028 and Si008 could be the source of chromium compounds like Cr VI, a known clastogen that can induce MN in various in vivo and in vitro test systems (De Flora et al., 1990; Ueno et al., 1995; Lemos et al., 2001). Another kind of influence is that of metallurgy which can include zinc, iron and chromium in its effluents, the last two known inductors of genotoxicity (Godet et al., 1996).

A longer study should be performed to improve the diagnosis of this area for the genotoxic effects detected by preliminary analysis using micronuclei in the pulmonary cells of hamsters (V79) and the DNA damage shown by this biomarker. This evaluation is important in water quality diagnoses in areas potentially contaminated by genotoxic agents, Since cytogenetic damage evaluation is a valuable step in the tier of different approaches used in genetic toxicology (Brusick et al., 1992), it is important diversify and broaden the levels of genotoxic response obtained by other assays (Cardozo et al., 2006).

The ecotoxicological evaluation of Sinos river samples showed improvement of the river quality between Km 8 and 188 in the period from Mar./01 to Mar./05.

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References

ANKLEY, GT. Predicting the toxicity of bulk sediments to aquatic organisms with aqueous test fractions: pore water vs. elutriate. *Environmental Toxicology and Chemistry,* 1991, vol. 10, no. 10, p. 1359-1366.

BRASIL. CONAMA - Conselho Nacional do Meio Ambiente. Resolução n0 357 [online]. Diário Oficial da União de 17 de abril de 2005. Available from: < http://www.mma.gov.br>.

BRUSICK, DJ., ASHBY, J., DE SERRES, FJ., LOHMAN, PHM., MATSUSHIMA, T., MATTER, BE., MENDELSOHN, ML., MOORE, DH., NESNOW, S. and WATERS, MD. A method for combining and comparing short-term genotoxicity test data: Preface. *Mutation Research,* 1992, vol. 266, no. 1, p. 1-6.

BURTON JR, GA. Critical issues in sediment bioassays and toxicity testing journal of aquatic ecosystem health. *Journal of Aquatic Ecosystem Health* 1995, vol. 4, no. 3, p. 151-156.

BURTON JR., GA. *Sediment quality assessment.* In Proceedings of the Conference on Assessment and Treatment of Contaminated Sediments in the North Branch Chicago River, Oct. 19-20, 1992. Boca Raton, Florida: Lewis Publishers, 1992. p. 167-182.

CARDOZO, TR., ROSA, DP., FEIDEN, IR., ROCHA, JAV., OLIVEIRA, NCA., PEREIRA, TSP., PASTORIZA, TF., MARQUES, DM., LEMOS, CT., TERRA, N.R. and VARGAS, VMF. Genotoxicity and toxicity assessment in urban hydrographic basins. *Mutation Research,* 2006, vol. 603, no. 1, p. 83-96.

COUNTRYMAN, PI. and HEDDLE, JA. The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. *Mutation Research,* 1976, vol. 41, no. 2-3, p. 321-332.

COWGILL, UM., HOPKINS, DL., APPLEGATH, SL., TAKAHASHI, IT., BROOKS, SD. and MILAZZO, DP. *Brood size and neonate weight of Daphnia magna produced by nine diets*. 1985. [Authorized reprint from special technical publication 89s copyright].

DE FLORA, S., BAGNASCO, M., SERRA, D. and ZANACCHI, P. Genotoxicity of chromium compounds. *Mutation Research,* 1990, vol. 238, no. 2, p. 99-172.

ELENDT, BP. and BIAS, WR. Trace nutrient deficiency in *Daphnia magna* cultured in standard medium for toxicity testing. Effects of the optimization of culture conditions on the life history parameters of *D. magna*. *Wat. Res*., 1990, vol. 24, no. 9, p. 1157-1167.

FENECH, M. The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations. *Mutation Research,* 1993, vol. 285, no. 1, p. 35-44.

GERSICH, FM. and MAYES, MA. Acute toxicity tests with *Daphnia magna* straus and *Pimephales promelas* Rafinesque in support of National Pollutant Discharge Elimination Permit Requirements. *Wat. Res*., 1986, vol. 20, no. 7, p. 939-941.

GILLIS, PL., CHOW-FRASER, P., RANVILLE, JF., ROSS, PE. and WOOD, CM. Daphnia need to be gut-cleared too: the effect of exposure to and ingestion of metal-contaminated sediment on the gut-clearance patters of *D. magna*. *Aquatic Toxicology*, 2005, vol. 71, no. 2, p. 143-154.

GODET, F., BABUT, M., BURNEL, D., VEBER, A. and VASSEUR, P. The genotoxicity of iron and chromium in electroplating effluents. *Mutation Research,* 1996, vol. 370, no. 1, p. 19-28.

INGERSOLL, CG., ANKLEY, GT., BENOIT, DA., BRUNSON, EL., BURTON, GA., DWYER, FJ., HOKE, RA., LANDRUN, PF., NORBER-KING, TJ. and NINGER, PV. Toxicity and bioaccumulation of sediment-associated contaminants using freshwater invertebrates: a review of methods and applications. *Environmental. Toxicology and Chemistry*, 1995, vol. 14, no. 11, p. 1885-1894.

LEMOS, CT., RÖDEL, PM., TERRA, NR. and ERDTMANN, B. Evaluations of basal micronucleus frequency and tetravalent chromium effects in fish erythrocytes. *Environmental Toxicoogy Chemistry* ,2001, vol. 20, no. 6, p. 1320-1324.

NEBEKER, AV., ONJUKKA, ST. and CAIRNS, MA. Chronic effects of contaminated sediment on *Daphnia magna* and *Chrinomus tentans*. Bulletin of environmental contamination and toxicology, 1988, vol. 41, no. 4-6, p. 574-581.

OLMSTEAD, AW. and LE BLANC, GA. 2000. Effects of endocrine-active chemicals on the development of sex characteristics of *Daphnia magna*. *Environmental Toxicology Chemistry,* 2000, vol. 19, no. 8, p. 2107-2113.

PIETERS, BJ. and LIESS, M. Material nutricional state determines the sensitivity on *Daphnia magna* offspring to short-term exposure. *Aquatic Toxicology*, 2006, vol. 76, no. 3-4, p. 268-277.

REYNOLDSON, TB, DAY, KE., CLARKE, C. and MILANI, D. Effect of indigenous animals on chronic end points in freshwater sediment toxicity tests. *Environmental Toxicology Chemistry,* 1994, vol. 13, no. 6, p. 973-977.

SCHUYTEMA, GS., NEBEKER, AV. and CAIRNS, MA. Comparison of recirculation static and elutriate aquatic sediment bioassay. *Bulletim of Environmental Contamination and Toxicology,* 1996, vol. 56, no. 5, p. 742-749.

SMOLDERS, R., BAILLIEUL, M. and BLUST, R. Relationship between the energy status os *Daphnia magna* and its sensitivity to environmental stress. *Aquatic Toxicology*, 2005, vol. 73, no. 2, p. 155-170.

SUEDEL, BC., DEAVER, E. and ROGERS JR., JH. Experimental factors that may affect toxicity of aqueous and sediment-bound copper to freshwater organisms. *Archives of Environmental Contamination Toxicology,* 1996, vol. 30, no. 1, p. 40-46.

TERRA, NR. and FEIDEN, IR. Reproduction and survival of *Daphnia magna* Straus, 1820 (Crustacea: Cladocera) under different hardness conditions. *Acta Limnologica Brasiliensia,* 2003, vol. 15, no. 2, p. 51-55.

TERRA NR., FEIDEN IR., MOREIRA JS. and NUNES EA. Reproductive inhibition in *Daphnia magna* Straus, 1820, exposed to sediment samples of an area under impact from the petrochemical industry. *Acta Limnologica Brasiliensia,* 2006, vol. 18, no. 3, p. 229-237.

TERRA, NR., FEIDEN, IR. and CORREA, LM. *Ação tóxica do sedimento do rio dos Sinos na sobrevivência e na reprodução de Daphnia magna Straus, 1820 (Crustacea)*. In Congresso de Ecologia do Brasil, 6., Fortaleza, 2003. Anais de Trabalhos Completos. Fortaleza: Universidade Federal do Ceará. p. 397-399.

TERRA, NR., FEIDEN, IR. and NUNES, EA. *Efeito do sedimento do rio Gravataí na reprodução e na sobrevivência de Daphnia magna, 1820, Straus (Crustacea: Cladocera).* Simpósio Internacional de Qualidade Ambiental, 4, 2004. Tema 4, trabalho 436.

TERRA, NR., FEIDEN, IR., FACHEL, JM., MOREIRA, JS. and LEMKE, C. Chronic assays with *Daphnia magna*, 1820, Straus in sediment samples from Caí River, Rio Grande do Sul Brazil. *Acta Limnologica Brasiliensia*, 2007, vol.19, no.1, p. 31-39.

TERRA, NR., LEMOS, CT., FEIDEN, IR. and CORRÊA, LM. *Ensaios biológicos na avaliação da qualidade ambiental.* In: Tucci, CEM., Marques, DMLM. (eds). Avaliação e controle da drenagem urbana. Porto Alegre, Rio Grande do Sul: ABRH, 2001. p. 87-95.

UENO, S., SUGIYAMA, M., SUSA, N. and FURUKAWA, Y. Effect of dimethylthiourea on chromium (VI)-induced DNA single-strand breaks in Chinese hamster V-79 cells. *Mutation Research,* 1995, vol. 346, no. 4, p. 247-253.

VARGAS, VMF., MIGLIAVACA, SB., MELO, AC., HORN, RC., GUIDOBONO, RR., FERREIRA, ICF. and PESTANA, MHD. Genotoxicity assessment in aquatic environments under the influence of heavy metals and organic contaminants. *Mutation Research,* 2001, vol. 490, no. 2, p. 141-158.

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