Reproductive inhibition in Daphnia magna Straus, 1820, exposed to sediment samples of an area under impact from the petrochemical industry.

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ABSTRACT: Reproductive inhibition in Daphnia magna Straus, 1820, exposed to sediment samples of an area under impact from the petrochemical industry. Bom Jardim is one of the streams that form the Caí river basin and it has been influenced by petrochemical industry wastes since 1982. Therefore we studied the effect of sediment on the cladoceran Daphnia magna Straus, 1820, in the stream to observe the impact so far caused by the industrial complex on the environment. Three stations in the stream were sampled to perform chronic tests on sediments. Long duration tests in semi-static flow were performed with D. magna. At the beginning of the observations, the microcrustaceans were 2 to 26 hours old. Each sampling station received ten individuals, placed individually in beakers containing one part of sediment to four parts of culture medium (v:v). We observed that reproduction at all sites was below the minimum expected limit to maintain the species (20 individuals/offspring), sometimes reaching zero at the site with the most impact, such as the one located next to the area where the treated effluents from the petrochemical complex were sprinkled. Both survival and reproduction data suggested that stream sediment quality decreases towards the mouth. Looking at reproduction and survival, we observed that the former was more sensitive to identify environmental quality in the assays performed.
Key-words: Chronic assay, Cladoceran, Epibenthic microcrustacean, Sediment.

RESUMO: Inibição reprodutiva em Daphnia magna Straus, 1820, expostas a amostras de sedimento de uma área sob impacto de indústria petroquímica. O arroio Bom Jardim é um dos formadores da bacia hidrográfica do rio Caí e recebe influência de rejeitos de um complexo industrial petroquímico desde 1982. A ação do sedimento deste arroio foi estudada sobre o cladócero Daphnia magna Straus, 1820 para observar o impacto que o complexo industrial causou até o momento. Para avaliar a ação desta área, três locais foram definidos para realização de testes crônicos do sedimento. Foram planejados testes de longa duração em fluxo semi-estático, sendo que ao início das observações os microcrustáceos possuíam entre 2 a 26 horas de vida. Dez indivíduos, foram dispostos individualmente, por amostra, em béqueres contendo uma parte de sedimento para quatro partes de meio específico utilizado para cultivo de Daphnia magna (v:v). Em todos os locais, a reprodução apresentou-se abaixo do limite mínimo esperado para a manutenção da espécie (20 indivíduos/ninhada), chegando algumas vezes a zero no local mais impactado, como aquele situado junto à área de aspersão dos efluentes tratados deste complexo petroquímico. Tanto a sobrevivência quanto à reprodução sugerem que o arroio apresenta qualidade decrescente do sedimento, em direção a foz. Considerando a reprodução e a sobrevivência, observamos que a primeira foi mais sensível para a identificação da qualidade ambiental nos ensaios realizados.
Palavras-chave: Ensaios crônicos, Cladóceros, Microcrustáceos epibênticos, Sedimento.

Introduction

The impacts to which natural environments have been constantly submitted due to anthropic action have led the State Foundation for Environmental Protection, the environmental agency of the state of Rio Grande do Sul, Brazil, to monitor, continuously, several hydrographic basins in the state. One of the studied basins is Bom Jardim, one of the streams forming the Caí river basin, which discharges into the Guaíba Lake, a source of public supply and a primary recreation site for the population of Porto Alegre, the state capital, and surroundings. Since 1982 this stream has been affected by a petrochemical industry complex (consisting of a first generation industry and eight second generation ones) whose effluents are sprayed on the land after primary, secondary and tertiary treatments. After rainfall surface runoff and the remnants of these discharges washes out the land into the stream.

Studies were performed on the Caí River (close to the mouth of Bom Jardim), about genotoxicity was made throughout a Salmonella mutagenicity assay (Vargas et al., 1993; 1995) as well as in human lymphocytes (Lemos et al., 1994; Lemos & Erdtmann, 2000), exposed to samples of water from this river. Another study performed in the treatment lagoons of this industrial complex found an alteration in the morphology and survival of Moina micrura and in the survival of Ceriodaphnia dubia (Bohrer, 1995).

Chronic assays were planned to evaluate the impact caused by this petrochemical complex in order to find out the significance of the toxic agents. The bioavailability must be evaluated by means of bioassays (Lemos & Terra, 2003), because many environmental aggressors only become visible when they are present in high doses. However, when they in small amounts, they silently affect an individual's genetic baggage, interfere in their physiologic functions, change the reproductive frequency or quality and quantity of generated organisms (Terra & Feiden, 2003).

This study exposed Daphnia magna Straus, 1820 to sediment samples submitted to petrochemical impact. The assessment of sediment quality by bioassays is important, because, besides showing the influence on the organisms exposed, the effect on the organisms that feed on these sediments can be inferred. In recent years more methods have been created to assess contaminants in sediments, since these are potential sources of water contamination (Terra & Schäfer, 2000). Accumulated pollutants, such as PAHs (Ankley et al., 1996; Ireland et al., 1996; Gewurtz et al, 2000)) are deposited in the sediment and later they or their by-products are released in living organisms. When heavy rains occur in the headwaters, there is torrential flow downstream, disturbing the bed all the way to the mouth, At such times the bed load containing substances such as metals or chemicals may be carried a long way and deposited downstream.

The microcrustacean D. magna has shown good responses in sediment exposure tests (Nebecker et al., 1984; 1988; Suedel et al., 1996; Terra et al., 2004; Gillis et al., 2005).

The purpose of this study was to assess the influence of the sediment of three sites in Bom Jardim brook on Daphnia magna.

Material and methods

Eight sediment samplings at each of the three points were performed during the period of the study and used as substrate for D. magna (Clone A) between June 2001 and May 2002. Ten replications were used per sample moment, and each replication received a single microcrustacean. In order to collect information on the influence of the petrochemical complex installed in this area, three sampling stations were defined, located at sites close to the zone where the final liquid effluent is sprinkled, in landfarming (an area where the wastes are disposed of on the land), and at the stream mouth. Landfarming may result in contamination of adjacent areas due to rainfall or water. This flow method of waste disposal, common in petrochemical areas, may endanger local fauna (Schroder et al., 2000).

The three sampling stations (Fig.1) are described below:

BJ000- (29º50'11"S and 51º21'01"W)- This receives drainage water from the final disposal areas for liquid effluent and drainage from the landfarming of the integrated system of solid waste treatment in this complex. It is located downstream from BJ02;

BJ01- (29º50'33"S and 51º24'96"W)- This is located upstream from the drainage region of the final liquid effluent disposal areas;

BJ02- (29º50'27"S and 51º23'37"W)- This is located next to one of the areas used for disposal of the final liquid effluent. It is also influenced by drainage from the sludge farm and the solid waste disposal area.

The chronic assay (21 days) was used, with exposure of organisms aged 2 to 26 hours at the beginning of the observations. The period of exposure was defined so that the compounds added to the sample would act from the initial phase of microcrustacean life until the senile phase, passing through the reproductive period. At the same time as the assays with sediment samples, observations were performed using microcrustaceans exposed only to the culture medium (control group), in order to evaluate the health of the lots used for the observations. This group was submitted to the same forms of medium substitution, feeding and observations.

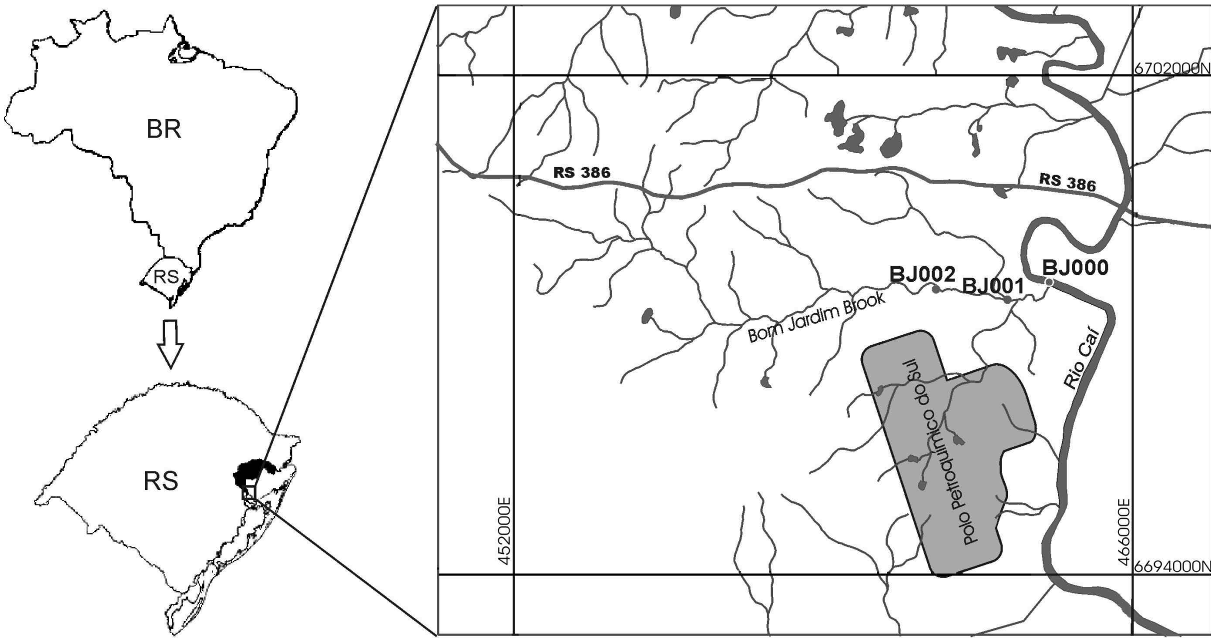

Figure 1: Location of the study site in Brazil, Rio Grande do Sul state; BJ000, BJ01, BJ02, sampling stations.

All experiments were conducted in incubators at 20±1°C under a 16h light/8h dark photoperiod. Ten microscrustaceans disposed individually in 50 mL beakers were used for each period of the study. Each beaker received one part of sediment and four parts of liquid medium (v:v), in the same ratio used in other experiments (Nebeker et al., 1986; 1988; Burton, 1992; Suedel et al., 1996; Terra et al., 2001). The beakers were covered with Parafilm®. We used culture medium M4 (Eledent & Bias, 1990) so that the organisms exposed would not be weakened by nutrient deficiency, during the period of exposure. The sediments were collected with a Petersen grab sampler and kept cool at 4°C from collection to time of use (Nebeker et al., 1988; Borgmann & Norwood, 1993; Ingersol et al., 1995; Terra et al., 2004). All tests were performed with bulk sediment samples (Ankley, 1991; Burton, 1992; Schuytema et al., 1996), without sieving the sample, but removing the large organisms with pincers (Ingersol et al., 1995), because the presence of indigenous organisms may greatly affect the chronic end point in sediment toxicity tests (Reynoldson et al., 1994). Horn et al. (2004) analyzed the grain size in the Bom Jardim brook area, classifying them as gravels, sands, silt and clay.

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 in 0.9 ±0.11 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 LC50-24h.

The liquid medium was removed on Mondays, Wednesdays and Fridays using a microcrustacean collector to take out the individuals, and new M4 medium was introduced with a washing flask in order to avoid damage or stress. At the time of medium removal, the microcrustacean was taken out, avoiding stress caused by lack of water, and it was placed back into the beaker with new culture medium. The same sediment sample remains throughout each experiment. Whenever the medium was changed, the survival of adults was observed, and from the reproductive period onwards, the young individuals were counted and eliminated. The food supplied consisted of the Chlorophyceae alga, Scenedesmus subspicatus at a concentration of 10^7cells mL^{-1}, ad libitum, complementing the food available in the sediment. Scenedesmus subspicatus was supplied on days when the medium was removed, in a sufficient volume (1 mL) to ensure food supply until the new removal. The alga was cultivated in our laboratory from monospecific cultures, without contamination, kept in a CHU nutrient medium (Chu, 1942).

The Duncan test (SPSS 9.0 for Windows) and the reproductive mean were applied to analyze the data on births. The minimum satisfactory limit for births was considered to be the mean of 20 live individuals per brood (Cowgill, 1985) at each assay. To evaluate reproduction we also applied the Spearman correlation between sampling sites, besides the deviations from the reproductive mean (chronic toxicity).

At least 80% of survivors were expected at each sample moment in order to evaluate survival (acute toxicity).

Indexes (from zero to three) were established, for each studied parameter (reproduction and survival). The lowest index identified the best quality, and zero indicated the ideal condition; a weight of three represents the most critical situation for the ecosystem. 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 number of births per offspring, obeying the following schedule: 0= ³20 neonates, 1= 15 to 19 neonates, 2= 10 to 14 neonates and 3= £9 neonates.

The indexes were used to identify the most critical site and the most sensitive parameter (survival or reproduction).

Results

Less than 80% survival was observed in seven of the eight samplings in BJ000, since of the 80 individuals exposed per sampling site, there was 97% survival in BJ01, 91% at BJ02, and only 40% in BJ000 (Fig. 2).

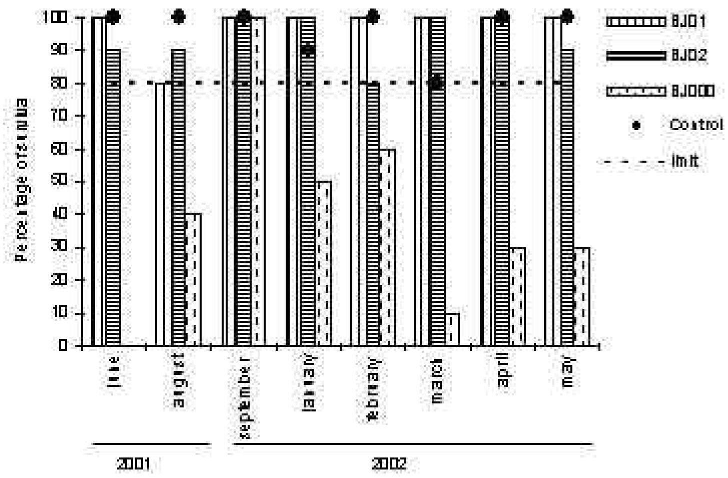

Figure 2: Percentage of survival of D.magna exposed to sediment samples from Bom Jardim brook, control group and expected survival from June 2001 to May 2001.

Survival in BJ000 reached zero in June 2001, and total mortality occurred 72 hours after the beginning of exposure. In March 2002 the same site showed only a single surviving individual at the end of experiment, 50% mortality having occurred in the first 72 hours, another 30% up to 120 hours and another 10% by the seventh day of exposure. The only surviving individual presented retarded growth, beginning reproduction only on the tenth day of the assay. At BJ000, mortality occurred during the initial half of the observations (Fig. 3).

Applying the Duncan test (a=0.05), we find that in BJ000 the production of young was lower in 100% of the samplings compared to the other stations. The absolute number of births indicates a depression at all times for BJ000 (Fig. 4).

The total of neonates shows the reproductive inhibition at BJ000 compared to the other sites, since in this site 1,104 individuals, in BJ01, 4,502 and in BJ02, 3,992 cladocerans respectively were born.

The Spearman correlation was positive (a=0.01) for reproduction between BJ01 and

BJ02 sites. Table I presents the mean and the standard deviation (CI=95%) of the neonates per sampling sites, and the standard deviations between births were rather high at the three sites.The reproductive mean per month sampled was lower in BJ000, followed by BJ02.

The survival indexes are high for BJ000, confirming site involvement, whereas at all other sites they were always zero, since survival was higher than 80% at all times (Fig. 5).

D. magna presented reproductive deficiency which can be verified from the high indexes presented (Fig. 6).

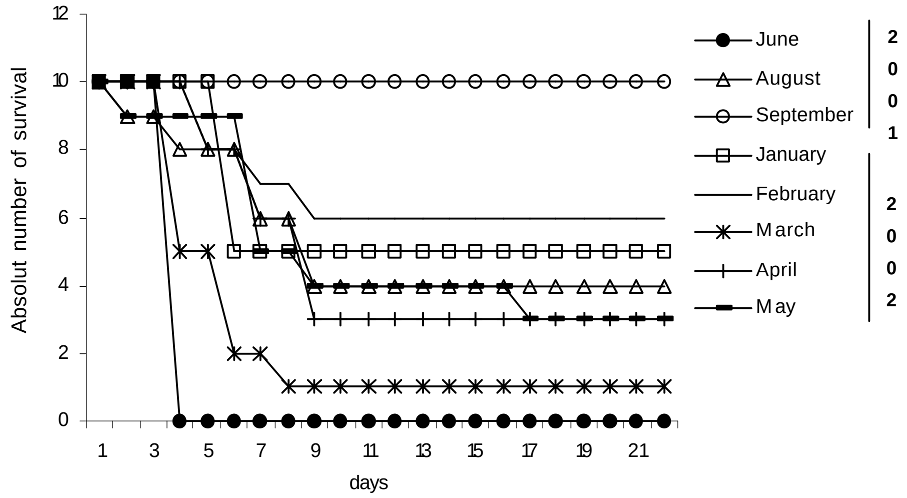

Figure 3: Monthly survival curve of D.magna exposed to sediment samples from Bom Jardim brook during 21 days of exposure from June 2001 to May 2002.

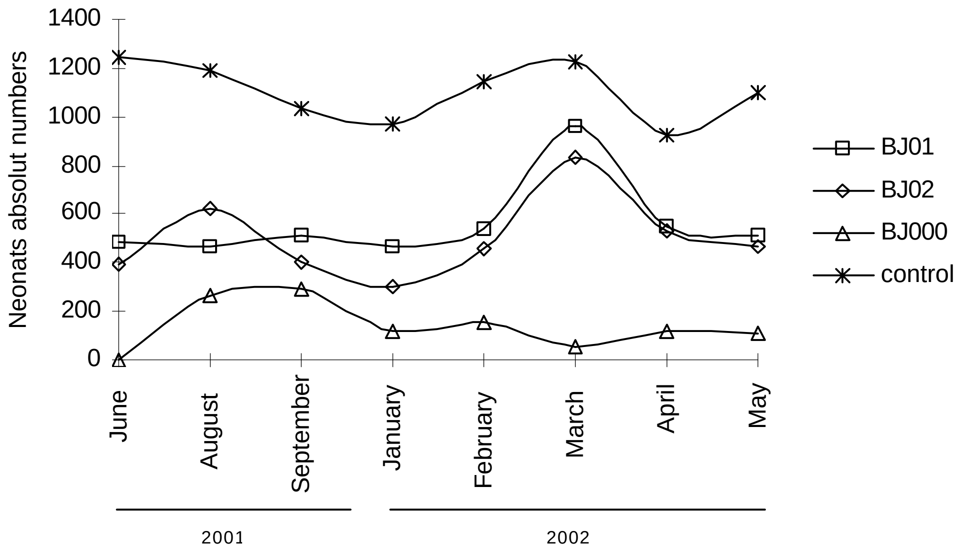

Figure 4: Absolute number of neonates per site and month from June 2001 to May 2002.

Table I: Means and standard deviations of the births, per sampling site, from June 2001 and May 2002

SITES	MEAN		SD
BJ000	138	±	98.3274
BJ02	499	±	164.9580
BJ01	563	±	165.0305

N=8

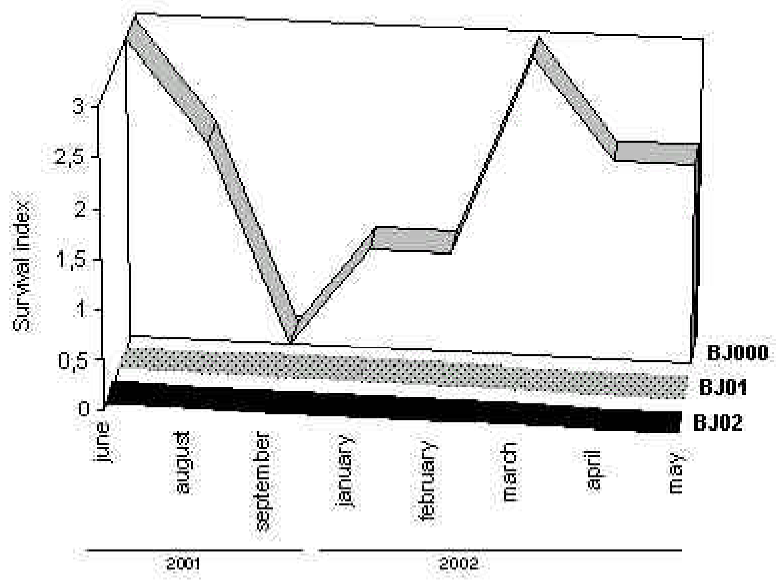

Figure 5: Survival index of D.magna exposed to sediment samples from the three sites evaluated in Bom Jardim brc

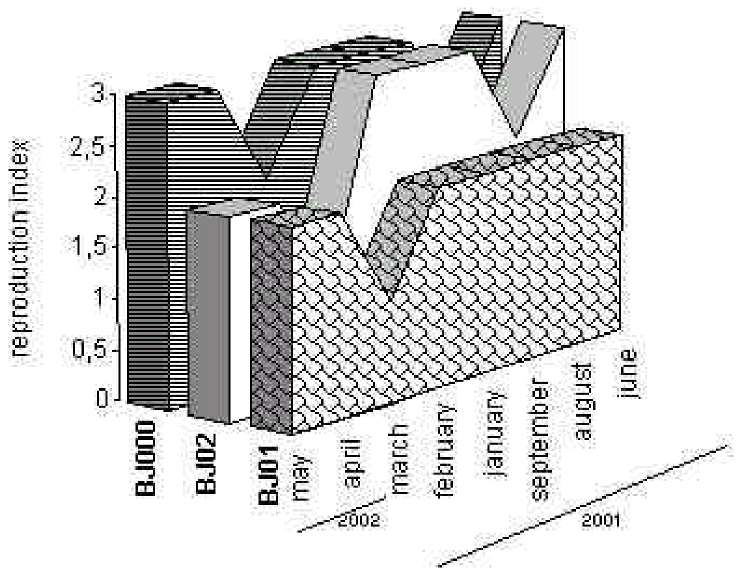

Figure 6: Reproduction index of D.magna exposed to sediment samples from Bom Jardim brook, according to month and site sampled from June 2001 to May 2002.

Discussion

Small cladocerans present rapid growth during the ontogenetic cycle, and therefore it is easier to get answers in a shorter time in stress-causing environments (Terra et al., 2001).

Survival (less than 80%) in seven of the eight samplings in BJ000, indicate the low quality at that site. The percentages of survival shown in Fig. 2 identify the BJ000 sediment as the one that has been altered most in Bom Jardim brook. Studies developed in the industrial complex area identified a genotoxic effect on human lymphocytes through SCE (Lemos et al., 1994), micronuclei induction in human cells (Lemos & Erdtmann, 2000) and mutagenic action (Vargas et al., 1993; 1995) in samples in this region show a change in the quality of the study area.

Analyzing the sequence of deaths in BJ000: 100% in 72 hours in June 2001; a single surviving individual in March 2002, 50% of the mortality having occurred in the first 72 hours, another 30% up to 120 hours and another 10% by the seventh day of exposure, the site may be considered inappropriate to sustain microcrustacean life. The only surviving individual presented retarded growth, beginning reproduction only on the tenth day of the assay. In BJ000, mortality occurred during the initial half of the observations, remaining stable until the end of the assays, a case of acute toxicity (Fig. 3). A natural selection process of cladocera individuals occurred at the initial moment of exposure, and only the stronger ones survived and continued the reproductive process, although less satisfactorily than expected.

Evaluating the reproductive aspects using the Duncan test (a=0.05), we find that in BJ000 the production of young was lower in 100% of the samplings compared to the other stations.

Considering reproduction, in all sampling stations, an average of less than 20 individuals per offspring was observed. Cowgill et al. (1985) found that the mean of 20 neonates per brood is expected, and that stressed animals have been known to produce small broods. The toxicity of aromatic hydrocarbons, in particular those of the polyaromatic group (PAH), is clearly identified in aquatic organisms (Trucco et al., 1983). This by–product of oil, present in petrochemical activity, may alter the life of cladocerans (Trucco et al., 1983). PAHs are prevalent pollutants in the aquatic environment and may cause a broad range of toxic effects (Ireland et al., 1996). According to Gewurtz et al. (2000) PAHs may accumulate in the trophic system, and be transferred directly to higher levels. These contaminants are potentially able to increase the toxicological stress in aquatic ecosystems. Sediments serve as the ultimate repository of PAHs (Ankley et al., 1996). In the study area, besides this priority pollutant, there are other factors and pollutants that could alter environmental quality, such as chloride, nitrates, transparency, turbidity, iron, aluminum, titanium, chromium, conductivity, total residue and elevation of conductivity.

The absolute number of births indicates a depression at all times for BJ000, probably because the material carried from upstream areas is accumulated on the bed at this site (Fig. 4). While in BJ01, 4,502 and in BJ02, 3,992 cladocerans were born, respectively, in BJ000 this number was reduced to 1,104.

BJ000 always presented the worst reproductive level, and in June 2001 no reproduction occurred due to the high mortality at the beginning of the observations. Impact on the reproduction of the daphnids exposed to samples from sites contaminated by a petrochemical industry was also observed by Nikunen (1985). Because of test duration, besides being exposed longer to pollutants through food and gill filtration, D. magna absorbs harmful agents available in the environment, since the cladocerans, (epibenthic cladocerans), excavate the sediment surface (Suedel et al., 1996) releasing substances adsorbed to them, even if they receive food ad libitum. The presence of sediment particles in the gut indicates that D. magna used in whole sediment bioassays ingest sediment (Gillis et al., 2005).

The BJ01 and BJ02 sites showed a positive Spearman correlation (a=0.01) for reproduction, possibly because these two sites were close to each other and did not suffer direct influence of drainage water from the final disposal areas for liquid effluent and drainage from the landfarming of the integrated system of solid waste treatment, present in BJ000.

The mean and the standard deviation (CI=95%) of the neonates presented in Tab. I show a high variability for births. This variability is common, occurring even at sites that have not been altered, as previously observed (Terra & Feiden, 2003). However, environmental pressure may increase this variability, as seen in BJ000, the site that suffered the most impact, followed by BJ02.

The elevated survival indexes observed in BJ000 confirm the negative influence of the environment on the survival of microcrustaceans since whereas at all other sites they were always zero, in BJ000 survival was higher than 80% at all times (Fig. 5).

A study performed using water samples from the Caí River at the mouth of Bom Jardim brook showed that some environmental parameters presented a correlation with micronucleus induction. When negative with dissolved oxygen and positive with air temperature and rainfall, they showed a tendency to associate with the genotoxic effect observed (Lemos & Erdtmann, 2000). The altered sites assessed suggest the onstant presence of small amounts of pollutants that could interfere in the development of D. magna, due to the reproductive deficiency expressed by the high indexes (Fig. 6).

In this study we identified reproduction as the most sensitive parameter involving survival. This had already been observed by Nikunen (1985). When the indexes dealing with survival are added to reproduction, BJ1000 is considered the worst site sampled (total of indexes, 36), followed by BJ02 (total of indexes: 19).

Reproduction data suggest that the quality of Bom Jardim decreases towards the mouth, according to the decreasing gradient of survival at BJ000 in relation to the other sites sampled.

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