Taquari River (Rio Grande do Sul, Brazil) sediment ecotoxicology, using *Daphnia magna*, 1820, Straus as test organism

Ecotoxicologia do sedimento do Rio Taquari (Rio Grande do Sul, Brasil) utilizando *Daphnia magna*, 1820, Straus como organismo-teste

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Abstract: The Taquari River is socio-economically and environmentally important in Rio Grande do Sul State, Brazil. This study evaluates the influence of sediment samples from the river on the survival and reproduction of *Daphnia magna.* For this purpose, four sites were monitored from the middle course of the river downstream to its mouth using semi-static chronic bioassays (21 days). Sediment samples were collected ten times between December/2000 and September/2005. Survival rates were analyzed to evaluate acute toxicity and the reproductive responses were examined for chronic toxicity. Reproductive activity was affected at the beginning of the observations returning to the expected limits for healthy environments only during the last month of the study. Reproduction was more sensitive than mortality to evaluate environmental quality, since the reproductive process was affected even on occasions when mortality was within acceptable standards. The data showed that in the last two and half years of the study, the quality of this river gradually recovered independently of the season.

Keywords: bioassay, reproduction, survival, cladocerans.

Resumo: O Rio Taquari é importante para o estado do Rio Grande do Sul, Brasil quanto as suas características sócio-econômicas e ambientais. Este estudo avalia a influência de amostras de sedimento deste rio, considerando a sobrevivência e a reprodução de *Daphnia magna.* Foram monitorados quatro locais do curso médio deste rio até a foz, utilizando bioensaios crônicos (21 dias) semi-estáticos, sendo realizadas dez amostragens de sedimento em cada um dos pontos, entre Dezembro de 2000 e Setembro de 2005. As porcentagens de sobrevivência foram analisadas para avaliar a presença de toxicidade aguda e as respostas reprodutivas a presença de toxicidade crônica. Foi observado efeito agudo em apenas três pontos no mês de Abril/2003 e em um ponto no mês de Setembro do mesmo ano. Os demais resultados indicaram efeito crônico no início das observações retornando ao limite esperado para ambientes saudáveis somente no último mês de estudo. A reprodução foi o parâmetro mais sensível comparado a mortalidade, considerando a avaliação da qualidade ambiental, uma vez que a reprodução foi afetada muitas vezes e a mortalidade esteve, normalmente, dentro dos padrões aceitáveis. Os dados mostraram que nos últimos dois anos e meio de estudo, a qualidade deste rio recuperou-se gradativamente, independente da estação do ano.

Palavras-chave: bioensaios, reprodução, sobrevivência, cladóceros.

1. Introduction

Biological tests to classify the capacity of the environment to preserve aquatic life and human health have been essential to evaluate the quality of environmental compartments.

The Taquari river basin, in the northeast of Rio Grande do Sul State, covers 26,428 km^2, and flows through ten municipalities (Ferreira and Both, 2004). Being about 500 km long, it is used as a source of power, leisure, food and water supply. However, it is often under the influence of several sources of pollution, including sanitary and industrial sewage, sand dredging, navigation and lumber processing. The Taquari river valley is essentially an agricultural region where large amounts of organophosphorated compounds are used. Due to local terrain geology and deforestation, rainfall carries to watercourses a large portion of the agricultural, livestock and domestic products derived from human activity (Zanotelli et al., 2004). This river meets the Jacuí River and others to form the Guaíba lake basin. Porto Alegre, the capital of Rio Grande do Sul, the southernmost state of Brazil, lies on the banks of the lake, from which it gets its water supply.

Taquari River is Class 2 from the headwaters to the mouth and its water can be used for human consumption after conventional treatment, to protect aquatic communities, being suited to primary contact recreation according to Brazilian Law (Brasil, 2005).

Daphnia magna, a microcrustacean bioindicator for environmental quality, was used to evaluate the studied area. It is frequently employed in ecotoxicological assays to assess the quality of river sediments. The techniques involving whole sediment were developed with good responses in exposure tests (Suedel et al., 1996; Terra et al., 2004, 2006; Gillis et al., 2005). Bioavailable toxic compounds in the sediment may be absorbed by cladocerans through the ingestion of organic particles used as food. Laboratory experiments can detect adverse effects resulting from the exposure of microcrustaceans to sediment samples, according to the time of exposure and the feeding habits of the species. This is an important evaluation for detecting the effects of many chemical compounds using survival and reproduction as endpoint, since substances that are not detected by chemical methods may interfere in fauna.

The main purpose, of this study was to evaluate the Taquari River quality by analyzing sediment samples, using *D. magna* as a test organism. The biological parameters used for this evaluation were survival and reproduction of these microcrustaceans.

2. Material and Methods

To perform this study, ten samples were collected between December 2000 and September 2005 at four sites in the Taquari River (Figure 1), which were named according to the initial letters of the river name (TA), followed by how many kilometers there were from the mouth. The sites are described below with the geographical coordinates and the main pollution sources.

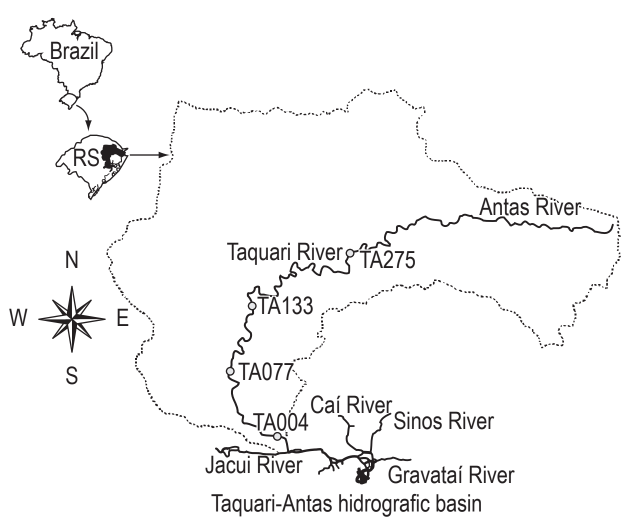

Figure 1. Location of the study site in Rio Grande do Sul, Brazil: TA004, TA077, TA133, TA275 are the sampling stations.

Ta275 - (29° 00' 45,2" S and 51° 22' 00,5" W) - Industrial and sanitary effluents, agriculture and livestock;

Ta133 - (29° 13' 35,1" S and 51° 51' 06,4" W) - Sanitary sewage, tannery effluents and vegetal oil wastes;

Ta077- (29° 30' 41,9" S and 51° 58' 47,5" W) - Sanitary sewage, tannery effluents, meat-packing plants, brewery and navigation;

Ta004 - (29° 55' 45,2" S and 51° 43' 50,4" W) - Navigation, sand dredging, wood processing and sanitary sewage.

Sediment was collected with a Petersen grab sampler at a depth of 1.20 m, transported on ice to the laboratory, and stored in darkness at 4 °C until its use in the bioassays (Burton, 1995; Ingersol et al., 1995; Terra et al., 2001). According the methodology the experiments were carried out at most after one month from sampling to the beginning of exposure.

All tests were performed with whole sediment samples (Ankley, 1991; Burton, 1992a; Schuytema et al., 1996; Cardozo et al., 2006; Terra et al., 2006; 2007), without sieving the sample, but removing the large organisms (Ingersoll et al., 1995), because the presence of indigenous organisms may greatly affect the chronic endpoint in sediment toxicity tests (Reynoldson et al., 1994). The specimens of *D. magna* (Clone A) were cultured 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.

Since these are long duration assays, before the exposure began all lots of animals were submitted to tests of sensitivity to $K_2Cr_2O_7$ and those that presented LC50-24 hours in 0.91 ± 0.19 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.

Ten assays were randomly performed for each site, exposing the microcrustaceans neonates (2-26 hours old), as already done successfully in other experiments (Burton, 1992b; Suedel et al., 1996; Terra et al., 2001; 2006; Cardozo et al., 2006). A control group with ten replicates was exposed only to the M4 medium (pH: 7.8; hardness: 230 mg $CaCO_3$/L). The cladocerans were maintained individually in beakers with a nominal value of 50 mL containing one part of sediment to three of culture medium (v:v). The beakers were covered to avoid contamination and evaporation. All experiments were conducted in incubators at 20 ± 2 °C with a 16 hours light/8 hours dark photoperiod (Gersich and Mayes, 1986; Nebecker et al., 1988; Smolders et al., 2005; Pieters and Liess, 2006). The cladocerans were monitored on alternate days, for mortality (total lack of movement) and the production of offspring. After this procedure the young individuals were discarded, and new culture medium were introduced carefully to avoid disturbing the stability of the surface sediment layer. After replaced the female adult was returned the beaker. The daphnids were fed, after each observation, with a culture of the green algae *Scenedesmus subspicatus* (0.6 mL; 10^7cells.cm^{-3}) and food prepared using fermented fish feed complemented by biological yeast (0.1 mL), to supply the nutrients needed. The food prepared with fish chow and yeast followed the methodology recommended in ABNT NBR 12713 (2004).

The data obtained in the experiments with *D. magna* defined the level of alteration (acute or chronic) of the sites evaluated in the Taquari River, by analysis of the brood size, where a minimum of 20 individuals are expected per brood (Cowgill et al., 1985), and the percentage of survival (acute) which must be between 80 and 100%.

The two-factor ANOVA test was used in statistical analysis to compare the points in the river and the months for the reproduction variable. The data were weighted by the inverse of the variance of each group in order to reduce the variability. In this analysis, the factors "month" and "site" were fixed and the "beakers" were the repetitions (n = 10). The SLICED option of SAS software was used to present the interaction of factors sliced by month. The MSD (Minimum Significant Difference) multiple comparisons test was used, as well as a 5% level of significance.

The dry weight of the survivors only of the Sept./05 sampling was evaluated to complement the data. In order to calculate the dry weight, the cladoceran survivors were initially grouped by collection site, washed in distilled water and then taken to the oven at 105 °C for 2 hours (Kusk, 1996). After weighing on an electronic analytic balance (QUIMIS, Q -500L210C), the mean was calculated taking into account the number of individuals at the end of exposure, to compare the mean weight among the exposed organisms, in relation to control group.

Water samples were collected for verification of the presence of total heavy metals (Acid Digestion of Samples and Determination by Flame Atomic Absorption Spectrophotometry). Samples for heavy metal dosage were kept in glass flasks with a nominal capacity for 1000 mL, containing 5 mL of nitric acid. Total P and Phenol were also analyzed, since both are indicators of anthropic pollution. While the first followed the Method of digestion with potassium persulphate and ascorbic acid, the second used ABS spectrometry by ABS – Molecular UV-V and extraction with CHC13.

3. Results

The data analysis showed that the Taquari River quality improved over time. Figure 2 shows the survival percentage of cladocerans during the exposure period (Dec./00 to Sept./05). The interrupted line shows the minimum limit expected for survival (80%) in healthy pollutant-free environments, while the values below this line indicate the pressure of the environment on the microcrustaceans. It can be observed that in 20% of the samples, the responses were below the minimum limit expected for these parameters. We found that TA004 always presented appropriate results, showing the absence of an acute ecotoxicological effect, while at TA077 and at TA275 this effect (mortality) was detected in 30% of the samples and 20% at TA133.

The number of cladocerans born during the exposure period is shown in Figure 3. In the first months of observations a smaller generation is observed, followed by an ascending curve, indicating the recovery of the river water quality with statistical significance. The same figure shows Dec./03 and Apr./03 as the most critical moments, with the generation of only 64 individuals at TA077, 193 at TA133 and 195 at TA245.

Brood sizes were above the expected value of 20 neonates per brood only in the last sampling (Sept./05) considering the studied sites, indicating that *D. magna* was affected by the environment during almost the whole studied period (Figure 4).

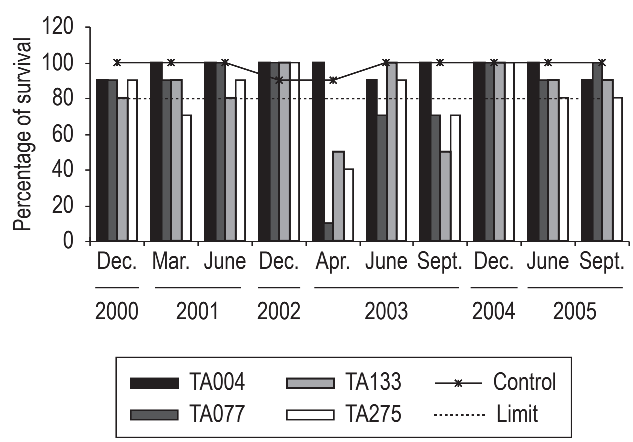

Figure 2. Survival percentage of *Daphnia magna* exposed to sediment samples from Taquari River, of the control group and the expected survival, from December 2000 to September 2005.

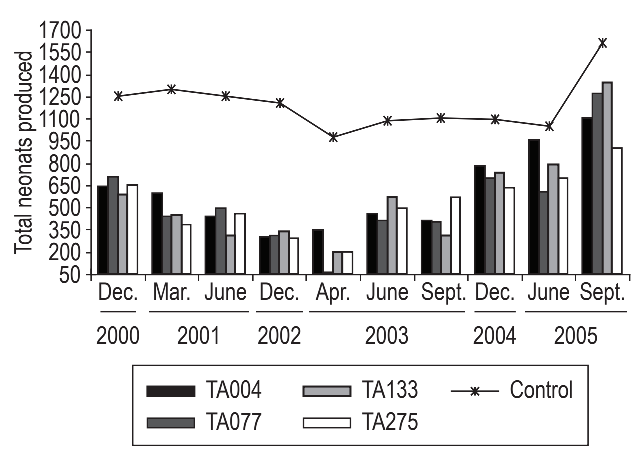

Figure 3. Total neonates per site and month from December 2000 to September 2005.

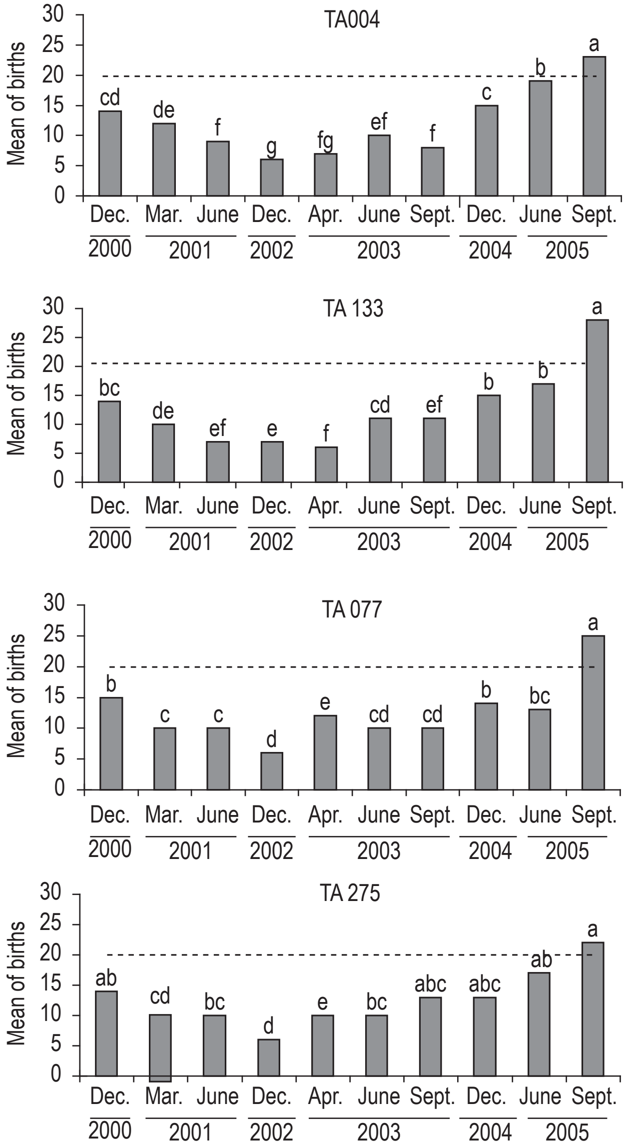

Figure 4. Cladoceran mean brood sizes observed in bioassays using sediment river for each sample site from December 2000 to September 2005 (means followed by the same letter do not differ significantly).

Comparing the evolution of each site over time, a similar tendency is observed with a decrease in the quality during the first half of the study followed by later recovery (Figure 5).

The ANOVA test showed that the interaction between month and site was significant ($p < 0.001$). In other words, the differences found in mean brood sizes observed for the different months studied depends on the site location (Table 1).

Multiple comparisons of means among months at each sampling site are shown in Figure 4. Analyzing the four graphs presented we observed that the quality of water decreased between June 2001 and June 2003. However, the environment recovered from June 2005 onwards at almost all sampling sites.

Complementary data of final dry weight were used to compare the biomass production among the sites, and the mean

Table 1. Multiple comparisons of mean brood size (MDS test). Means with the same letter do not differ significantly.

Mar./01				Dec./04			
Site	N	Mean	Comparisons	Site	N	Mean	Comparisons
Ta004	10	59.4	a	Ta004	10	77.7	a
Ta133	10	44.9	b	Ta133	10	72.8	a
Ta077	10	43.5	b	Ta077	10	69.1	a b
Ta275	10	38.6	b	Ta275	10	63.0	b
June/01				June/05			
Site	N	Mean	Comparisons	Site	N	Mean	Comparisons
Ta077	10	49.6	a	Ta004	10	95.4	a
Ta275	10	45.7	a	Ta133	10	78.9	a b
Ta004	10	44.0	a	Ta275	10	69.7	a b
Ta133	10	30.7	b	Ta077	10	60.4	b
Abr./03				Sept./05			
Site	N	Mean	Comparisons	Site	N	Mean	Comparisons
Ta004	10	34.2	a	Ta133	10	134.0	a
Ta133	10	19.5	b	Ta077	10	127.1	a b
Ta275	10	19.3	b	Ta004	10	110.0	b c
Ta077	10	6.2	c	Ta275	10	90.2	c

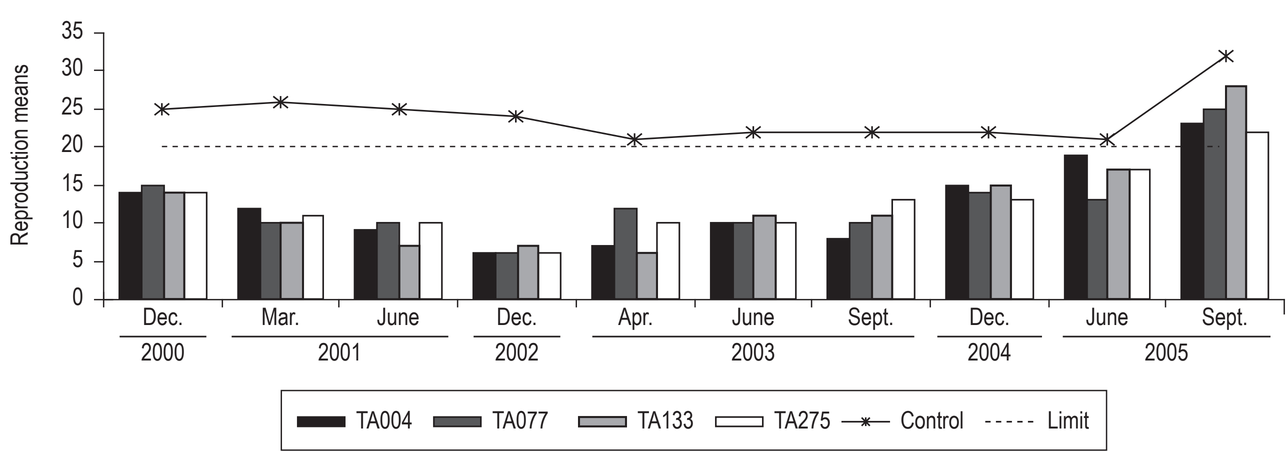

Figure 5. Brood sizes of *Daphnia magna* exposed to sediment river samples per site from December 2000 to September 2005.

dry weight values of the individuals exposed to the Sept./05 sediment samples are shown (Figure 6). Microcrustaceans exposed to the sediment of site TA004 produced a smaller biomass compared to the other studied sites.

The analyses of heavy metals, performed in water during the study period, did not show a direct relationship with survival and reproductive changes, although sometimes the responses went beyond the values allowed by Brazilian legislation for Class 2 waters. The same was found for Total P and Phenol. This absence of a relationship may have occurred because the evaluations were performed in different compartments.

4. Discussion

The ecotoxicological assays performed with sediment samples from Taquari River, in most observations indicated good conditions for *D. magna* survival, but not for reproduction. Although an acute effect on survival was expected at the site close to the mouth (TA004), the strong current acting in this area may have prevented toxic compounds from settling, and the results found were similar to the other sites. However, less biomass production of individuals exposed to the sample collected at TA004 and measured after the end of the assay in Sept./05 was found compared to the other sites. The low dry weight value found at TA004 was probably due to the pollution from lumber processing upstream and adjacent to this site, since we observe that at the other sites the tendency was an increase in biomass, the more distant the sites were from headwaters.

As in the other sites evaluated, at TA077 it was found that the presence of tannery effluents, meat-packing plants, brewery and navigation also influenced the area, and this impact was expressed in reproduction.

Taquari River studies between sites TA004 and TA077 found that the benthonic fauna of this region presented an altered composition (Perico et al., 2004), which is compatible with the results obtained in the present study.

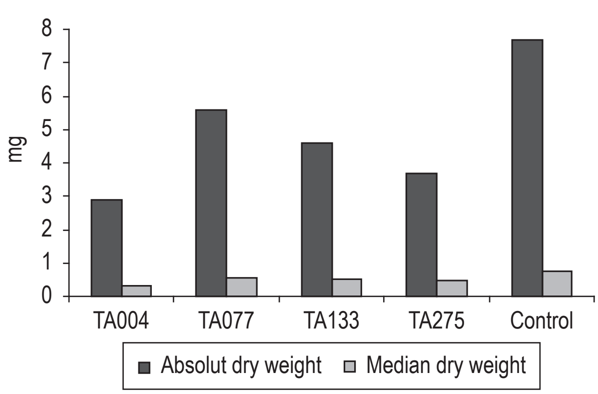

Figure 6. Dry weight (mean and absolute values) of *Daphnia magna* exposed to sediment samples from Taquari River in September 2005.

Although site TA133 has sources of pollution that are different from those at TA004, both behave similarly when the mean number of births is considered. This may be a result of the presence of strong environmental aggressors, such as the tannery effluents at the former and lumber processing at the latter.

However TA275 is located upstream from the main sources of pollution in Taquari River, it should be taken into account that the degradation shown by the D. *magna* assays could be due to the point contaminant sources that exist in the area, such as sanitary sewage and industrial effluents, besides non-point sources such as farming areas and the presence of domestic animals. Work with samples from regions close to TA275 revealed low water quality values attributed to the significant presence of coliforms, especially in the creeks flowing into the river, as a consequence of the great impact of anthropic activity in the region (Michaelsen and Freitas, 2004). In the same area, the presence of vineyards (Tonietto et al., 2004) may have contributed to bad water quality because of pesticides.

Fewer individuals were born between the samplings of Dec./00 and Dec./02, revealing a chronic ecotoxicological effect of the samples during this period. From Apr./03 onwards, the number of births gradually increased, indicating less ecotoxicological influence of the sediment on the individuals. The reproductive capacity continued to rise until the end of the samplings, but it was only in Sept./05 that the mean number of neonates per brood was above the line that delimits the minimum level of responses appropriate to maintaining the fauna, and characterizing the absence of chronic ecotoxicity.

Among the statistical factors considered, six sites of the ten studied presented significant differences between mean brood size per sampling point. On the other hand, a significant difference was observed among them when sites were analyzed individually, independent of season of the year, supporting the information from Michaelsen and Freitas (2004) that seasonality was not a decisive condition for the water quality in Taquari River.

Survival, over time, showed low impact from the sources of pollution on the test organisms. However, in April and September 2003, except for TA004, an acute ecotoxicological effect was observed on the cladocerans exposed to the sediment samples from this river. A likely reason for this result may be the excess rainfall that commonly occurs in the State during the autumn and winter months, raising the volume of the water mass and the water flow, causing pollutants to be carried from the headwaters to the mouth.

5. Conclusion

According to the data, this part of the river recovered along the studied years. This process was gradual and took about two and a half years for the sites sampled to recover its quality. It is expected that this recovery process will continue, due to tighter environmental control maintained by FEPAM and by law enforcement authorities. There is a schedule for the improvement of the treatment of effluents discharged into the environment. However, this watercourse continues being monitored, since it is a dynamic structure with a capacity for self-depuration, and is continually affected by external agents.

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