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Water Quality Assessment in Piracicamirim Creek Upstream and Downstream a Sugar and Ethanol Industry Through Toxicity Tests With Cladocerans

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ABSTRACT

An environmental impact study was conducted to determine the Piracicamirim's creek water quality in order to assess the influence of effluents from a sugar industry in this water body. For this, toxicity tests were performed with a water sample upstream and downstream the industry using the microcrustaceans Daphnia magna, Ceriodaphnia dubia *and* Ceriodaphnia silvestrii *as test organisms, as well as physical and chemical analysis of water. Results showed that physical and chemical parameters did not change during the sampling period, except for the dissolved oxygen. No toxicity was observed for* D. magna *and reproduction of* C. dubia *and* C. silvestrii *in both sampling points. Thus, the industry was not negatively impacting the quality of this water body.*

Key words: *Daphnia magna*, *Ceriodaphnia dubia*, *Ceriodaphnia silvestrii*, water quality

INTRODUCTION

Pollution of aquatic environments by the industrial and municipal wastewater has been a growing problem in many countries (Wang and Freemark 1995). In Brazil, the sugarcane sector plays an important role in the development of the country, since it is the largest sugarcane producer. Effluents discharged by this sector can produce changes in both aquatic fauna and flora, besides endangering the human health who use river water for domestic and agricultural purposes (Ayyasamy 2008). As regards the negative impact of industrial wastewater in aquatic environments, physical and chemical parameters are insufficient to assess the damage in this environment (Dalzell 2001). It is, therefore, essential to use the biological systems on toxicity tests using the living organisms since they interact with the pollutants, thus giving an effective response on the water quality (Wang and Freemark 1995; Gibson et al. 2000). Therefore, given the importance of both the methods, Cairns (2002) emphasized that there must be an association between the physical/chemical and biological monitoring, thus allowing a better assessment of the water pollution.

Studies have shown that the industrial discharges despite meeting the criteria and regulations established based on the physico-chemical and microbiological parameters exhibit high toxicity to

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aquatic biota (Sotero-Santos et al. 2005). In order to evaluate such toxicity, ecotoxicological tests are used, since they are able to evaluate the toxicity of substances or mixtures, such as industrial effluent (Eckenfelder 2000).

Microcrustaceans are commonly used in toxicity tests because they have high sensitivity, availability and abundance, knowledge of their biology and ease of cultivation in the laboratory (Rand and Petrocelli 1985).

As the use of organisms at the same or different trophic levels may have different results for the toxicity (Chapman 2000), the objective of this work was to evaluate the Piracicamirim´s creek water quality upstream and downstream which has discharge from an ethanol industry for physico-chemical and ecotoxicological tests with *D. magna*, *C. dubia* and *C. silvestrii*.

MATERIALS AND METHODS

Characterization of study area

Piracicamirim creek is a tributary of the Piracicaba River and its watershed spans an area of 133 km^2 (Ometto et al. 2000). Climate is subtropical and the meteorological characteristics define a rainy season (October to March) and a dry season (April to September) (Ometto et al. 2000; Silveira et al. 2000). Located in the municipalities of Piracicaba, Rio das Pedras and Saltinho, the Piracicamirim creek has 24.6 km length from its source (located in Saltinho-SP) to its mouth in the Piracicaba River. It is 4.26m wide and has 1.56 m depth on average (Ometto et al. 2000). Currently, this watercourse is considered polluted, even after the construction of a sewage treatment plant in 1997 due to illegal sewage spills and chemical discharges of sugar cane crops (Ometto et al. 2004).

Sampling stations, methods of collection and preservation

Surface water samples were collected upstream and downstream an ethanol industry in the municipality of Rio das Pedras-SP. Samples were collected monthly from April to November 2010, during the harvest period. The collected water was stored in appropriate containers, identified, placed in polystyrene boxes with ice and taken to the laboratory. Physical and chemical parameters evaluated at each sampling included pH, conductivity, dissolved oxygen, water temperature and room temperature.

Acute toxicity test with *D. magna*

Organisms used in the test (6-24 h old) were obtained from the cultures performed in the laboratory at 20 ± 2°C and16h:8h photoperiod (light: dark). Reconstituted water with conductivity less than 10 μS/cm-1 was used for the medium (medium M4) developed by Elendt and Bias (1990), prepared from the purified water plus salts, with 250 ± 25 mg/L $CaCO_3$ total hardness and 7-8 pH and with 300 ± 25 μS/cm-1 conductivity. Algae *Pseudokirchneriella subcapitata* were provided for feeding (3 x 105 cells/mL).

The acute toxicity test was done following the ABNT standard (2004). Samples were processed at the following dilutions: 100% (1:1), 50% (1:2) and 33.34% (1:3), using the medium M4 as dilution water according to Table 1. At each dilution 10 neonates were added using two replicates. Test duration was 48 h in the dark, without food at 20°C ± 2. Results of effects observed for *D. magna* were expressed as toxicity factor (TF) that corresponded to the lowest dilution, which occurred immobility or up to 10% death. The result was expressed in integer equivalent to the test solution's dilution factor (Knie and Lopes 2004).

Table 1 - Sample dilution factor for acute toxicity test (48 h) with *Daphnia magna.*

Sample Dilution	Dilution Factor (FD)	Sample (mL)	Dilution Water (mL)	Final Volume (mL)	Test Solution Concentration (%)
Control	-	-	50	50	-
1:1	1	50	-	50	100
1:2	2	25	25	50	50
1:3	3	16.67	33.33	50	33.33

Toxicity tests with *C.dubia* and *C. silvestrii*

To maintain the growth of *C. dubia* and *C silvestrii* ABNT (2005) protocols were followed. Organisms were kept in the glass bottles containing 500 mL MS medium (Keating 1985) and 40 adult organisms in each culture. They were placed in incubator at 25°C ± 2, 16 h light and 8 h dark period. The culture medium was renewed twice a week. *P. subcapitata* was provided for feeding (approximately 1 x 10^5 cells per organism) three times a week. To implement the chronic toxicity test, the ABNT standards (2005) were followed. For each sampling point (upstream and downstream), 15 mL sample in its natural concentration (100%) was added to the containers of 20 mL capacity. A control group with MS medium was also used. Each point and the control contained 10 repetitions and one organism aged between 6 to 24 h at each replicate. The test duration was 7 days and solutions were renewed every 2 days. The test was conducted in an incubator at 25ºC ± 2, 16 h light and 8h dark photoperiod. *P. subcapitata* was provided for feeding. At each renewal and end of the test, the adult organism was transferred to another container of equal volume and the number of born neonates was counted at each replicate.

Statistical analysis

Data obtained for *C.dubia* and *C. silvestrii* were analyzed by ANOVA followed by Tukey test with $\alpha = 0.05$.

RESULTS AND DISCUSSION

Among the physical and chemical parameters analyzed, pH was the one which had minor variation over the sampling period, both upstream (5%) and downstream (1%), followed by the conductivity, air and water temperatures and final dissolved oxygen, which had a larger variation (Table 2).

Table 2 - Physical and chemical variables (means, standard deviation and coefficient of variation) of water upstream (A) and downstream (B) an sugar and ethanol industry from April to November 2010.

A	Parameters	Mean	Standart Deviation	Coefficient of Variation (%)
	Water temperature	17.90	±3.37	18
	Air Temperature	25.91	±4.25	16
	pH	6.84	±0.39	5
	Conductivity	96.45	±12.25	12
	Dissolved Oxygen	5.13	±1.81	35
B	**Parameters**	**Mean**	**Standart Deviation**	**Coefficient of Variation (%)**
	Water temperature	21.34	±2.58	12
	Air Temperature	27.80	±4.87	17
	pH	6.89	±0.07	1
	Conductivity	155.33	±18.70	12
	Dissolved Oxygen	5.68	±1.49	26

For the toxicity test with *D. magna*, no acute effect on mobility was observed in all the tested dilutions of the water samples upstream and downstream from April to November 2010. Thus, the toxicity factor value for *D. magna* obtained for all the samples was equal to 1, meaning that there was no toxicity even in the pure sample.

About the chronic toxicity test with *C. dubia*, only the reproduction values in the control and downstream were statistically equal in every month (capital letters) (Table 3). Upstream, the average number of reproduction was statistically the same from April to October 2010 and different in November 2010 (capital letters) (Table 3).

For *C. silvestrii*, only the number of control's neonates was statistically equal during the months analyzed (capital letters) (Table 4). Upstream, the reproduction values observed were statistically equal from April to September 2010, differing in October and November 2010. However, the average reproduction in these two months was equal to those in April, July, August and September (capital letters) (Table 4). Downstream, April, May, June, July, September, October and

November had the same average reproduction, differing only in August, which was statistically equal to May, July, October and November (capital letters) (Table 4).

Analyzing at each sampling site plus the control month after month (lowercase), it was clear that the control, upstream and downstream were statistically equal for *C. dubia* between April and October 2010 (Table 3), whereas the reproduction in the control and downstream were statistically identical for *C. silvestrii* in April, May, July, September and November (Table 4).

Tables 3 and 4, show the results water toxicity upstream; Tables show the same for downstream for *C. dubia* and *C. silvestrii*, respectively.

Table 3 - Average number of *C. dubia* neonates after 7 days of exposure to Piracicamirim's creek water (Piracicaba-SP) upstream and downstream an sugar and ethanol industry.

TRE	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV
C	15.30aA	15.10aA	15.00aA	15.20aA	15.70aA	15.30aA	15.80aA	15.00aA
U	20.00aA	14.10aA	16.60aA	11.40aAB	14.20aA	8.50aAB	9.10aAB	0.90bB
D	10.80aA	19.10aA	16.00aA	19.10aA	17.40aA	12.00aA	20.20aA	14.40aA

C = Control, U = Upstream, D = Downstream. Means followed by the same letters do not differ by Tukey test with $\alpha = 0.05$.

Table 4 - Average number of *C. silvestrii* neonates 7 days after exposure to Piracicamirim's creek water (Piracicaba-SP) upstream and downstream an sugar and ethanol industry.

TRE	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV
C	15.40aA	18.20aA	21.40aA	19.20aA	16.20aA	15.70abA	15.00abA	15.80aA
U	17.70aA	23.30aA	21.40aA	14.50aAB	13.80bAB	11.50aAB	7.50bB	7.50aB
D	12.20aB	18.50aAB	4.50bB	23.80aAB	29.70aA	11.20aB	22.90aAB	17.90aAB

C = Control, U = Upstream, D = Downstream. Means followed by the same letters do not differ by Tukey test with $\alpha = 0.05$.

Table 5 - Comparison of toxicity tests results with *C. dubia* from the water sample collected upstream and downstream an sugar and ethanol industry from April to November 2010 compared to the control.

T	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV
U	NT	NT	NT	NT	NT	NT	NT	TO
D	NT	NT	NT	NT	NT	NT	NT	NT

T = Treatment, U = Upstream, D = Downstream.
TO = Toxic, NT = Non Toxic.

Table 6 - Comparison of toxicity tests results with *C.silvestrii* from the water sample collected upstream and downstream an sugar and ethanol industry from April to November 2010 compared to the control.

T	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV
U	NT	NT	NT	NT	TO	NT	TO	TO
D	NT	NT	TO	NT	NT	NT	NT	NT

T = Treatment, U = Upstream, D = Downstream.
TO = Toxic, NT = Non Toxic.

Cladocerans, especially daphnids, are widely used as test organisms in ecotoxicological studies, and its known sensitivity is certainly one of the reasons for its acceptance (Viganò et al. 1996). Several studies have compared the sensitivity of these organisms with other groups of aquatic organisms (Viganò et al. 1996). Overall, it has been observed that daphnids have a similar sensitivity to toxic substances (Mount and Norberg 1984; Kline et al. 1989) and *C. dubia* is sometimes more sensitive than the *D. magna* (Winner 1988; Mokry and Hoagland 1990; Kszos et al. 1992).

The toxicity of a river whose basin is heavily industrialized and urbanized can be caused by many unknown contaminants (Viganò et al. 1996). Although toxicity tests could not provide information of probable toxicants present in the environment, Kimerle et al. (1985), using this type of test with at least two different species, reported reliable results in relation to the danger of toxic substances in the environment.

Concerning the physical and chemical analyses of water, comparing the parameters of both the sampling sitess no change was observed. There was no toxic effect of water for *D. magna*, *C. dubia* and *C. silvestrii*, showing that the three species had similar sensitivities in relation to water of the sampling points.

Several studies have reported the influence of industrial discharges on water quality and aquatic organisms. For example, Knie and Lopes (2004) reported the quality of effluents from the pharmaceutical, textile, metallurgical, electroplating and drinks industries and observed a 64 toxicity factor for the *V. ficheri* in the textile industry, while for the *D. magna* the same industry had toxicity factor equal to l6, which proved the high toxicity of these effluents. According to these authors, when the result of acute toxicity presented toxicity factor value higher than one, sample had toxicity, but if this value was equal to 1 (as in this study) the sample was not toxic, since it was unnecessary to be diluted in order to not cause effect to the organisms. In another study, Nedeau et al. (2003) analyzed the water samples collected downstream an industrial effluent and it had negative effect on the benthic community. However, Gómez et al. (2008) evaluated the effect of effluents from a textile industry on the water quality and habitat through the benthic community. Results showed that the water after downstream the industry decreased species richness and abundance. Arimoro (2009) reported a drastic reduction of benthic macroinverbrate species after the discharge effluent of a rubber industry. Yadav et al. (2009) correlated the effect of effluents from the fertilizer industry with reduced levels of acetylcholinesterase from various organs of the fish species *Channa striatus*. Assessing the toxic effects of industrial effluents, Radic' et al. (2010) observed growth inhibition and chlorophyll levels in duckweed (Lemna L. minor). Liu et al. (2002) found that the effluent of an electroplating industry was highly toxic to *Daphnia similis*. Ayyasamy et al. (2008) evaluated the toxicity of effluents from a sugarcane industry on the terrestrial and aquatic plants, and observed that the concentrations above 60% affected the growth and there was decrease in the levels of chlorophyll, protein and carbohydrates with increasing concentrations.

Although no toxic effects was observed from the samples collected after the discharge of the effluent from the alcohol industry on *D. magna*, *C. dubia* and *C. silvestrii*, it would be necessary to give greater importance to long-term monitoring studies in aquatic environments near the industrial activity using the aquatic species as test organisms.

CONCLUSION

According to the results of physical and chemical parameters and acute toxicity tests with *D. magna* and chronic tests with *C. dubia* and *C. silvestrii*, the industry was not impacting this aquatic environment since most of the observed toxicity occurred upstream industry.

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