Chronic assays with Daphnia magna, 1820, Straus in sediment samples from Caí River, Rio Grande do Sul, Brazil.

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ABSTRACT: Chronic assays with Daphnia magna, 1820, Straus in sediment samples from Caí River, Rio Grande do Sul, Brazil. Caí river which is of major socioeconomic and environmental importance in Rio Grande do Sul has been monitored by FEPAM, regarding for antropic impact on the sediment of the middle and upper thirds of its course, since 2001. The purpose of this study was to assess the influence of the sediment from four sites along the Caí River on the health of the epibenthic cladoceran Daphnia magna, by performing semi-static chronic bioassays. Sediment was used because this compartment has the capacity to retain toxic substances that may be available later due to the organism metabolism or to the action of environmental physical agents. In the bioassays, microcrustaceans were exposed to environmental stress situations for 21 days from the beginning of their life (2-26h). The response with no mortality was often accompanied by a response showing reproductive deficiency. ANOVA (Analysis of Variance) and MSD multiple comparison tests were used to analyze the data. The percentage of survivors and the mean number of births per brood were analyzed. Indexes were defined to compare the parameters considered (reproduction and survival). Reproduction proved to be more sensitive than mortality, indicating lack of acute toxicity and the presence of chronic toxicity. Recovery of quality is observed is this river reach considering the reproductive data obtained during the study.
Key words: Cladoceran, bioassay, ecotoxicology, survival, reproduction.

RESUMO: Ensaios crônicos com Daphnia magna, 1920, Straus em amostras de sedimento do rio Caí, Rio Grande do Sul, Brasil. O rio Caí devido a sua importância sócio-econômica e ambiental tem sido monitorado pela FEPAM considerando os impactos que vem sofrendo no sedimento dos terços médio e superior de seu curso, desde 2001. A proposta deste estudo foi a de avaliar a influência do sedimento de quatro locais do rio Caí sobre a saúde do cladócero epibêntico Daphnia magna, por meio da realização de bioensaios crônicos, semi-estáticos. Utilizou-se sedimento, pois este compartimento tem a capacidade de reter substâncias tóxicas, as quais podem ser disponibilizadas posteriormente devido ao metabolismo dos organismos ou à ação de agentes físicos ambientais. Os bioensaios expuseram microcrustáceos a situações de estresse ambiental por 21 dias desde o início da vida (2-26 h) dos dafnideos. Muitas vezes a resposta negativa para a mortalidade foi acompanhada por resposta positiva para a deficiência reprodutiva. Para análise dos dados, utilizou-se ANOVA (Análise de Variância) e MDS (Diferença Mínima Significativa) com comparações múltiplas. Também foram analisadas a porcentagem de sobreviventes e a média de nascimentos por ninhada. Foram definidos índices para a comparação dos dois parâmetros considerados (reprodução e sobrevivência). A reprodução mostrou-se mais sensível que a mortalidade, indicando ausência de toxicidade aguda e presença de toxicidade crônica. Considerando os dados reprodutivos, observa-se no decorrer do estudo recuperação na qualidade deste trecho do rio.
Palavras chave: Cladoceros, bioensaios, ecotoxicologia, sobrevivência, reprodução.

Introduction

Water bodies are constantly disturbed by man, who exploits them indiscriminately or excavates bottom sediment in search of food This has modified ecosystems, triggering changes in environmental quality and in the capacity of rivers to auto-depurate and recover their equilibrium. Sediments are the ultimate repository of recalcitrant contaminants, such as organochlorines, polycyclic aromatic hydrocarbons (PAHs) and metals that enter the aquatic ecosystem. Thus, there has been great interest in developing methods and models to predict the effects of sediment-associated contaminants on benthic communities (Ankley et al., 1996). Sediments have proved to be one of the best tools to obtain biological answers on environmental quality, due to the accumulation of metals and adsorption of organic products to particulate matter. Sediment retains toxic substances that can adhere to their components and later become available to aquatic organisms through runoff, metabolic activity or the effects of storms. These factors cause the re-suspension of upper sediment layers into the water column because of currents, after heavy rainfall or intense navigation. These products can thus be made available to the trophic chain through organism ingestion and metabolization, altering their health.

Cladocerans are microcrustaceans which are among the primary consumers in the trophic chain; Daphnia magna ^{3} 48h old, actively graze on sediment surfaces (Suedel et al., 1996) releasing sedimented substances, and they are therefore an important instrument to evaluate sediment quality.

The Fundação Estadual de Proteção Ambiental of the state of Rio Grande do Sul(RS), Brazil, began ecotoxicological studies in several areas of the state, specifically in the water bodies that form the Guaiba lake watershed, due to anthropic actions that can trigger changes in the natural development of biocenoses, leading to loss of environmental homeostasis. This lake bathes Porto Alegre, the capital of Rio Grande do Sul, and is used as a source of public water supply after conventional treatment, and as a primary recreation area.

Chronic toxicity evaluation studies, based on environmental samples, are important to identify sites that have been even only slightly altered, so that measures can be proposed to recover environments that suffer negative interferences from anthropic activity.

Biological tests are essential to evaluate the quality of the ecosystem for its capacity to maintain aquatic life. In this study the Caí river sediment quality was evaluated by observing reproductive changes and survival of D. magna exposed to sediment samples from the selected sites. This 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 (Burton-Jr., 1992a; Burton-Jr. et al., 1995; Olmstead & LeBlanc, 2000; Terra et al., 2001; Terra et al., 2003; Terra & Feiden, 2003; Terra et al., 2004; Burton-Jr. et al., 2005; 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. Daphnia magna have been considered the most sensitive animal for sediment assays, especially in the presence of metals (Nebecker et al., 1984).

Chronic assays show alterations and give positive responses to apparently innocuous variables when living beings are exposed to them for a short time, which are not detected by chemical methods due to low concentration. Biological tests are therefore essential to make decisions on environmental quality. Data indicate that amphipods in direct contact with contaminated sediment were severely affected, compared with amphipods exposed only to overlying water (Ingersoll et al., 2000), and it is therefore important to perform bioassays using epibenthic organisms.

The purpose of this study was to assess the influence on reproduction and survival of D. magna when exposed to the Caí River sediment.

Material and methods

Between Mar/01 and Dec/04, 14 sediment samplings were performed at four Caí river sites in order to preserve the biota, considering Class 2 according to CONAMA Resolution No.357 (Brasil, 2005). The sites were located 70 (S29°37'48,6" W51°22'45,6"), 92 (S29°30'18,8" W51°21'36,4"), 136 (S29°19'31" W51°10'50") and 245 (S29°21'46,5" W50°31'16,8") kilometers from the mouth, and therefore they are called CA070CD000 (extension CD000, indicates the Cadeia River mouth), CA092, CA136 and CA245 (Fig.1). These are official FEPAM monitoring network sites. The four sites will be referred to as A, B, C and D, from upstream to downstream. A is site CA 245; B is CA 136; C is CA092 and D is CA70CD000.

Sediment was collected by a grab sampler (Petersen), transported on ice to the laboratory, and stored in the dark at 4°C until it was used (Burton-Jr., 1995; Ingersoll et al., 1995; Terra et al., 2001). Organism exposure began at most one month after collection.

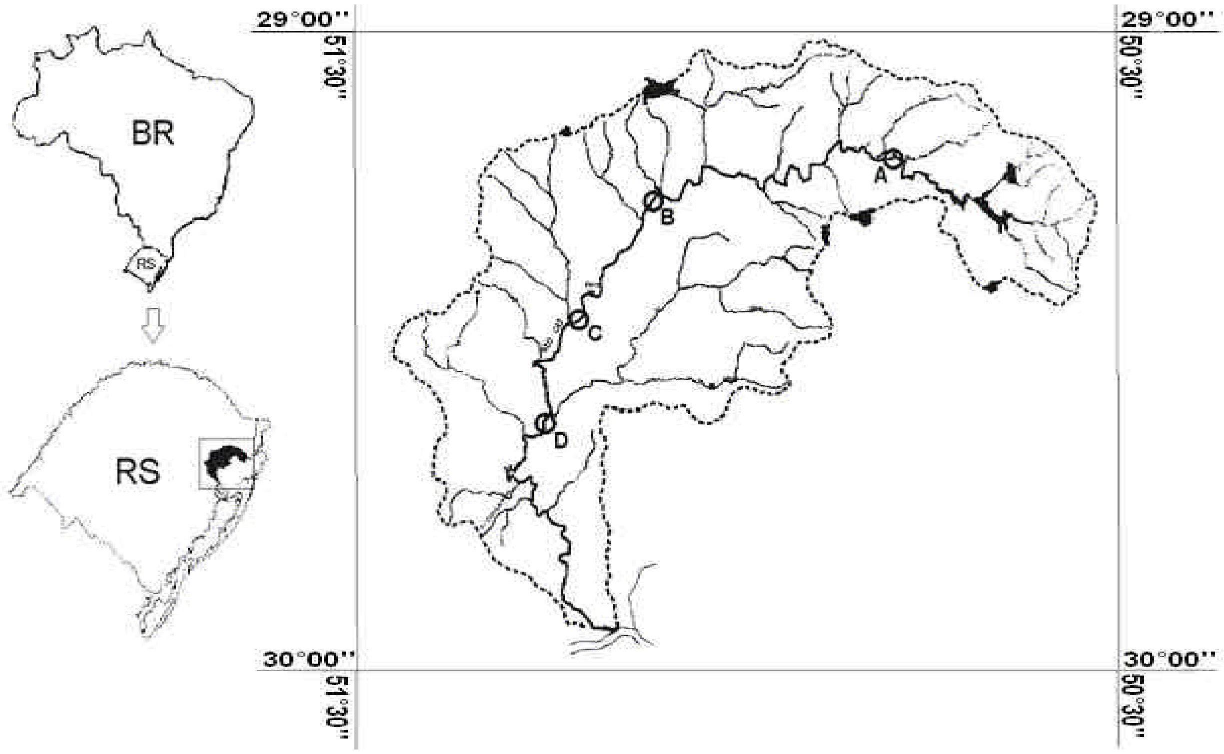

Figure 1: Location of the study site in Brazil, Rio Grande do Sul state, Cai River basin, Sites A, B, C and D.

The glassware was carefully washed in order to eliminate any organic toxic or metal residue. Therefore, neutral liquid soap, nitric acid p.a. diluted at 50% and acetone p.a. were used.

A total of 560 D. magna juveniles were exposed to sediment samples.

D.magna (clone A) used in all experiments in this study were brought from the Institut für Wasserboden und Lufthygiene des Bundesgesundheitsamtes, Germany, and have been successfully cultured under controlled laboratory conditions for over 10 years. Cultures and tests were kept in separate incubator chambers to minimize contamination.

The daphnids used in the assays were obtained from cultures kept at a density of 25 adult individuals per 1000mL in M4 medium (Eledent & Bias, 1990). These culture conditions maintained the microcrustaceans in the parthenogenetic reproductive stage. All daphnids in this study were from the third or later brood. Before exposure to sediment, the organisms were submitted to tests for sensitivity to potassium dichromate, and lots with LC50-24h of 0.97 ± 0.24mg$K_2Cr_2O_7$ were used. The Trimmed Spearman-Karber Method (Toxstat version 1.5) was applied to calculate LC50-24h.

We started experiments adding ten newborn water fleas, each maintained in its own glass beaker, comprising the number of replicates for each sample. The beakers were covered with ParafilmMâ to prevent medium evaporation or contamination.

Fourteen 21-d semi-static tests were conducted, randomly exposing D. magna newborns, 2-26 hours old, to control and to Caí sediment samples, in a beaker with a nominal value of 50 mL, using 30mL of fresh M4 medium, at pH 7.8, and with a total hardness of 230mg$CaCO_3$/L, for 10mL of bulk sediment, a proportion used successfully in other experiments (Burton-Jr., 1992b; Suedel et al., 1996; Terra et al., 2001). All experiments were conducted in incubators at 21±1°C under cool white fluorescent light with a 16h light / 8h dark photoperiod.

The cladocerans 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 cladoceran and the culture medium were removed with a microcrustacean collector (an object similar to a 25-ml volumetric pipette, cut at both ends, and fresh M4 medium was introduced through the inner wall of the beaker, preventing a breakdown of sediment stability. After introducing recently cultured medium, D.magna was placed in the same beaker again. The daphnids were fed three times a week with a culture of green algae, Scenedesmus subspicatus, at a concentration of 10^7 cells/cm^3, a sufficient amount (1mL) to ensure feeding until the next handling.

An ANOVA test with two factors was used for statistical analysis, to detect significant differences between the river points observed every month for the reproduction variable. In this analysis, "month" and "site" factors were fixed and the "beakers" were the repetitions (n=10). The SLICED option of SAS software was used to present the ANOVA solution for the interaction of factors distributed by month. To confirm that the last five months showed an improvement in the reproduction index we also evaluated the sliced option of month distributed by site Since the data are very variable, the assumption of homogeneity (constant variance) was achieved for the ANOVA test, using the transformation of the data that consists in multiplying the data 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 (14 months), totalizing 56 groups, with ten repetitions (beakers) each. The multiple comparison test MDS (minimum difference significant) was also used. The level of significance was 5% for all tests.

A complementary evaluation of the data defined the level of ecosystem alteration (acute or chronic), using the reproductive mean per brood (chronic), where a minimum of 20 individuals expected per brood, and the percentage of survival (acute), where the expected value was to be equal to or higher than 80.

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 = ^{3}20 neonates, 1 = 15 to 19 neonates, 2 = 10 to 14 neonates and 3 = £9 neonates.

Water samples were collected for verification of the presence of total heavy metals (Acid Digestion of Samples and Determination by FlameAtomic Absorption Spectrophotometry) and coliforms (Multiple Tubes and Chromogenic Substrate Test). Dissolved oxygen (DO) and pH assessments were obtained from direct readings performed with appropriate equipment, Oximeter and Potentiometer, respectively. Samples for heavy metal dosage were kept in glass flasks with a nominal capacity for 1000 mL, containing 5mL of nitric acid, while the samples for microbiological analyses were collected in a borosilicate flask with a wide mouth and a 100 mL capacity.

Sediment samplings were accompanied by analyses of biological, physical, chemical and metal parameters. It should be highlighted that, whereas these analyses were performed in water, the cladocerans were exposed to sediment samples which different from water are a cumulative compartment, and it is possible that the content of metals and other persistent chemicals is higher there than those detected in the water samples.

The microbiological analyses were performed with the Multiple Tubes Technique until Sep/02 and with the Chromogenic Substrate test beginning in Jun/03.

Results

Survival was affected at Site D, during the months of Dec/01, Apr/03 and Sep/04, when the percentage of survivors was respectively 70%, 10% and 60%; at Site C in Sep/04 when there were 60% of survivors; at Site A in Mar/02 when there were 50% and in Abr/03 and Dec/04, when there were 40% survivors. It was found an average of eight surviving individuals (d±2.4) at Site D, nine (d±1.1) at Site C, nine (d±0.6) at Site B and eight (d±2.0) at Site A.

Survival indexes were high at Site D (Dec/01, Apr/03 and Sep/04), Site C (Sep/04) and Site A (Mar/02, Apr/03 and Dec/04).

Concerning 56 responses referring to mean births, a satisfactory result was observed only in Jun/04, in Site B. Site A generally presented the lowest reproduction means, whereas Site B presented the highest means (Fig.2). Figure 2 also shows that the river improved in the last few months, except for Site A in the last month. The final number of neonates indicated Site A as the site that produced the smallest number of neonates and Site B, the largest. Table I presents the absolute value of individuals generated among all observations, the mean and standard deviation.

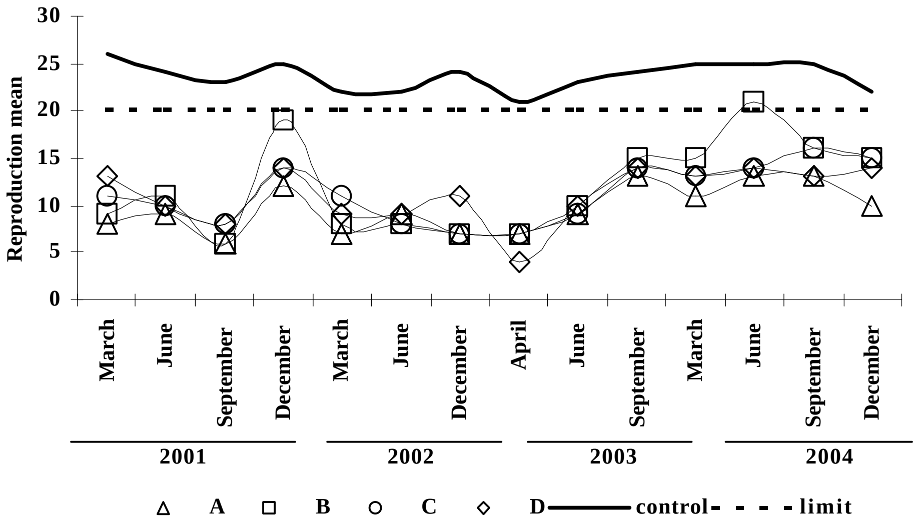

Figure 2: Behavior of the cladoceran reproduction means observed in tests using sediment for each sample site (A, B, C and D).

Table I: Total number of neonates, mean and standard deviation of births per site sampled.

Site	Neonates	mean	SD
A	5496	393	±143
B	8088	578	±242
C	7653	547	±139
D	6844	489	±163

Figure 3 shows the indexes obtained for reproduction at each sampling moment and the monthly sum total of these indexes, with the evolution of sediment quality in the river over time.

Figure 4 shows that the sums of the survival and reproduction indexes are different from each other, and the survival indexes are lower than the reproduction ones. Analysis of variance showed that the main factors (month and site) and their interaction present significant differences between reproduction means ($p<0.001$). In other words, the differences between the reproduction means observed at each point depend on the month when they were observed.

When the interaction between factors (site and month) was analyzed, distributed by months, as can be observed in Tab. II, the months with $p < 0.05$ (in boldface) presented significant differences in the reproduction means at each point.

Table III presents multiple comparisons using the MDS (minimum difference significant) test, where the means with the same letter do not differ significantly to a 5% level of significance.

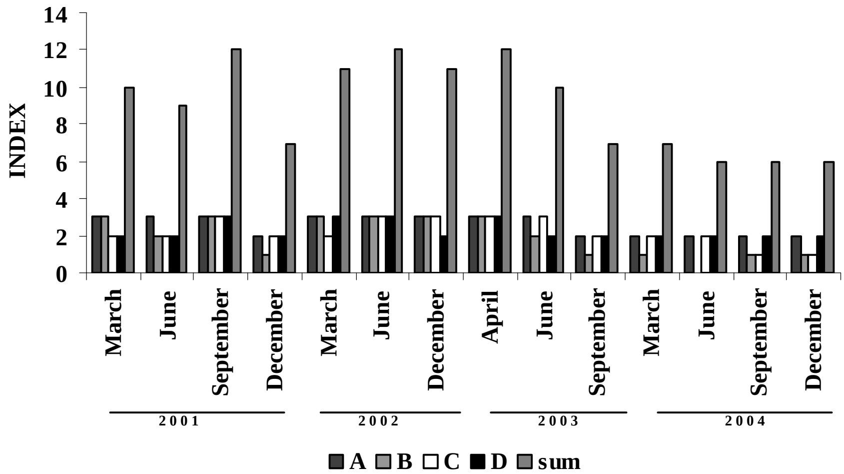

Figure 3: Indexes for reproduction at each sampling moment and monthly sum of these indexes demonstrating the evolution of sediment quality in Cai River at the sample sites (A, B, C and D).

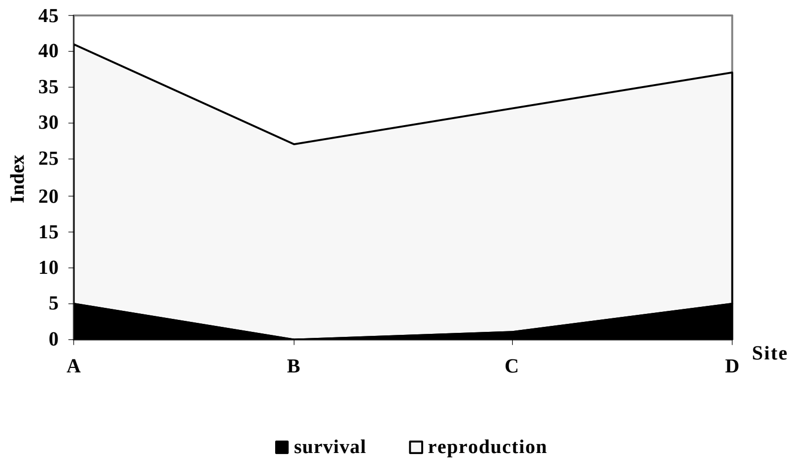

Figure 4: Sum of the survival and reproduction indexes, per sample site (A, B, C and D). Survival indexes are lower than the reproduction.

Table II: Site × month interaction effect, sliced by month for reproduction.

site*month Effect Sliced by month for reproduction					
month	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**P > F**
1	3	20,771	6,924	6.92	**0.0001**
2	3	1,090	0,363	0.36	0.7794
3	3	24,879	8,293	8.29	**<.0001**
4	3	40,463	13,488	13.49	**<.0001**
5	3	43,536	14,512	14.51	**<.0001**
6	3	6,745	2,248	2.25	0.0819
7	3	7,320	2,440	2.44	0.0636
8	3	217,303	72,434	72.43	**<.0001**
9	3	0,634	0,211	0.21	0.8883
10	3	5,995	1,998	2.00	0.1133
11	3	8,221	2,740	2.74	**0.0428**
12	3	66,079	22,026	22.03	**<.0001**
13	3	9,125	3,042	3.04	**0.0286**
14	3	23,176	7,725	7.73	**<.0001**

Table III: Multiple comparisons of the total reproduction means using MDS test. Means with the same letter do not differ significantly.

month 1 — Mar/01

Site	N	Mean	comparisons		
D	10	64	a		
C	10	51	a	b	
B	10	44		b	c
A	10	36,3			c

month 2 — Jun/01

Site	N	Mean	comparisons		
B	10	50,2	a		
D	10	44,7	a		
C	10	44,5	a		
A	10	43,2	a		

month 3 — Sep/01

Site	N	Mean	comparisons		
C	10	40,1	a		
D	10	39,2	a		
B	10	30		b	
A	10	25,7		b	

month 4 — Dec/01

Site	N	Mean	comparisons		
B	10	95,3	a		
C	10	72		b	
A	10	56,1		b	
D	10	54,1		b	

month 5 — Mar/02

Site	N	Mean	comparisons		
C	10	55,3	a		
D	10	46,5	a		
B	10	33,7		b	
A	10	18,3			c

month 6 — Jun/02

Site	N	Mean	comparisons		
A	10	47,7	a		
C	10	42,3	a		
B	10	41,1	a		
D	10	40,9	a		

month 7 — Dec/02

Site	N	Mean	comparisons		
D	10	51,5	a		
C	10	35,1	a		
B	10	33,5	a		
A	10	30,4	a		

month 8 — Apr/03

Site	N	Mean	comparisons		
C	10	37,5	a		
B	10	37,2	a		
A	10	17,8		b	
D	10	1,8			c

month 9 — Jun/03

Site	N	Mean	comparisons		
D	10	48	a		
C	10	46,4	a		
B	10	46,4	a		
A	10	41,8	a		

month 10 — Sep/03

Site	N	Mean	comparisons		
C	10	71,1	a		
B	10	70	a		
D	10	56,5	a		
A	10	50,3	a		

month 11 — Mar/04

Site	N	Mean	comparisons		
B	10	72,8	a		
C	10	66,8	a	b	
D	10	60,2	a	b	
A	10	51,5		b	

month 12 — Jun/04

Site	N	Mean	comparisons		
B	10	104,6	a		
C	10	71,4		b	
D	10	59,2		b	
A	10	58		b	

month 13 — Sep/04

Site	N	Mean	comparisons		
B	10	80,3	a		
C	10	61,5	a	b	
A	10	51,9	a	b	
D	10	46,6		b	

month 14 — Dec/04

Site	N	Mean	comparisons		
D	10	71,2	a		
B	10	69,7	a		
C	10	69,3	a		
A	10	20,6		b	

In Tab. IV, pH values higher than the levels allowed by CONAMA Resolution no. 357 (Brasil, 2005) were obtained only in 2001 and 2003. The DO was below the value allowed by the same Resolution in 2001 and 2002, whereas the microbiological analyses were altered in most of the observations. The same Tab. IV shows that total heavy metals with values above the levels permitted were concentrated in 2001. Only Cu was present in 2002 and 2004. Despite a survey on the presence of mercury, this metal was not detected in any of the samplings.

Table IV: Presence of heavy metals, bacteria, pH and DO, outside the standards allowed by CONAMA, from 2001 to 2004.

	2001				**2002**				**2003**				**2004**			
Site Parameter	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
pH		Mar									Jun	Jun				
DO		Dec	Dec	Dec		Jun	Mar	Mar								
Bacteria			Mar Jun	Mar to Dec		Sep	Mar Sep	Jan to Sep	Sep	Dec	Dec	Dec			Sep	Jun Sep Dec
Cadmium (mg/L)	Mar Jun	Mar	Jun	Mar												
Lead (mg/L)	Jun	Jun	Jun	Mar Jun												
Copper (mg/L)			Jun	Mar Jun				Mar							Sep	Sep

Discussion

The toxicity levels along the course of the Caí River vary because of the different types of stress to which it is submitted, the water volume or bed width. The river bed gradually becomes wider downstream. Close to the mouth (Site D) Caí River is 100 m wide, and close to the source (A), it is only 20 m wide. At the sites between the two, the width is 30 m (Site B) and 60 m (site C). The riverbed is rocky, with bends at sites D and A, and straight at the others.

The evaluations performed during this period sometimes showed an acute effect of the samples on the microcrustaceans. The more intense effects were observed at Site D in Dec/01, Apr/03 and Sep/04, when survival was 70%, 10% and 60% respectively. Site D is located at the confluence of Cadeia and Caí rivers, the former under heavy impact from the tanneries, which is probably the reason for the high mortality of microcrustaceans exposed to the samples. Although mortality was high at this site, especially in Apr/03, the final mean number of survivors, considering all moments evaluated, was at least 80% at all sites, indicating that the high mortality is due to isolated events. Considering all observations site by site, there is visible homogeneity in the mean number of survivors. Site D and Site A presented the same mean number of survivors (eight), with a variation of ±2.4 individuals at the first site and ±2.0 at the second. On the other hand, at Sites C and B, the mean was nine individuals with a variation of ±1.1 and ±0.6, respectively. This variation may occur due to the different levels of stress caused by the influence of the sites sampled.

Considering the survival indexes, site D presented the highest value, probably because of the source of pollution to which it is exposed. This was expected, since Site D is located close to the Cadeia mouth, and receives the impact of this river polluted by tannery discharges.

Reproduction is more sensitive to environmental aggressions than the survival/mortality relation, and it should be evaluated. Often negative responses for mortality are accompanied by positive responses for reproductive deficiency, and this requires that data obtained from the reproductive activity in chronic bioassays be further explored statistically. Among the 56 responses responses referring to the mean number of births, a satisfactory result was observed on only one occasion (Site B-Jun/04) (Fig. 2), identifying the presence of chronic toxicity at different levels in the other observations. The evaluated sites suffered different impacts. While at Site D contamination occurs mainly due to chromium-rich tannery effluents, at Site C the most persistent source is metallurgical activity; at Site B the contribution is from viticulture or industrial wastes and at Site A livestock is the main source of pollution. The reproductive data show a decline in the number of neonates at the sites nearer the mouth, except for Site A, which, although located at the source presents the smallest number of neonates (Tab. I). We have observed that the sites located at sources of other rivers in the state do not present the expected reproductive yield either. This may be due to the characteristics of the rocks where the rivers begin, and a more detailed study of these sites is required, which has not yet been performed. Table I also shows the high variability in the number of neonates, which is common among cladocerans. The reproduction indexes obtained show a decrease in these values from Jun/03 onwards, suggesting that the river quality recovered (Fig. 3), although at Site A there was an elevation during Dec/04, possibly due to the dry season that usually occurs in the south of the country during this season (summer). The comparison of reproduction and survival indicates that the former is more highly affected, since it presents higher indexes, confirming the absence of acute toxicity and showing the presence of chronic toxicity in the reach studied (Fig. 4).

The reproduction means presented a very different behavior for the factors observed. Of the 14 months analyzed, nine showed differences in the reproduction means among the sediment collection sites (Tabs. II and III). Site A, which is near the river mouth, presented the lowest reproduction means, because the soil is rocky and the cladocerans do not reproduce well in this type of formation, as seen in a previous study (unpublished data). Site D showed the least variability (except for Apr/03), with an intermediate level in the reproduction means. The sites with the best yields were Site B and Site C, with an advantage for the former. Except for site A, close to the source, with rocky soil, we observe a decreasing gradient in the reproduction means towards the mouth. According to Tab IV, which shows the environmental quality indicators, we systematically find the worst situations at the sites closest to the mouth.

An improvement in the reproduction means of the sites can be noted in the last few in recent months. This finding is in accordance with the information concerning the physical, chemical, microbiological and heavy metal parameters obtained in this area (Tab. IV). In 2001, the year in which it presented low reproductive means, except for the month of December, the values of physical, chemical, microbiological and heavy metal parameters are concentrated outside the standards allowed by CONAMA. While the metals appear only a single time in 2002 (Site D) and in 2004 (Sites C and D), in 2003 they are absent and in 2001 they are present on 13 occasions.

A significant improvement in the reproduction means of the sites can be noted in the last five months, according to the multiple comparisons test.

Transferring the results obtained by exposure of D. magna to this river, we observed that the sites sampled presented slightly inadequate conditions for the development of cladocerans, although it has been found that the river quality is improving.

Studies performed with biological assays, especially those using chronic damage suffered by organisms after long periods of exposure as a tool for identification, are becoming increasingly important to study and recover freshwater quality. Early diagnosed damage can be reversed with less effort and at a lower economic and social cost. It is appropriate to reinforce the idea that water resources have been deteriorating rapidly, and that places with good quality water are becoming rare due to the misuse of these resources by the human population. Due to lack of foresight in using and controlling water quality, this natural asset, formerly abundant, may become rare for all living beings and extremely costly for man. The recovery observed in the reach studied is a sign that joint work performed by the environmental protection agency of Rio

Grande do Sul with the business community who have been working seriously to treat the effluents of industries established in the state, is already beginning to show positive results for ecosystem quality.

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References

Ankley, G.T., Liber, K., Call, D.J., Markee, T.P., Canfield, T.J. & Ingersoll, C.G. 1996. A field investigation of the relationship between zinc and acid volatile sulfide concentrations in freshwater sediments. J. Aquat. Ecosyst. Health, 5:255-264.

Brasil. CONAMA. 2005. Resolução CONAMA, n° 357. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes e de outras providências. Diário Oficial da União, Brasília, 17 de março de 2005.

Burton Jr., G.A. 1992a. Sediment quality assessment. In: Proceedings of the Conference on Assessment and Treatment of Contaminated Sediments in the North Branch Chicago River. Chicago, p.1- 9.

Burton Jr., G.A. 1992b. Plankton, macrophyte, fish and amphibian toxicity testing of freshwater sediments. In: Burton Jr., G.A. (ed.) Sediment toxicity assessment. Lewis Publishers, Boca Raton. p.167-182.

Burton Jr., G.A. 1995. Critical issues in sediment bioassays and toxicity testing. J. Aquat. Ecosyst. Health, 4:151-156.

Burton Jr., G.A., Greenberg, M.S., Rowland, C.D., Irvine, C.A., Lavoic, D.R., Brooker, J.A., Moore, L., Raymer, D.F.N. & McWilliam, R.A. 2005. In situ exposures using caged organisms: a multi-compartment approach to detect aquatic toxicity and bioaccumulation. Environ. Pollut.,134:133-144.

Eledent, B.P. & Bias, W.R. 1990. Trace nutrient deficiency in Daphnia magna cultured in standard medium for toxicity testing. Effects of the optimization of culture conditions on life history parameters of D. magna. Water Res., 24:1157-1167.

Gillis, P.L., Chow-Fraser, P., Ranville, J.F., Ross, P.E. & Wood, C.M. 2005. 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. Aquat. Toxicol., 71:143-154.

Ingersoll, C.G., Ankley, G.T., Benoit, D.A., Brunson, E.L., Burton, G.A., Dwyer, F.J., Hoke, R.A., Landrun, P.F., Norber-King, T.J. & Ninger, P.V. 1995. Toxicity and bioaccumulation of sediment-associated contaminants using freshwater invertebrates: a review of methods and applications. Environ. Toxicol. Chem., 14:1885-1894.

Ingersoll, C.G., Ivey, C.D., Brunson, E.L., Hardesty, D.K. & Kemble, N. 2000. Evolution of toxicity: whole-sediment versus overlying-water exposures with amphipod Hyalella azteca. Environ. Toxicol. Chem., 19:2906-2910.

Lemos, C.T. & Terra, N.R. 2003. Poluição: causas, efeitos e controle. In: Silva, J., Erdtmann, B. & Henriques, J.A.P. (eds.) Genética toxicológica. Alcance, Porto Alegre. p.119-138.

Nebeker, A.V., Cairns, M.A., Gakstatter, J.H., Malves, K.W., Schuytema, G.S. & Kranczyk, D.F. 1984. Biological methods for determining toxicity of contaminated freshwater sediments to invertebrates. Environ. Toxicol. Chem., 3:617-630.

Olmstead, A.W. & Le Blanc, G.A. 2000. Effects of endocrine-active chemicals on the development of sex characteristics of Daphnia magna. Environ. Toxicol. Chem., 19:2107-2113.

Suedel, B.C., Deaver, E. & Rodgers, J.H.J. 1996. Experimental factors that may affect toxicity of aqueous and sediment-bound copper to freshwater organisms. Arch. Environ. Contam. Toxicol., 30:40-46.

Terra, N.R. & Feiden, I.R. 2003. Reproduction and survival of Daphnia magna Straus, 1820 (Crustacea: Cladocera) under different hardness conditions. Acta Limnol. Bras., 15:51-55.

Terra, N.R., Feiden, I.R. & Correa, L.M. 2003. Ação tóxica do sedimento do rio dos Sinos na sobrevivência e na reprodução de Daphnia magna Straus, 1820 (Crustacea). Ecossistemas aquáticos, costeiros e continentais. Anais do VI Congresso de Ecologia do Brasil. Fortaleza, p.397-399.

Terra, N.R., Feiden, I.R. & Nunes, E.A. 2004. Efeito do sedimento do rio Gravataí na reprodução e na sobrevivência de Daphnia magna, 1820, Straus (Crustacea: Cladocera). Anais do IV Simpósio Internacional de Qualidade Ambiental. Porto Alegre, p.436-443.

Terra, N.R., Lemos, C.T., Feiden, I.R. & Correa, L.M. 2001. Ensaios biológicos na avaliação da qualidade ambiental. In: Tucci, C.E.M., Marques, D.M.L.M. (eds.) Avaliação e controle da drenagem urbana. ABRH, Porto Alegre. p.87-95.

Terra, N.R., Feiden, I.R., Moreira, J.S. & Nunes, E.A. 2006. Reproductive inhibition in Daphnia magna Straus, 1820, exposed to sediment samples of an area under impact from the petrochemical industry. Acta Limnol. Bras., 18:229-237.

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