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Evaluation of the Atibaia River water quality using *Lecane bulla* as a test organism

Avaliação da qualidade das águas do Rio Atibaia usando **Lecane bulla** *como organismo teste*

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ABSTRACT: In view of the diversity of environments found in the Brazilian territory, it is understandable that the use of native species can provide more relevant information for ecotoxicological studies. The purpose of this work was to evaluate the quality of water samples from the Atibaia River in an area that is under the influence of petroleum refinery using a native test-organism and submitting the data to PCA statistical analysis. Therefore, acute toxicity assays with *Lecane bulla* (Rotifera) were performed in four locations of the river, as well as physical-chemical analyses. Sampling was drawn in the dry and rainy seasons. The bioassays were static and lasted 48 hours; dead organisms were quantified at the end of the tests. Toxicological differences among the samples/per location and control were compared by means of the Analysis of Variance. Physical-chemical and mortality variables were simultaneously analyzed by multivariate analysis of the principal components and the Pearson correlation coefficient. Water samples from the exit of the refinery stabilization pond (location S.1) were toxic to *L. bulla* in both seasons, with significant differences in relation to the control and between the seasons. The statistical treatment of data showed that mortality was strong and positively correlated with total hardness, chlorides and EC, which together with pH presented higher values in location S.1, in the dry and in the rainy seasons. Due to its sensibility to the quality of the Atibaia river water samples, the potential use of *L. bulla* for ecotoxicological studies as an alternative test organism could be demonstrated.

KEYWORDS: multivariate analysis; PCA; bioassays; acute toxicity; rotifer.

RESUMO: Em virtude da diversidade de ambientes encontrada no território nacional, o uso de espécies nativas em estudos toxicológicos constitui assunto de extrema relevância. Este trabalho objetivou avaliar a qualidade das águas do rio Atibaia, em uma região sob influência de uma refinaria de petróleo utilizando-se um organismo-teste nativo, e aplicando-se o método estatístico multivariado análise de componentes principais (PCA). Ensaios de toxicidade aguda com o rotífero *Lecane bulla* (Rotifera) e análises físico-químicas foram realizados com amostras obtidas em quatro diferentes pontos do rio em questão. As amostragens ocorreram nas estações seca e chuvosa. Os bioensaios foram estáticos e duraram 48 horas; organismos mortos foram quantificados no final dos testes. Diferenças toxicológicas entre as amostras de cada ponto e o controle foram comparadas por análise de variância. As variáveis físico-químicas e a mortalidade foram simultaneamente submetidas à análise multivariada dos componentes principais e pela correlação de Pearson. Amostras de água retiradas da saída da lagoa de estabilização (ponto S.1) foram tóxicas ao organismo *L. bulla* nas duas estações, com diferenças significativas em relação ao controle e entre as estações. O tratamento estatístico dos dados demonstrou que a mortalidade correlacionou-se forte e positivamente com parâmetros como dureza total, cloretos e EC, e, com o pH, apresentou valores maiores no ponto S.1 em ambas as estações. A sensibilidade apresentada pela espécie *L. bulla* em relação à qualidade das amostras da água do rio Atibaia indicou que este organismo apresenta potencial de uso para estudos de ecotoxicologia, podendo ser futuramente empregado como organismo-teste alternativo.

PALAVRAS-CHAVE: análise multivariada; PCA; bioensaios; toxicidade aguda; rotífera.

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INTRODUCTION

The Atibaia River runs through a large industrial and metropolitan area and several cities use the waters of this river as source of public water supply. Along its course, the river receives discharges of urban sewage and effluents of many industries, including a petroleum refinery (Hoshina et al., 2008).

Ecotoxicological assays aiming at monitoring bodies of water generally use internationally standardized exotic species. It is known, however, that determining such toxic levels using native species would provide the ecotoxicological studies with higher representation and reliability. Rotifers prove to be a suitable alternative for test organisms, since they present parthenogenetic reproduction, short life cycle and gestation periods, besides occurring in Brazilian aquatic ecosystems.

The abundance of rotifers shows how important they are as one of the largest groups of freshwater zooplanktons in limnological studies, together with Cladocera (Anomopoda) and Copepoda. They make up organisms that are frequently used in aquaculture and are strongly connected with aquatic habitats in virtually all of their active stages, and only their resting stages are drought-resistant (Segers, 2008)

In a water quality assessment, different approaches can be used, including chemical analysis and toxicity tests. When applied individually, they can lead to great uncertainties; however, if used together, they generate safer data on environmental conditions (César et al., 2007). The use of multivariate analysis comes as a valuable tool for interpreting the obtained environmental data. Principal component analysis (PCA) provides information about the most significant parameters which describe all the interpretation of the mass of data, data reduction, besides summarizing the statistical correlation among constituents in the water with minimum loss of the original information (Helena et al., 2000).

The aim of this study was to evaluate the water quality of the Atibaia River using the rotifer *Lecane bulla* as an indicator-organism, showing its potential for this use. The principal component analysis was performed to show the parameters measured in the water that might interfere with the same quality and mortality of the rotifer. This kind of analysis aims at identifying the variables that better explain seasonal and temporal variations and the relationship between them.

MATERIAL AND METHODS

Sampling

Water samples were collected close to a petroleum refinery in the state of São Paulo, as follows: site (S.1) corresponds to the output of the stabilization pond effluent from the petroleum refinery, biologically treated, stabilized for about seven days and discarded in the Atibaia River; site (S.2) 200 m upstream of the discharge of effluents refinery stabilization pond; site (S.3) 200 m downstream from the effluent disposal; and site (S.4) 800 m downstream from discharge, as shown in Table 1. Sampling was conducted during Brazil's dry (April, May, June and August, 2008) and rainy (October, December, 2008 and Februbary, 2009) seasons. All samples were stored at 4ºC up until the moment the assays were carried out.

Water quality analyses

Physical-chemical analyses were carried out using the methodology described by the Standard Methods (Clesceri et al., 1998). The analyzed parameters were hydrogenionic potential (pH), electrical conductivity (EC) in µS/cm, dissolved oxygen (DO) in mg/L of O_2, total hardness in mg/L $CaCO_3$, apparent color in Pt-CO/L and chlorides in mg/L of Cl^{-1}. Previous analyses on ammonia concentration of the studied sites (data not shown) were found between 2.28 – 2.65 mg/L with no statistical influence on the living organisms.

Test organism

L. bulla was obtained in Lobo-Broa Reservoir (Itirapina, Brotas, SP; 22º15'S, 47º49'W), bred in laboratory, being fed with microalgae *Pseudokirchneriella subcapitata* containing approximately 1 x 10^6 cells/mL. The animals were kept in clean places with no substance or toxic vapor, under a 16-hour photoperiod and an 8-hour darkness at a thermoperiod of 20 ± 2ºC. Breeding and test were carried out with dilution water, which had pH between 7.4 ± 0.2, total hardness of 44 ± 4 mg/L in $CaCO_3$ and EC of approximately 160 µS/cm.

Sensitivity test of *L. bulla* to a reference toxicant

Tests were conducted using juvenile rotifers. There is a clear distinction in size between adults and juvenile rotifers. Thus rotifers that appeared much smaller in size in comparison to full-fledged adults were collected and used directly in the testing.

Table 1. Sampling sites in the Atibaia River

Sites	W	S
	Grade min seg.	Grade min seg.
1	22 44 22.0	47 07 43.0
2	22 44 43.0	47 07 25.3
3	22 44 25.3	47 07 35.2
4	22 44 22.3	47 07 40.8

GPS equipment: Garmin, and Trex Summit; W: longitude West; S: latitude South

A light intensity of 600 lux, a light cycle of 16-hour light and 8-hour dark, and a temperature of 20.0 ± 2ºC was used in all tests, which were conducted in two well culture plates with a test well volume of 1 mL. Juvenile rotifers were transferred from their culture dishes to each test well using a glass micropipette so that there were animals in each well, the ones that had previously been fasting for 48 hours. Rotifers were transferred under a stereoscope (used at 40X magnification). Dilution water transferred with the rotifers was drained from each well with the use of a glass micropipette, and test solutions were added to the wells.

Stock solutions were serially diluted with dilution water to achieve the required test concentrations. A definitive toxicity test was undertaken using 2.5, 4.5, 6.5, 8.5 and 10.5 mg/L cadmium chloride (Sigma-Aldrich, purity 99%). Testing also included a negative control (dilution water only, 0 mg/L cadmium chloride). Ten replicates were used for each concentration.

After a 24-hour exposure period, mortality was recorded for each well. Rotifers were considered dead if they were not moving. Tests were accepted if there was at least 90% survival in the controls.

The LC50 value was calculated using JSPEAR, the Trimmed Spearman-Karber method (Hamilton et al., 1977). Thereafter, rotifers were exposed to this calculated LC50 concentration on a further ten occasions over an eleven-month period.

Acute toxicity test using *L. bulla* as test organism

Toxicity tests were performed using the same methodology for testing for sensitivity to cadmium chloride, by 48 hours of exposure to the Atibaia River water samples. Twelve replicates were used for each sampling.

Statistical analysis

L. bulla mortality data was statistically analyzed on the R platform (R Development Core Team, 2009). By means of the Analysis of Variance (ANOVA), considering the interaction of seasonal effects on factorial experiments, toxicity differences of sampled locations were compared with the control carried out in the dry season, which did not present mortality rate as in the rainy season. The data regarding physical-chemical variables, along with toxicity data, underwent a multivariate analysis of PCA using the aforementioned software. The multivariate factor analysis is an analytical technique resulting from a set of uncorrelated variables called factors, which explain the variation observed in the original set (Anazawa; Ohmori, 2005; Brown, 1998). Correlated analyses were developed for combined data of all sample locations for physical-chemical variables and mortality, which were calculated with the Pearson correlation coefficient.

RESULTS

Physical-chemical variables in the Atibaia River waters analyzed in the four sample locations (S.1; S.2; S.3 and S.4) in the dry and rainy seasons can be found in Table 2.

In all sample locations, the color variable presented concentrations above the water quality standards for Class II Rivers — CONAMA Resolution n. 357/05 (CONAMA, 2005) — and all of the values recommended by the World Health Organization (WHO) for drinking water quality (Who, 2004). The same was observed for DO values in locations S.2 and S.4 in the rainy season, and for chloride in location S.1 in both seasons. Total hardness parameters, EC, pH and chlorides presented higher values for location S.1 in comparison to the others in both seasons.

The 24-h LC50 average was calculated to be 3.86 mg/L cadmium chloride, which suggests the rotifers used in this study are reasonably tolerant to cadmium chloride.

The mean mortality rate of *L. bulla* was higher in the rainy season for all ocations but S.4. The highest mean mortality rates were found in location S.1 in both seasons (Fig. 1).

The Analysis of Variance results verified the influence of the location on mortality; S.1 and S.4 presented differences from the control ($p = 0.00264$; $p = 0.00324$). Seasonal effects were verified only for location 1 ($p = 0.0148$).

The correlation coefficient of the seven analyzed variables (Table 3) distinguished several relevant relationships (indicated by underlined values):

- strong, positive correlation between total hardness, pH, chlorides and EC ($r = 0.71 – 1.0$) was attributed to contributions of residuary waters;
- strong, negative correlation between color and pH, color and OD ($r = -0.88 – 0.91$);
- high positive correlation between pH and DO, pH and chlorides ($r = 0.68 – 0.72$); d) mortality rate was strongly and positively correlated with total hardness, chlorides and EC ($r = 0.51 – 0.73$). Concentrations of most physical-chemical parameters were highly correlated.

Analyses of PCA were simultaneously applied to physical-chemical and ecotoxicological data of surface water, from the four sampling locations, in order to extract the main factors corresponding to the different variation sources in water quality; the purpose was to determine the main controls for assessing surface water. The results obtained from the two main factors of PCA showed significant associations of different variants. Liu et al. (2003) classified load factors as 'strong', 'moderate' and 'weak' corresponding to the absolute load values > 0.75, 0.75 – 0.50 and 0.50 – 0.30, respectively. Both factors explained 86.21% of the variance in the set of original data, which was considered very good, and capable of identifying the main variance sources of the analyzed parameters.

Table 2. Water quality parameters at different sites of the Atibaia River.

Parameters	Rainy season				Dry season			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Total hardness (mg/L $CaCO_3$)	132.33 ± 35.08	27.33 ± 8.33	28.00 ± 5.57	28.67 ± 7.57	171.85 ± 51.23	37.40 ± 2.30	39.00 ± 0	34.75 ± 2.50
Electrical conductivity (μS/cm)	2558.33 ± 816.56	280.60 ± 162.73	247.39 ± 208.76	306.57 ± 207.00	1998.08 ± 631.11	202.55 ± 59.57	267.68 ± 64.83	214.93 ± 69.16
Dissolved Oxygen (mg/L O_2)	5.25 ± 1.26	4.33 ± 1.65	5.08 ± 1.53	4.08 ± 1.95	5.83 ± 0.19	6.27 ± 0.44	7.19 ± 1.21	7.85 ± 0.69
pH	7.86 ± 0.80	7.05 ± 0	7.35 ± 0.08	7.22 ± 0.31	7.93 ± 0.54	7.56 ± 0.27	7.46 ± 0.05	7.66 ± 0.25
Color (Pt-Co/L)	205.33 ± 53.67	693.67 ± 782.59	542.67 ± 537.24	673.67 ± 727.47	130.75 ± 43.83	141.50 ± 52.35	172.25 ± 42.15	164.50 ± 34.61
Chloride (mg/L)	590.05 ± 141.76	13.11 ± 6.98	17.04 ± 9.95	17.70 ± 10.22	766.13 ± 233.39	9.64 ± 1.33	16.78 ± 9.43	18.77 ± 6.01

SD: standard deviation.

Table 3. Correlations of chemical parameters and toxicity in the River (all sites and seasons combined).

	Mortality	Total hardness	Electrical conductivity	Dissolved Oxygen	pH	Color	Chloride
Mortality	1.00						
Total hardness	0.51*	1.00					
Electrical conductivity	0.73*	0.94*	1.00				
Dissolved Oxygen	-0.07	0.32	0.16	1.00			
pH	0.41	0.74*	0.71*	0.68*	1.00		
Color	-0.05	-0.47	-0.37	-0.91*	-0.88*	1.00	
Chloride	0.56*	1.00*	0.95*	0.25	0.72*	-0.41	1.00

The given correlation are the Pearson correlations. *Relevant correlations.

The first main factor was prevalent and represented 64.21% of total variance, with strong positive loads of total hardness, EC, pH and chlorides; and a strong negative load for color, indicating an intense correlation with these parameters, and moderate positive loads for mortality and DO. This can be attributed to a mineral component of the river water and could be called a "geogenic" factor. The second main factor represented 26.0% of total variance, showing a strong negative load for DO, and moderate positive loads for mortality rate and color, so that it could be called "anthropogenic factor" (Table 4).

With the aim of identifying seasonal and spatial tendencies of the concentration, the sampling locations were plotted (Fig. 2). A line over the ordinal plan showed that the samples in the rainy season tended to converge towards the upper left part of the plan, where the variables color and mortality had higher relative importance for the y-axis in this region; and along the x-axis there was high correlation with color, except for location S.1, presented in the upper right part, in which the variables pH, EC, total hardness and chlorides better explained its location in the x-axis. The samples in the dry season were located in the lower part, slightly directed towards the left, where greater importance was placed to DO in relation to the y-axis and towards color in the x-axis. Location S.1, however, was presented in the upper right part, in which the variables pH, EC, total hardness and chlorides better explain its location in the x-axis.

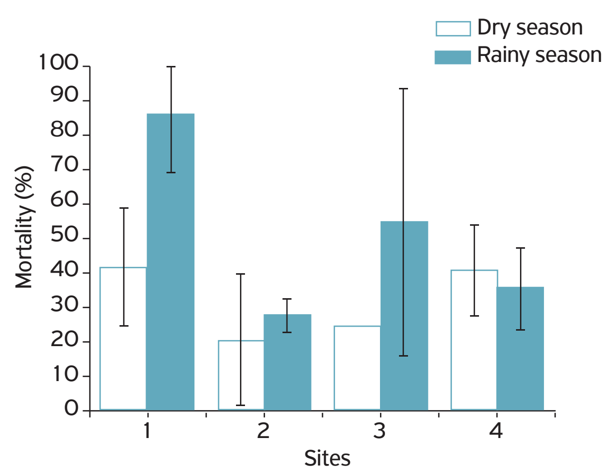
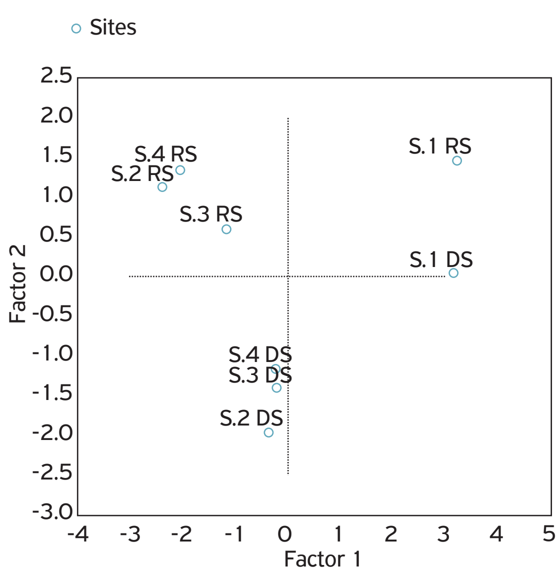


Figure 1. Mortality of *Lecane bulla* in water in different season and sites of Atibaia River

Table 4. Values of two extracted factor loadings for physical-chemical parameters and toxicity in the Atibaia River.

Variable	Factor 1	Factor 2
Mortality	0.576831*	0.582826*
Total hardness	0.928254*	0.226565
Electrical conductivity	0.905364*	0.401774
Dissolved Oxygen	0.541506*	-0.790965*
pH	0.920625*	-0.288813
Color	-0.709463*	0.686359*
Chloride	0.914781*	0.296130
Variablility (%)	64,21	26,00

*Absolute component loadings > 0.5. Relevant loadings, indicating significant contributors to the water quality.



RS: Rainy season; DS: Dry season.

Figure 2. Principal component analysis of the physical-chemical parameters and toxicity in the S.1 as S.4 sites in Atibaia River in dry and rainy seasons

When it comes to spatial distribution, scoring parts for the sample locations were better explained by factor 1 (y-axis), in which the variables pH, EC, total hardness, chlorides and color presented higher loads. Locations S.2, S.3 and S.4 were near the left axis, with high correlation with color, whereas location S.1 was located next to the right side of the axis, having a high correlation with the variables pH, EC, total hardness and chlorides.

DISCUSSION

High levels of EC, due to the significant amount of dissolved salts, were observed in all river locations in both dry and rainy seasons during the study; location 1, though, was in evidence, since it presented higher values than the other locations. The EC values were attributed to the mineral content in all sampled locations, in which ion concentrations were higher.

The values of pH in the collected water samples met the 6.5 – 8.5 standards specified by the WHO (WHO, 2004).

The highest chloride concentrations were found in location 1. Such values were higher than the ones recommended by WHO and CONAMA 357/05, which suggest concentrations that are lower than 250 mg/L of Cl. The concentrations in the dry season were higher than those in the rainy season.

Total hardness presented high concentrations in location S.1, being the concentrations in the dry season higher than the ones in the rainy season. High values of hardness may affect acceptability in water consumption concerning taste and deposition levels; however, ecological and epidemiological studies have shown inverse relationships between the water hardness and cardiovascular diseases (WHO 2004).

DO levels in locations S.2 and S.4 in the rainy season were in disagreement with CONAMA 357/05, which recommend DO values higher than 5 mg/L of O_2. Oxygen concentration monitoring in the aquatic system is important for the biological, chemical and physical systems. The variables involved in both the increase and the decrease of oxygen levels in the river water are so numerous and complex that there is no model capable of being used without carrying out a careful analysis of local characteristics.

Color results were high in all sample locations – higher than the 15 and 75 mg/L limits established by WHO and CONAMA 375/05, respectively, being the highest values found in the rainy season. Color is generally caused by the presence of organic matter (mainly humic and fulvic acids), especially associated with the fractions of humus found in soils of river banks. Color is also strongly influenced by the presence of iron salts and other metals, which might indicate sources of water contamination with industrial effluents and, consequently, a risky situation (WHO, 2004).

The results of susceptibility testing with $CdCl_2$ is higher than those found by Nandini et al. (2007), who reported a 24 LC50 of 0.19 mg/L for the freshwater rotifer *Brachionus macracanthus,* but lower than that reported for the water frog *Rana ridibunda* a 96 h LC50 of 51.2 mg/L (Selvi et al., 2003), and that determined for guppy *Poecilia reticulata* the 96 h LC50, 30.4 mg/L in a static bioassay test system, and 30.6 mg/L with Behrens–Karber's method (Yilmaz et al., 2004).

Surface water samples from location S.1 proved to be highly toxic for *L. bulla* in both seasons, presenting significant differences from the control by the ANOVA, as well as significant toxicity differences for this location between the two seasons. Values in this location were found higher than the ones recommended by WHO and CONAMA 357/05 for physical-chemical parameters of color and chlorides, besides presenting higher pH, EC and total hardness values. Location S.4 also showed significant differences in relation to control;

however, seasonal variation was not significant. The other locations did not show significant differences from the control or between the dry and the rainy seasons. Water toxicity of this area might be due to effluent inputs from fertilizer industries, petrol refineries and chemical companies located in the region.

The correlation analysis supported these results, since strong positive correlation was found between mortality rates and total hardness, chlorides and EC and between the variables themselves, which can reflect the inflow of either sediments or others related to anthropogenic activities. Strong, negative correlation between color and pH, color and DO may indicate contamination by organic matter from households, agriculture, industries or discharges of other pollutants into industrial effluents. However, color and DO slightly correlated with total hardness, chlorides and EC, which may suggest different or multiple sources.

There are several studies in which the PCA is used to integrate environmental physical-chemical data with toxicity parameters (César et al., 2007; Riba et al., 2004a, b; Delvalls et al., 2002; Delvalls; Chapman, 1998). The results of both the PCA and the Pearson correlations showed strong relationship between variables that may have a common source of inflow in the field of study. Color, DO and pH association is mainly connected with anthropogenic activities. Chloride, total hardness and EC association is attributed to high salt discharges from industrial effluents or to the elements on rocky materials.

The first factor (F1) shows environmental degradation related to some parameters that show the presence of salt (total hardness, EC, pH and chlorides), since such variables were moderately linked to toxicity by the analysis of PCA. Toxicity data for *L. bulla* was also moderately associated with the second factor (F2), along with color and DO, meaning that the high level of color and the low level of DO are leading to environmental degradation in the places where this factor presents strong positive load. Plotting of PCA data due to the locations and seasons verify the differences in location 1 in relation to sampling locations and the dry and rainy seasons.

Studies on acute water toxicity assays are known for the species *Philodina acuticornis odiosa* (Hagen et al., 2009), *Brachionus havanaensis* (Juárez-Franco et al., 2007), *Brachionus macracanthus* (Nandini et al., 2007), *Brachionus patulus* (Sarma et al., 2006) *Brachionus calyciflorus* (Calleja et al., 1994), *Brachionus plicatilis* (Snell et al., 1991), *Brachionus rubens (*Halbach et al., 1983); however, there are no records of tests with rotifers in Brazil.

The species *L. bulla* fits the criteria of availability for laboratory culture, small-sized species and life cycle, organism availability, representation in the studied ecosystem and genetic stability for obtaining uniform organism stocks, being used as a test organism assessed in the present study, which proved to be positive to ponder the toxic potential of the analyzed surface water samples.

CONCLUSION

Different statistical techniques used in this study showed how useful they can be to monitor surface water, identifying the variables that better explain seasonal and temporal variations and the relationship between them. Multivariate analyses were in evidence, which allowed identifying the behavior of each season sampling and the correlation analysis that identified the relationship among them. The PCA revealed the factors or the sources accounting for the water quality variations, proving to be an efficient way to manage water resources. Due to environmental degradation in the Atibaia River, especially in the region of Paulínia, the PCA technique was used, and the inferences about water quality could be drawn without a reference area. The main reason for the Atibaia River degradation in step with the physical-chemical and the ecotoxicological parameter evaluated has been the input of industrial and agricultural effluents, as well as domestic sewage, resulting from great changes in the water characteristics. Specifically in location S.1, the refinery effluents analyzed in this study may contain chemical compounds that could compromise, in this collection, the quality of the waters where they are discharged. *L. bulla* is a widely distributed species in the Brazilian aquatic ecosystems. It plays an important role in preserving primary production in biomass for a large number of invertebrates, and may be a useful and economic tool for monitoring surface water, since it is easy to culture and carry out toxicity assays. Moreover, it is sensitive to differences in water quality.

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