Ecotoxicological evaluation of sediment from a river contaminated by industrial effluents, Sinos River (Rio Grande do Sul, Brazil) using *Daphnia magna* (Straus, 1820)

Avaliação ecotoxicológica do sedimento de um rio contaminado por efluentes industriais, Rio dos Sinos (Rio Grande do Sul, Brasil) usando *Daphnia magna* (Straus, 1820)

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Abstract: Aim: The Sinos River has been systematically studied because it is one of the tributaries of the Guaíba Lake Hydrographic Basin which bathes Porto Alegre, the capital of Rio Grande do Sul. The study aims at following river quality development and evaluating changes in the development of *Daphnia magna* (Straus, 1820), supplying information to plan the recovery of this water resource; **Methods:** During two and a half years (May/05 to October/07), sediment was monitored at six sites using *D. magna* in toxicity test. The ecotoxicological assays were performed for 21 days under controlled laboratory conditions. Percentage of survival and mean reproduction were recorded and data were submitted to statistical analyses; **Results:** Analysis of the results showed significant variations ($\alpha < 0.05$) in the mean number of births, and Si38 and Si56 were the sites that varied most. Si08, located close to the mouth had the worst mean reproduction rates, with only a small variation of the results. The results varied more during the dry months, since sewage settles in areas close to the discharge point; **Conclusions:** Although survival and reproduction are not directly related parameters, they are both important, since they indicate different pollution processes. The impact on micro-crustacea reproduction shows continuous chronic action. The study showed increasing degradation of the Sinos River from the source to the mouth, compatible with anthropic action.

Keywords: *Daphnia magna*, microcrustacean, ecotoxicology, pollution, sediment.

Resumo: Objetivo: o rio dos Sinos vem sendo estudado sistematicamente por ser um dos afluentes da Bacia Hidrográfica do Lago Guaíba que banha Porto Alegre, capital do Rio Grande do Sul. O objetivo deste estudo é acompanhar a evolução da qualidade deste rio e avaliar as alterações no desenvolvimento de *Daphnia magna* (Straus, 1820), fornecendo informações para o planejamento e recuperação deste recurso hídrico; **Métodos:** Durante dois anos e meio (maio/05 a outubro/07) o sedimento de seis locais deste rio foi monitorado utilizando *D. magna* em testes de toxicidade. Os ensaios ecotoxicológicos foram realizados por 21 dias em condições controladas de laboratório. Foram registradas a porcentagem de sobrevivência e a média reprodutiva; após os dados foram submetidos à análise estatística; **Resultados:** A análise dos resultados mostrou variações significativas ($\alpha < 0,05$) na média de nascimentos, sendo Si38 e Si56 os sites que mais variaram. Si08, localizado próximo à foz apresentou as piores médias reprodutivas com pequena variação dos resultados. Nos meses de estiagem, a variação dos resultados foi maior, pois os despejos sedimentam em áreas mais próximas ao lançamento; **Conclusões:** Embora a sobrevivência e a reprodução não sejam parâmetros diretamente relacionados, ambos são importantes, pois indicam processos poluentes diferenciados. Ficou evidenciada a ação crônica constante, através do impacto observado na reprodução dos microcrustáceos. O estudo mostrou degradação crescente do rio dos Sinos no sentido da nascente para a foz, compatível com a ação antrópica.

Palavras-chave: *Daphnia magna*, microcrustáceos, ecotoxicologia, poluição, sedimento.

1. Introduction

The Sinos River has been monitored since the 1990s (Vargas et al., 2001; Terra et al., 2003, 2008) because of its importance to the state of Rio Grande do Sul, and the impact of environmental stressors, including sanitary and industrial sewage, garbage, oil refinery, sand dredging and navigation. It is one of the five rivers that form the Guaíba Lake, and is used as a contact recreation area for the population of the state capital and surroundings, besides being the main source of water supply after conventional treatment.

The present study evaluates six river sites, one Class 2, located further upstream and five, Class 3. According to Brazilian legislation (Brasil, 2005) Class 2 waters are used for domestic supply after conventional treatment, primary contact recreation, and protection of the aquatic communities. Class 3 waters are for domestic supply, after conventional or advanced treatment, irrigation, fishing, watering animals and secondary contact recreation.

The responses in this study are based on cumulative effects after exposing *Daphnia magna* (Straus, 1820) to sediment, considering the time and species feeding habits. Sediment can retain heavy metals and other toxic substances that may be released due to the variation of currents or local biological activities, and it is a major source of water pollution.

D. magna has shown good responses to sediment exposure tests (Nebeker et al., 1988; Gillis et al., 2005; Terra et al., 2008), since individuals of this species, aged over 48 hours, graze the sediment surface mobilizing substances that have already been deposited (Suedel et al., 1996).

Changes in the reproduction and survival of these cladocerans were used as an assessment endpoint to characterize the area of study. The species was defined by reproducibility and sensitivity of the response to the agents. The tool applied to evaluate variation in the test-organisms response was statistical analysis for the purpose of comparing the responses of each point during the time sampled. The study aims to follow the evolution of the Sinos River quality, looking at changes in micro-crustacea development. This will supply information for water resource planning and recovery.

2. Material and Methods

Between May/05 and October/07, 14 sediment samples were collected at six sites in the Sinos River. These samples were evaluated by exposing 140 *D. magna* neonates (Clone A) per site, in the semi-static, chronic assay mode, with a total of 840 organisms exposed. This cladoceran was monitored for changes in reproduction and percentage of survival.

The sampling sites are named using the river name initials (Si) followed by the number of kilometers from the mouth (Figure 1).

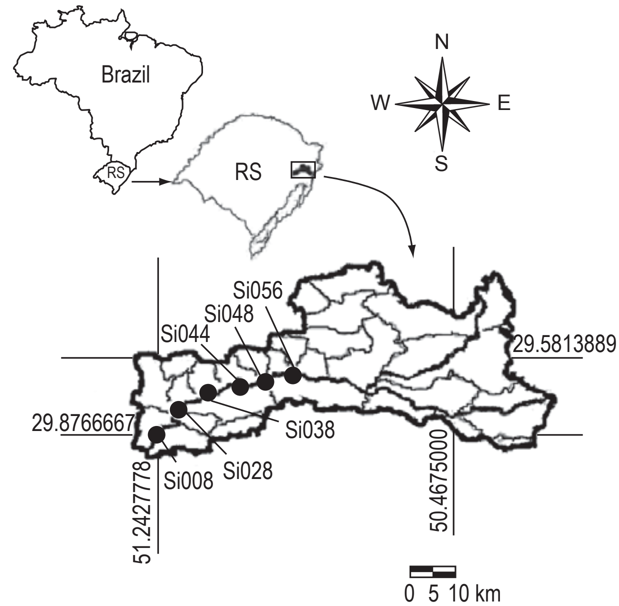

Figure 1. Location of the study site in Rio Grande do Sul, Brazil: Si56, Si48, Si44, Si38, Si28, Si08, are the sampling stations.

- Si56 (29° 43' 50" S and 51° 05' 00" W) – 50 m wide, with gallery forests and a sandy bed. Used for municipal water supply in the city of Novo Hamburgo. Sources of pollution are garbage, sanitary and industrial sewage (Class 2);
- Si48 (29° 44' 21" S and 51° 07' 22" W) – 50 m wide, with bends, gallery forests and a sandy bed. In this region sanitary and industrial sewage and garbage are discharged into the river. Sand dredging and navigation activities. It is located at the mouth of the Luiz Rau stream, which is contaminated by tanneries (Class 3);
- Si44 (29° 45' 24" S and 51° 08' 16" W) – 40 m long, with bends, gallery forests and a sandy bed. This is the water supply intake of the city São Leopoldo. Sanitary and industrial sewage and garbage are discharged into it. Sand dredging and navigation activities (Class 3);
- Si38 (29° 45' 50" S and 51° 10' 36" W) – downstream from the João Correa Canal, 60 m wide, a straight reach with gallery forest and a sandy bed. Sources of pollution are sanitary and industrial sewage, garbage, navigation, paper recycling and sand dredging (Class 3);
- Si28 (29° 47' 53" S and 51° 11' 24" W) – 60 m wide, straight, with grassed embankments, gallery forests, and a sandy bed. Sources of pollution are agricultural pesticides, tanneries, and sand dredging (Class 3);
- Si08 (29° 52' 36" S and 51° 14' 34" W) – 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 (Class 3).

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 Jr., 1995; Ingersol et al., 1995; Terra et al., 2007).

All tests were run with bulk sediment samples (Burton Jr., 1992; Schuytema et al., 1996; Terra et al., 2007), 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 treated in the same way as in previous studies (Terra et al., 2001, 2006).

The cladocerans were cultured in controlled conditions in the laboratory under cool white fluorescent light (16 hours light/8 hours dark), temperature (20 ± 2 °C) (Pieters and Liess, 2006; Smolders et al., 2005; Nebeker et al., 1988), at a density of 25 individuals per 1000 mL of M4 medium (Elendt and Bias, 1990), to keep them at a stage of parthenogenetic reproduction.

All daphnids used in this study were from stock maintained for at least three generations and additionally were from the third or later brood. The cladocerans used presented LC50-24h = 0.90 ± 0.14 mg $K_2Cr_2O_7.L^{-1}$. The LC-24h values were determined by the Trimmed Spearman-Karber method (Hamilton et al., 1977) using Toxstat 1.5 computer program.

The sediment was distributed in beakers (50 mL) that remained covered with laboratory film in a refrigerator until the tests began. On the day before the initial exposures M4 medium was placed on the sediment, at a 1:4 (v:v) sediment to M4 ratio (Burton Jr., 1992; Suedel et al., 1996; Terra et al., 2001). Ten replicas per site were used, with one cladoceran in each replica. At the same time, control groups were conducted, under the same conditions as the tests.

The cladocerans were observed on Mondays, Wednesdays and Fridays for mortality (total lack of movement) and reproduction, during 21 days. The neonates were counted from the beginning of reproductive activity. After the observations were recorded, the old M4 medium was replaced by fresh medium, taking care to avoid disturbing the upper layer of the sediment. As a last stage, after each observation the adult daphnids were returned to the original beaker and fed a culture of the *Scenedesmus subspicatus* algae (0.6 mL; 10^7cells.cm^{-3}) complemented with fermented fish chow and biological yeast (0.1 mL). The food prepared with fish chow and yeast followed the methodology recommended in ABNT-NBR 12713 (ABNT, 2004). This amount of food ensures a nutrient supply until the next reading.

The biological parameters, survival and reproduction, were used as an assessment endpoint. Duncan's test was applied, providing accurate responses in tests with an equal number of repetitions (n = 10) and a high degree of discrimination, with results similar to each other.

The extent to which the site was compromised was verified considering the data generated by statistical analysis, the mean number of neonates per brood, and the survival/mortality ratio. For mean reproduction, at least 20 neonates were expected per brood and at least 80% for survival.

Water samples were collected to verify the presence of heavy metals (Acid Digestion of Samples and Determination by Flame Atomic Absorption Spectrophotometry) and coliforms (Chromogenic Substrate Test). Dissolved oxygen (DO) was obtained by direct readings performed with appropriate equipment (Oximeter). Samples for heavy metal dosage were kept in glass flasks with a nominal capacity of 1,000 mL, containing 5 mL of nitric acid, while the samples for microbiological analyses were collected in a borosilicate flask with a wide mouth and a 100 mL capacity. The heavy metals analyzed were those expected in the area of study due to the sources of pollution (Cr, Cu). Sediment samplings were accompanied by analyses of biological, physical, chemical and metal parameters. It should be mentioned that whereas these analyses were performed in water, the cladocerans were exposed to sediment samples which, differently from water, are a cumulative compartment. Possibly the content of metals and other persistent chemicals is higher there than those detected in the water samples.

3. Results

The results produced by this study showed that the Sinos river quality varies according to the polluting anthropic processes, besides climatic conditions and river flow. Figure 2 compares each site to the control group, indicating the minimum percentage of survival accepted in healthy populations (broken line). Thus, the whole column beneath this line indicates that the sample had an acute ecotoxic effect on the microcrustacea.

Figure 3 shows a similar comparison to Figure 2, this time looking at reproduction. In this graph, the broken line indicates the expected value for the mean number of neonates per brood (20). All the values below this line indicate chronic ecotoxic action.

Si56 presented the best results for survival, which were only different from those expected in February/06 (Figure 2). On the other hand, cumulative effects were observed with an impact on reproduction in 79% of the samplings (Figure 3). Although this reach is included in Class 2 (Brasil, 2005), Copper (Cu), was present with values above the allowable level on four occasions. *Escherichia coli*, bacteria originating in anthropic action, exceeded the value determined by Brazilian legislation in Class 2 waters in 57% of the samplings. Other parameters such as Dissolved Oxygen (DO), Total Phosphorus (Total P) and Phenols were also found, that did not conform to Brazilian legislation in three, four and three samplings respectively.

The Si48 sample collected in June/05 presented 90% mortality in 48 hours, reaching 100% by the 4th day of

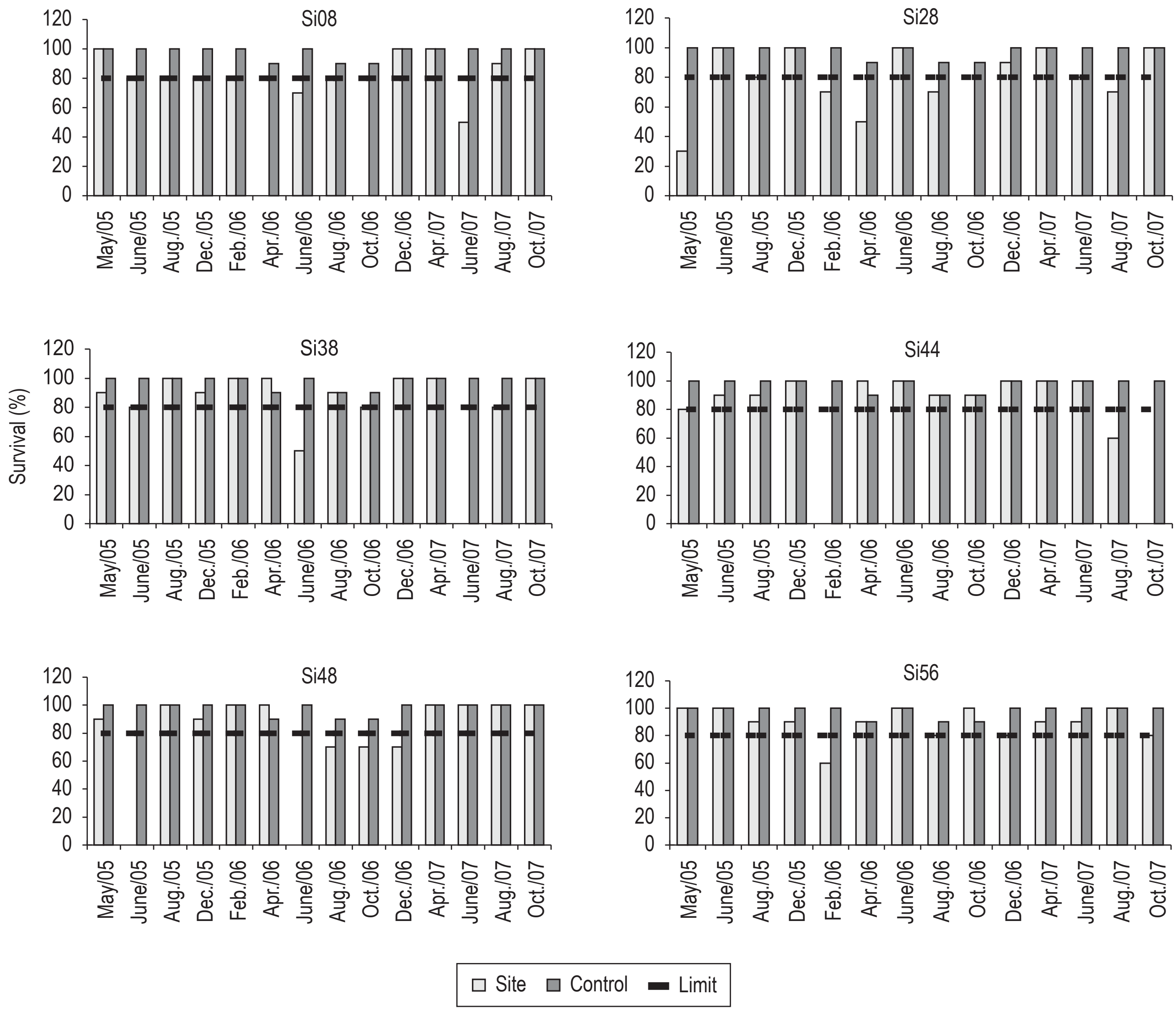

Figure 2. Percentage survival of *Daphnia magna* exposed to sediment samples from the Sinos River (May/05-October/07). The broken line indicates the minimum percentage of survival expected.

exposure. In the same month of the next year (2006), mortality began in the first 24 hours (40%) reaching 100% in 72 hours, while in August 2006 30% mortality occurred by the 10^{th} day, and in December the same percentage of deaths was observed by the 13^{th} day. Thirty-six percent of inadequate responses for survival were observed, while in reproduction the values were lower than expected in 86% of the samplings (Figures 2 and 3). The analysis of metals in water samples detected responses above the allowable level for Cu (9 samples) and Total Cr (6 samples). Total P (71%), *E. coli* (43% of analyses) and DO (50% of analyses) also did not conform to legislation.

Total mortality at Si44 occurred at two moments (February/2006 and October/2007). In February/06 the deaths began during the first 48 hours (90%) and became total in 96 hours, while in October/07 there were 50% of deaths in 48 hours, reaching 100% on the 5^{th} day. Seventy-nine per cent of the 14 samplings presented a mean reproduction lower than expected, while 21% of the samples were damaged for survival (Figures 2 and 3). In 2005, there were more *E.coli* than the values allowed by Brazilian legislation in 50% of the samplings. Similarly to the upstream site (Si48), inadequate responses were found for DO (14%), Total P (43%) and Cu (14%).

High flow periods influenced Si38 causing mortality (50%) at the beginning of observations (9^{th} day) in June/06. In the same month of the next year, mortality began in the first 24 hours (30%) reaching the maximum in 96 hours. An impact on reproductive activity is observed in 86% of the samples, while this occurs on survival in only 14% (Figures 2 and 3). Values above those allowed by Brazilian legislation were found for Total Cr, Cu and Chlorides (all 7%), Phenols, Total P (both 36%), *E. coli* (43%). DO was below 4 mg.L^{-1} in 21% of the collections.

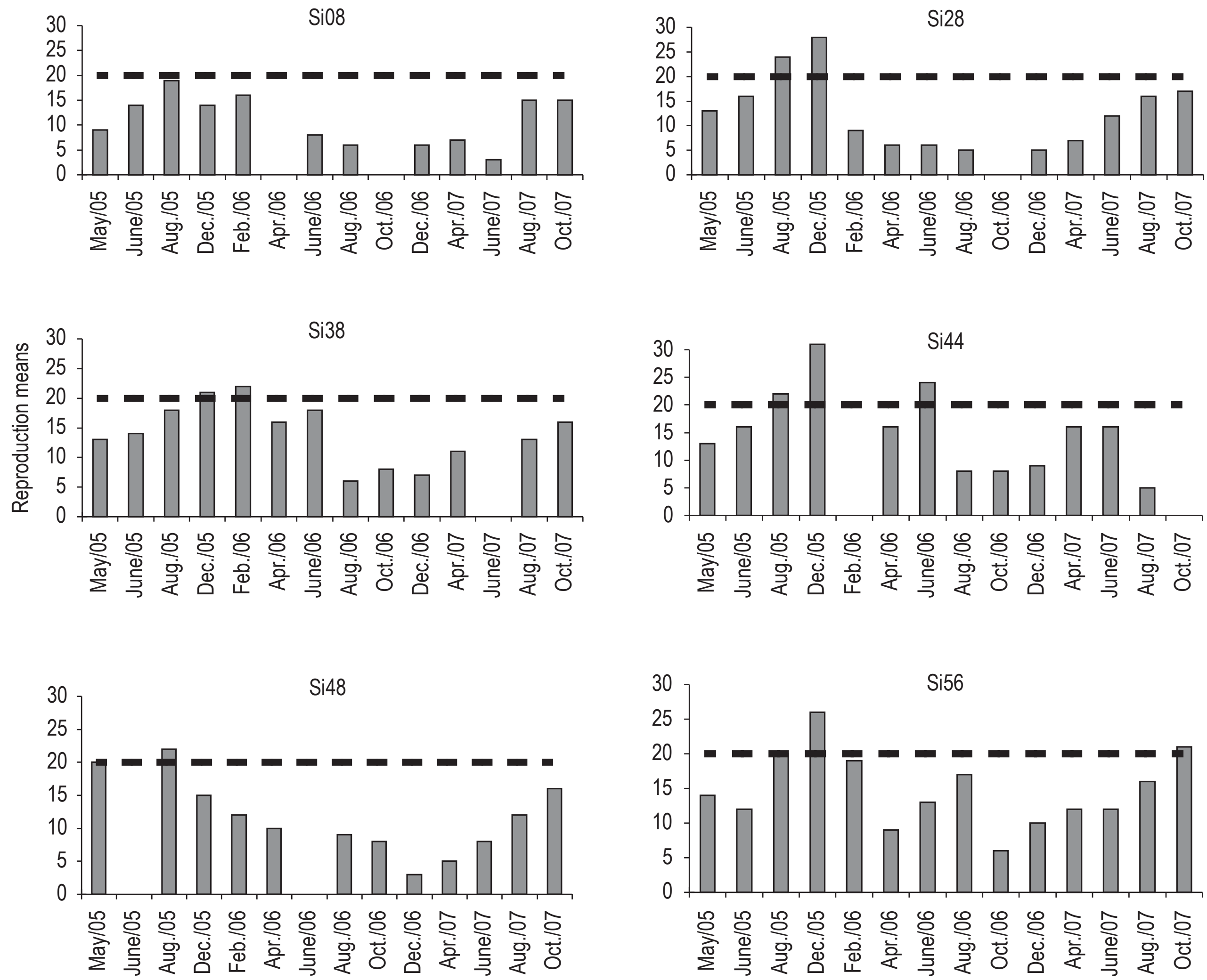

Figure 3. Mean reproduction per *Daphnia magna* brood (May/05 and October/07), in sediment samples from the Sinos River. The broken line indicates the minimum mean expected.

At Si28 the effect of pollution varied throughout the year. In May/05 the mortality began in 72 hours (20%), with another 40% on the fifth day, reaching 70% (12th day). The survivors (30%) presented delayed growth and a pale color until the end of observations. In February/06, although there was only 30% mortality (by the 8th day) 20% of the survivors presented delayed growth. In April the mortality that began on the seventh day continued until the 11th day (50%). On the other hand, in October of the same year, the mortality that began in the first 24 hours (50%) became total in 48 hours. In August/07 mortality reached 30% by the 9th day and remained at that level until the end of the observation cycle. Similarly to points Si38 and Si48, reproduction was low in 86% of the collections (Figure 3). At this site Cu exceeded the value allowed on three occasions, while Cr only exceeded it on one occasion. The indicators of anthropic pollution that are not in accordance with the law are represented by Phenols, Total P (both 21%) and DO (36%) and *E. coli* (29%).

At Si08 pollution was observed every year, in different months. In 2006, the mortality that began within 72 hours (20%) continued on the subsequent days (40% on the seventh day; 30% on the ninth) until it reached 100% (11th day). In April and October of the same year 80% mortality was recorded in the first 24 hours, which became total in 48 hours. Also in February of that year a delay in organism growth was observed. In June the next year (2007) 50% mortality occurred during the first 96 hours. Reproduction was below the level expected in all collections, although survival indicated only 29% of results that were not as expected (Figures 2 and 3). As at Si38, Cr and Cu were not according to standards in one of the samplings, while *E. coli*, Phenols, Total P were 7, 14 and 50%, respectively. DO was below the allowable amount in 43% of the samplings.

There was usually a reproductive variation between the points when the monthly mean was compared using Duncan's Test (Table 1). This variability was greater at Si56 with a difference (α = 0.05) in up to seven groups, showing the wide range of results over the months.

Si08 had the worst means compared to the other points and a small variation in the results over the period studied, showing that there is constant environmental degradation (Table 1).

The comparison of reproductive means between the points, taking each month alone, shows a lack of significant difference in August/05 (Figure 4). In August/06, August/07 and October/07, the difference occurred only at a single site compared to the others. During the other months, however, there was greater variability between the points.

4. Discussion

The present study supports some aspects evaluated in previous papers (Vargas et al., 2001; Terra et al., 2003, 2008), showing that the area of study presents alterations due to the presence of pollutants that interfere in the development of the test-organisms used. Persistent pollutants discharged by effluents or released from the river bed return to the trophic system, damaging the organisms that are part of it. These processes interfere in the aquatic biota, as seen in the analysis of data generated by the exposure of cladocerans to sediment samples from the Sinos River. *D. magna* are appropriate organisms for the study, since in assays using bulk sediment, sediment particles were identified in the gut (Gillis et al., 2005). On the other hand, the sediments absorb and bind certain xenobiotics, both in lentic and lotic systems. These toxic substances may affect organisms that take up contaminated sediment particles (Cairns et al., 1984). Factors such as body size, growth rate and physiological stage (juvenile or mature, spawning, molting, etc) strongly influence chemical absorption and excretion (Zitko, 1981).

Although Site Si56 is used for municipal water supply, it receives sanitary and industrial sewage. This information is supported by the presence of Cu, an indicator of industrial sewage. The high number of *E. coli*, Total P, Phenols, and DO deficiency characterize the discharge of domestic

Table 1. Comparison of monthly means for each site. Means followed by the same letter did not differ significantly (α = 0.05).

	Si08			Si28			Si38	
Month	Mean	Comparisons	Month	Mean	Comparisons	Month	Mean	Comparisons
Oct./06	0	a	Oct./06	0	a	June/07	0	a
Apr./06	1.7	a	Apr./06	16.9	a,b	Aug./06	26.6	b
June/07	7.2	a	May/05	20.0	a,b	Oct./06	34.0	b,c
Aug./06	29.0	b	Aug./06	23.3	a,b	Dec./06	36.1	b,c, d
June/06	29.7	b	Dec./06	23.5	a,b	June/06	45.7	b,c, d
Dec./06	31.9	b	Feb./06	30.2	b,c	Apr./07	54.7	c,d,e
Apr./07	33.7	b	June/06	31.8	b,c	June/05	58.7	d,e,f
May/05	43.7	b,c	Apr./07	36.7	b,c,d	May/05	60.3	e,f
June/05	56.9	c,d	June/07	54.7	c,d	Aug./07	61.1	e,f
Dec./05	58.3	c,d	Aug./07	57.7	d,e	Apr./06	79.5	f,g
Feb./06	64.4	c,d	June/05	80.4	e,f	Oct./07	79.9	f,g
Aug./07	67.0	c,d	Oct./07	82.8	f	Aug./05	91.9	g,h
Oct./07	73.0	d	Aug./05	108.5	g	Dec./05	102.2	g,h
Aug./05	77.4	d	Dec./05	140.2	h	Feb./06	111.9	h
	Si44			Si48			Si56	
Month	Mean	Comparisons	Month	Mean	Comparisons	Month	Mean	Comparisons
Feb./06	0	a	June/05	0	a	Oct./06	27.4	a
Oct./07	0	a	June/06	0	a	Apr./06	42.1	a,b
Aug./07	27.5	b	Dec./06	10.4	a,b	Dec./06	46.2	a,b
Oct./06	37.7	b,c	Apr./07	26.2	b,c	Apr./07	53.0	a,b,c,d
Aug./06	39.3	b,c	Aug./06	33.2	c,d	June/07	54.7	b,c,d
Dec./06	43.5	b,c	Oct./06	33.6	c,d	June/05	60.5	b,c,d,e
May/05	52.9	c	June/07	39.4	c,d	June/06	62.9	b,c,d,e
Apr./06	71.1	d	Apr./06	50.1	d,e	May/05	69.8	c,d,e
June/05	72.3	d	Aug./07	60.3	e,f	Aug./06	70.7	c,d,e
Apr./07	78.0	d	Feb./06	60.7	e,f	Feb./06	76.9	d,e,f
June/07	80.3	d	Dec./05	73.6	f,g	Aug./07	82.6	e,f
Aug./05	108.5	e	May/05	89.1	g,h	Oct./07	86.0	e,f
June/06	119.1	e	Oct./07	91.4	h	Aug./05	98.9	f,g
Dec./05	156.2	f	Aug./05	111.2	i	Dec./05	117.0	g

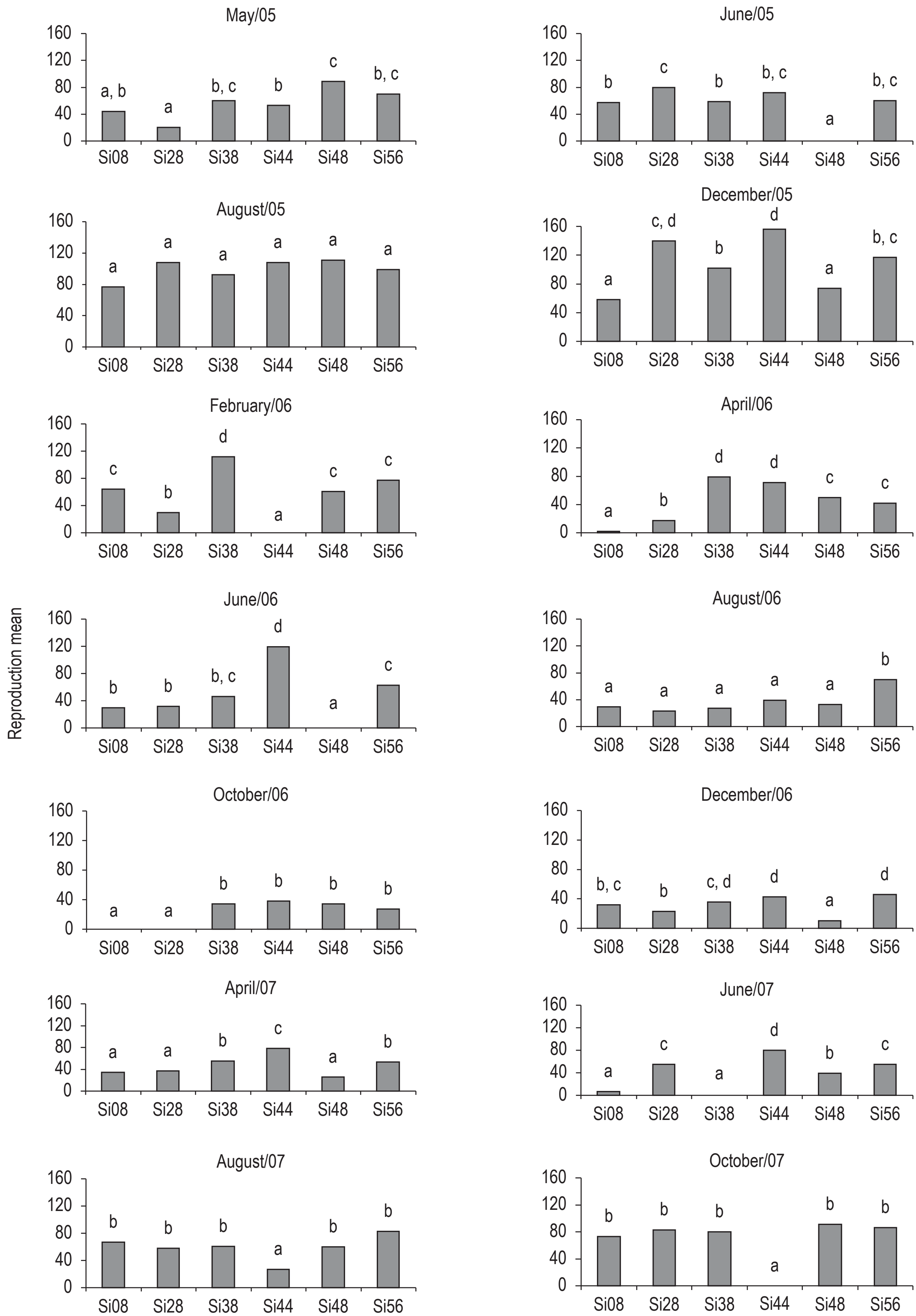

Figure 4. Monthly comparison of the reproductive mean in *Daphnia magna* exposed to sediment samples. The same letters indicate absence of a significant difference (α = 0.05) between the sites.

sewage. As in the present study, anthropic action had been previously found at this site (Terra et al., 2008; Vargas et al., 2001) through cytotoxic alterations, when water samples were examined. Current data show sediment recovery after October/06, the month in which the reproduction presented the most critical values.

Eight kilometers downstream from this site is Si48, which is influenced directly by the Luiz Rau stream, used to discharge domestic and industrial effluents. This site presented the greatest number of physical, chemical and biological parameters that did not conform to Brazilian legislation, as expected, due to the massive contribution of effluents discharged into the stream. Abnormal values of Cu, Cr, OD, Total P and *E. coli*, are common in areas with intense human activity, causing an impact on the environment and on local living organisms. The expected mean number of neonates was achieved only on two occasions. In June/05 and June/06, there was no survival and reproduction due to anthropic activity. The toxic effect normally occurs when Cu is mobilized from the solid phase (sediment) to the liquid phase (dissolved) (Cairns et al., 1984). Ecotoxicological damage may have been triggered by the release of previously deposited chemicals that returned to the trophic network. This usually happened when heavier rainfall, which often occurs in winter, caused a greater flow of the water mass, disturbing the river bed. Studies performed previously at Si48 presented positive results for cytotoxicity (Terra et al., 2008) and genotoxicity (Vargas et al., 2001). According to Terra et al. (2008), between the years of 2001 and 2005 sediment samples from this site already presented acute ecotoxicity in *D. magna*.

The site at the next location (Si44) suffers cumulative impact due to the discharge of pollutants from areas upstream. On the contrary of previous studies (Terra et al., 2008), here 79% of the reproductive means were below the expected rate, indicating a drop in the quality of this area. This did not happen at Si56, where the quality improved at the end of the study. Similarly to Si48, positive responses were found at Si44 for a few parameters indicating anthropic activity, such as Cu (industrial pollution), detected on two occasions with higher values than those allowed by the national legislation for Class 3 waters. According to Sundelin and Eriksson (2001) precipitated metals can be remobilized into the water column due to the change in physicochemical conditions or in urban activities. Parameters such as DO, Total P and *E. coli*, were not in conformity with the legislation in 14, 43 and 50% of the samplings. The poor conditions at this site were expected due to the proximity with Si48, a rather degraded area located 4 km upstream. The low availability of DO and high *E. coli* values coincided with the presence of Total P in the two and a half years of the study, due to sanitary sewage discharged at points close to the site.

At Si38 anthropic activity was also seen in the analyses of Total P, DO and *E. coli*. Human action changed the environmental quality, with a clear consequence for reproduction, where only two of the 14 samplings were within the expected standards. In a previous study (Terra et al., 2008), mortality at this site was elevated on June/03, during the first days of observation. This was similar to what occurred in June/07 when deaths began in the first 24 hours of exposure. The heavy rainfalls during the winter months can increase the current disturbing the sediment and mobilizing the chemicals that were previously deposited on the bed. The characteristics of Si38 are compatible with the presence of João Correia Canal, 60 km upstream, which discharges a load of pollution comprising sanitary and industrial sewage, garbage and paper recycling into the river. The load of nutrients discharged by domestic effluents raises the level of eutrophication, causing a drop in DO in the bottom sediment (Sundelin and Eriksson, 2001).

A cumulative impact was also observed at Si28, as was to be expected, since this site is affected by the entire area upstream of the river, besides being influenced by point sources of agricultural activity and tanneries. River bed dredging, common in this area, contributes to the recirculation of already settled pollutants. In most of the samplings (79%) mean reproduction was less than expected, indicating chronic ecotoxicological action with a marked change in river quality. Stressed organisms often produce smaller broods than the non-stressed ones (Cowgill el al., 1986). The action of xenobiotics alters the organic functions triggering undernourishment processes and stress in the cladocerans. Previous studies indicated an impact on this area, when the presence of signs of genotoxicity was confirmed (Vargas et al., 2001), besides signs of a positive response for the induction of micronuclei in V79 (Terra et al., 2008). Although genotoxic action does not interfere directly in the biota balance, it slowly disturbs the adjustment, breaking down system homeostasis. Mean reproduction per brood, which rose after May/05 and reached the expected amount in August and December of that year, fell again during the dry season and remained low throughout the next year (2006). We observed the slow and progressive degradation of the environment, reaching the most critical situation in October/06 with complete absence of reproduction. That month, the Sinos River near Si28 suffered an ecological disaster when leakage from industrial effluent treatment plants provoked massive death of fishes. Measures taken after this incident ensured the slow recovery of the river quality from that time on, which was observed by the end of the study.

Whenever ecosystem stability is disturbed, natural systems have to undergo a long recovery process, but in order for this to occur it is necessary to reduce the load of pollutants and allow time for the biota to recover.

Site Si08, located near the mouth, suffered a greater impact than the rest due to the influence of pollutants discharged all along the river. According to Terra et al. (2008) in a study performed in this area between 2001 and 2005, a cytotoxic effect was detected in water samples compatible with the strong anthropic influence found at this site. The same study reports that 54% of the samples analyzed presented a low reproductive level, while in the present study 100% of them presented diminished reproduction, indicating a drop in river quality. These changes may be caused by the presence of mixtures of pollutants, such as PAHs and metals found in areas strongly influenced by public and industrial waste or close to agricultural areas. The sediments function as a deposit for persistent contaminants such as PAHs and metals that enter the aquatic ecosystem (Ankley et al., 1996). The presence of Phenols, Total P and the low DO content confirm the discharge of sanitary sewage.

Duncan's test was used to compare the mean number of neonates month by month. It showed variations at each site (Table 1). The values were often close to each other (April/04) and no significant difference was found (α = 0.05), while in other months there was an occasional difference, as in August/06 (Si56), August/07 and October/07 (Si44). The slight variation may occur because the heavier rainfalls during the winter months cause stronger river flow displacing the wastes that had settled. During the dry months (December to April) there is greater variation because the wastes discharged into the river settle in limited areas close to their source.

5. Conclusions

Different pollution processes were identified by observing the survival and reproduction results, although these parameters are not directly related. While survival indicates acutely acting pollutants, reproduction indicates slow actions, which occur when the organisms spend more energy due to the reproductive processes or aging, and make individuals more susceptible to environmental aggression.

Currently, studies are being performed in the Sinos River to find the ecotoxicological responses to seasonal and longitudinal variations. In a previous study (Terra et al., 2008) increasing degradation was observed from the headwaters to the mouth, and this situation is repeated in the present study.

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