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Acute Ecotoxicological Evaluation at Icaraí Beach, Niterói – RJ, Brazil

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Icaraí beach, located in the municipality of Niterói, in the metropolitan region of Rio de Janeiro, Brazil, plays an important role in the social context, since it is very popular among the local population. However, it has been suffering negative impacts due to anthropic activities, which are enhanced by increasing urbanization. Ecotoxicological tests using the microcrustaceans *Artemia salina* as test organism are an important alternative in the evaluation of the toxic state of the water due to their sensitivity to chemical and toxic components of the environment. The present work aimed to evaluate the acute toxicity at two sampling sections (P1 and P2) using low cost methodology, technically simpler and with some adaptations. For this purpose, a number of physico-chemical parameters (temperature, pH, dissolved oxygen, salinity, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total coliforms and fecal coliforms) were analyzed, compared to the values established by CONAMA Resolution 357 of 2005, besides the performance of the acute ecotoxicological test with samples of water from the Icaraí beach and with the use of *Artemia salina* for the evaluation of the lethal dose (LD50). The samples showed a change in toxicity, because in some sample concentrations of the second collection, Section 2 presented a toxic effect. Therefore, the results were satisfactory, since the two ecotoxicological tests presented good answers, associated with their simplicity of execution and economic feasibility.

1. Introduction

The term ecotoxicology was created by the french researcher René Truhaut in 1969 to define "the study of the adverse effects of chemical substances in order to protect natural species and populations" (Truhaut, 1977). Its definition comes from two sciences, ecology and toxicology. Ecology accesses the diversity and representativeness of organisms and their meaning in ecosystems, and toxicology accesses the adverse effects of pollutants on the organism, being the toxic agent the substance capable of causing damage to the biological system.

Biological monitoring is based mainly on applications of biological indexes, evaluation protocols and multimetric indices. It can be subdivided into two groups: the liabilities that make use of biomonitors already existing in the site to be evaluated, and the assets where the biomonitors are inserted under controlled conditions, in laboratory or "in situ", including toxicity tests acute and chronic (Burton, 1994). With the population increase in the world, it has increased energy consumption and waste generation (Giuliano et al, 2018). In urban areas, the increase in the generation of domestic wastewater is a reality and biological monitoring can be used as an alternative method for measuring the toxicity generated and its influence on the health of the population (Sabeen et al, 2018).

The toxicity biotests have become an important tool in the monitoring of aquatic ecosystems, because through these it is possible to identify the impact caused by toxic agents (Magalhães, 2008). Based on the use of living organisms, which are sensitive to imbalances occurring in aquatic environments, where they live or are inserted, since marine environmental pollution refers to human activities that directly or indirectly launch pollutants into the ocean, thus causing damage to marine life, the marine environment, human health and fishing (Tian H, Wang R, 2018).

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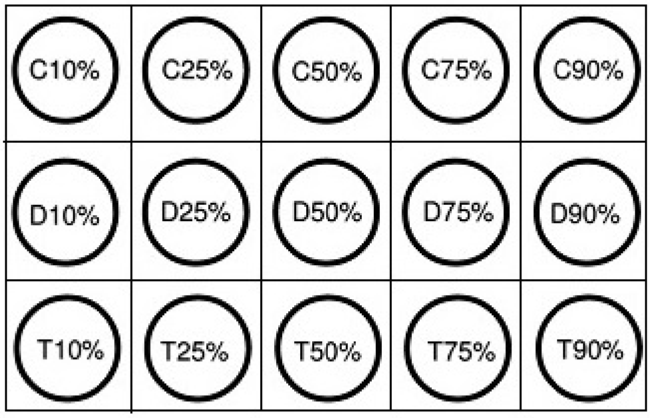
Lethality tests are widely used in preliminary analyses of general toxicity, which can estimate the mean lethal dose (LD50) (Luna et al., 2005). In toxicology, median lethal dose (LD50) is the required dose of a given substance or type of radiation to kill 50% of a population under test.
For an adequate survey of information that seeks ways of conserving water resources, the ecotoxicological methodology together with chemical analyses, allows a scientific study of the potential and effects caused by pollutants environmental impacts on organisms (Cairns, 1989), providing a detailed assessment of water quality and the magnitude of pollutants, using this information to elaborate proposals for water pollution control.
In this paper, it seeks to evaluate the acute toxicity in Icaraí Beach/RJ, using the microcrustacean *Artemia salina* as the test organism. Achieving This general objective, it will also be possible to contribute to the monitoring of water quality on this beach, which has great environmental, touristic, landscape and economical importance. Another aspect is to assess the mortality rate of *Artemia salina* in the samples under study according to the LD50, comparing the physicochemical parameters collected with the CONAMA Resolution 357/2005 and defining the acceptable concentration in the environment.

2. Materials and Methods

The collections were made at Icaraí Beach – in the southern region of the city of Niterói, RJ – in two points (named P1 and P2), in two dates. The points were selected to check the water quality that can be impacted by the launch of sewage and rainwater receiving.
Point P1 is located at the beginning of Icaraí Beach, next to the Pedra de Itapuca, where a gallery dumps pluvial water. Residential buildings are also located in their surroundings. Point P2 is located very close to the middle of the beach, approximately 550 meters from the first collection point.
The acute ecotoxicological assays with the use of *Artemias* were performed based on the methodology of Meyer et al. (1982) adapted. The water used for hatching was prepared in 1 liter of filtered water, with the addition of two drops of anti chlorine, to certify that the hatching will not be harmed by the presence of chlorine. And it was seen that the measured pH was outside the recommended range for hatching, between 8.5 and 9.0, that is, well alkaline. For pH adjustment, 60g of sodium bicarbonate ($NaHCO_3$) was added, and then a new measurement was performed to prove the water alkalinity. Once this was done, 30g of NaCl was added to achieve the necessary salinity.
The prepared water was mixed with the use of an air compressor for 60 minutes in the adapted plastic container. After the 60 minutes of the water mixing process, 0,3g of *Artemia salina* eggs of high hatch were added, together with the presence of artificial light constant of 20w in the first 24 hours, and, after that, during the photoperiod of 12h light/12h dark , at a temperature of 27°C, ideal period to reach the phase for the execution of the tests. After the period of 48 hours, the hatching of the náuplios was observed.
For the determination of the field parameters: temperature, PH and dissolved oxygen (OD), the equipment was immersed directly in the water, and the respective parameters were read at the site, in triplicate. These equipments were duly calibrated prior to the performance of the analyses. For the salinity analysis it was necessary to perform a dilution with a proportion of 1ml of the water sample collected for 9ml of distilled H_2O. Thus, the results obtained in triplicate were multiplied by ten to obtain the final value.
For the analysis of the LD50, the test tubes were organized in the bookcase divided into different concentrations: 10%, 25%, 50%, 75% and 90%. These concentrations are related to the amount of samples collected both in point 1 and in point 2, for the performance of the test. All of these concentrations were analyzed in duplicate, that is, each duplicate tube repeated the entire process described above. The controls referring to each concentration were filled with 10ml of hatching water in each one, to monitor the survival of *Artemias*. Next, 10 *Artemias* were inserted in each test tube, with the aid of a Pasteur pipette, for subsequent acute ecotoxicological evaluation, which was performed at a specified period of 24 hours. The assembly of the test tubes on the shelf for the ecotoxicological test of point 1 and point 2 is illustrated in Figure 1.
This procedure was performed in the same way for the samples of the two collections, on 18/10/2017 and 01/11/2017. The acute ecotoxicological tests were initiated, respectively, on 19/10/2017 and 03/11/2017, and the analyses of the results were performed, respectively, on 20/10/2017 and 04/11/2017.
The analyses of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), total coliforms and fecal coliforms were performed in the external laboratory following the recommendations described, respectively, in the methods SM 5210 B, SM 5220 D and MM-01-014 (APHA, 2012).



Caption:
C = Control
D = Duplicate
T = Tube
{C}% = Sample concentration

Figure 1. Illustration of the assembly of the vials on the shelf for the ecotoxicological test of point 1 and point 2.

3. Results

The water body studied was analyzed in relation to the physicochemical parameters for the best characterization of it. These parameters were: temperature, pH, dissolved oxygen, salinity, total coliforms, fecal coliforms, BOD and COD.
Table 1 below presents the results obtained for the parameters analyzed in the different samples.

Table 1. Physical and chemical parameters of the different samples used to perform the biotests.

Parameters	First collection		Second collection	
	P1	P2	P1	P2
pH	9,6	9,4	9,5	9,7
DO (mg/L)	5,92	6,11	6,30	6,52
Salinity (ppm)	35,3	34,8	31,0	34,5
Fecal coliforms (UFC/100 mL)	842	83	2575	1040

It was not possible to obtain total coliform parameter values for the second collection (day 01/11/17), since it was not possible to analyze it in the contracted external laboratory.
Some of these parameters were analyzed for posterior comparison with the CONAMA Resolution 357/2005 requirements, due to the water body frame analyzed to correspond to saline waters classified as Class 2, which are appropriate for recreation of secondary contact and amateur fishing (Petrobras, 2012). Thus, it also serves as the basis for the results obtained in the ecotoxicological tests. The results obtained in the analysis of the parameters: temperature, total coliforms, BOD and COD are exemplified in the following graphs:

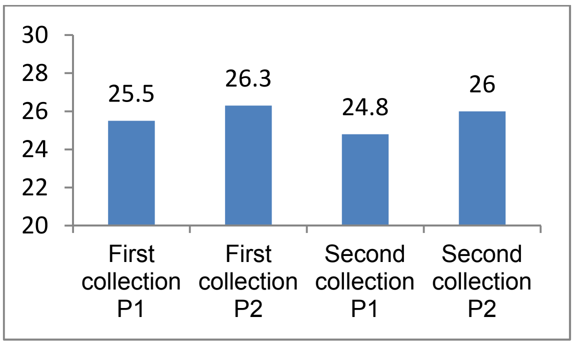


Figure 2a. Comparison of the temperature values obtained in the collections

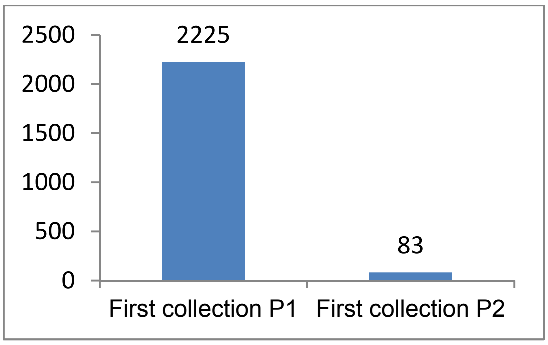


Figure 2b. Comparison of total coliform values obtained in the first collection.

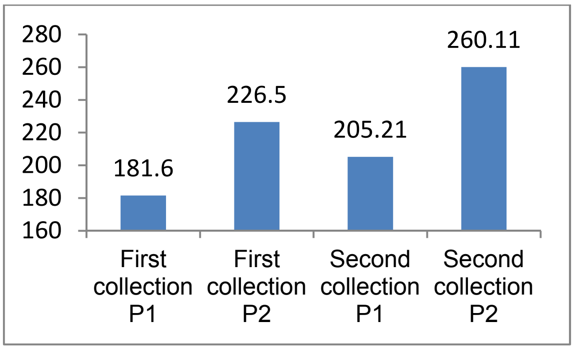


Figure 2c. Comparison of the BOD values obtained in the collections.

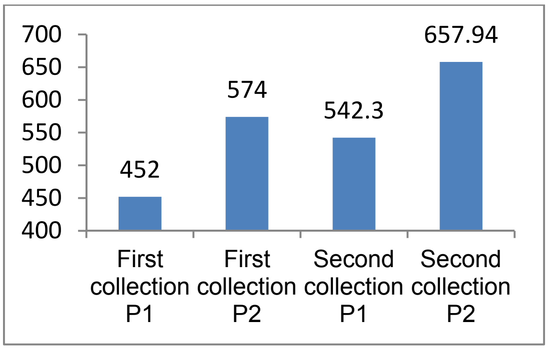


Figure 2d. Comparison of COD values obtained in the collections.

The comparison between the mortality rate in point 1 of the first collection and the same point in the second collection can be seen in Figure 3a. It shows that in the analyses of the two collections carried out in point 1, no signs of toxicity were identified for *Artemia salina*, since in the analyses of the two collections, the results of the mortality rate were lower than 50% in all concentrations (Hisem et al., 2011).

In Figure 3b It can be observed that the mortality rate of *Artemia salina* in point 2 showed a difference between the two collections performed. While in the first collection the results were satisfactory, with the mortality rate below 50%, in the second collection the results were satisfactory only in the concentrations of 75% and 90%, in the other concentrations the results were unsatisfactory, with mortality rate above 50%.

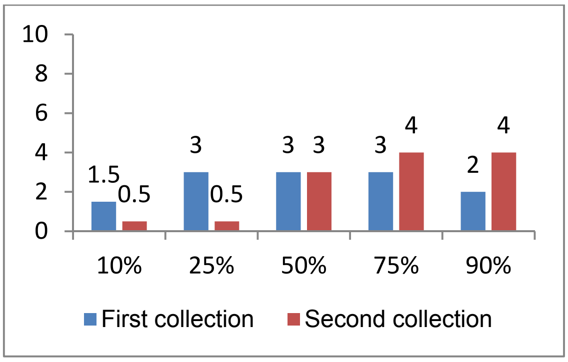


Figure 3a. Mortality rate of Artemia salina referring to point 1 in the analysis of the first and second collection.

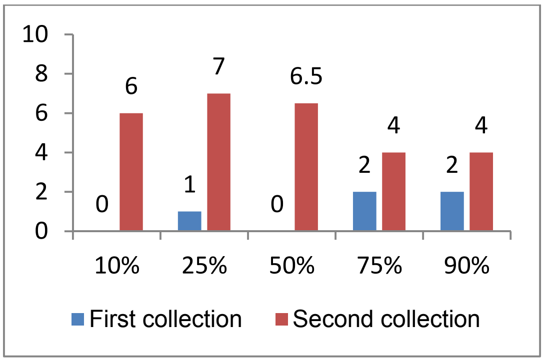


Figure 3b. Mortality rate of Artemia salina referring to point 2 in the analysis of the first and second collection.

The results obtained in the analysis of the parameters: pH, dissolved oxygen (DO), salinity and fecal coliforms were compared with the values established by CONAMA Resolution 357/2005, and are exemplified in the following graphs (Figure 4):

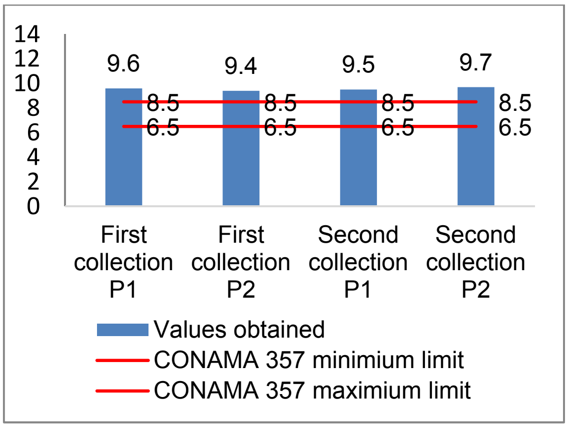


Figure 4a. Comparison of the pH values obtained in the collections with the limit established by CONAMA 357/05.

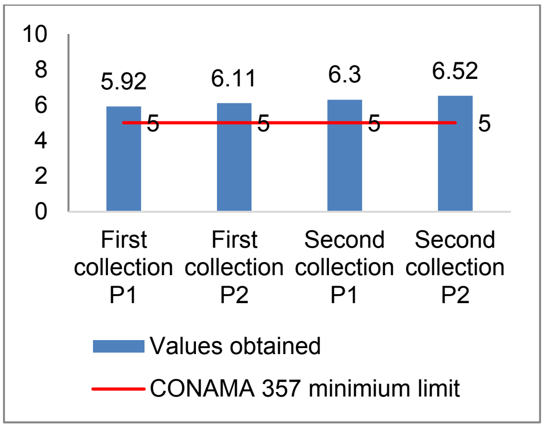


Figure 4b. Comparison of the DO values obtained in the collections with the limit established by CONAMA 357/05.

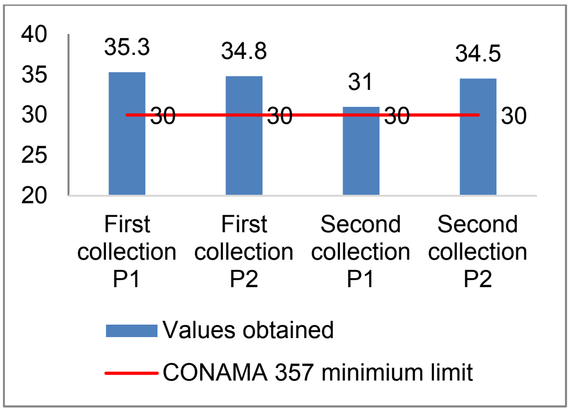


Figure 4c. Comparison of salinity values obtained in the collections with the limit established by CONAMA 357/05.

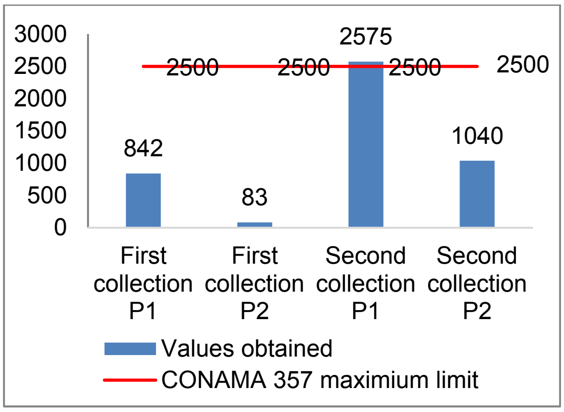


Figure 4d. Comparison of fecal coliform values obtained in the samples with the limit established by CONAMA 357/05.

4. Conclusions

The methodology adapted for the implementation and validation of the acute ecotoxicological assays showed that both tests are simple, reproducible and robust. The monitoring performed at the study site using this methodology, had simple procedures when comparing the procedure described in the literature in relation to the tests most used with the same bioindicator. In the tests proposed in this paper there was a need for cultivation of the organisms used, with no need to feed them, since they were being fed with the generated residue (sludge) it self, considerably reducing the time and total cost of the test. Reproducibility could be verified in the duplicates performed.

In the conditions under which the research was carried out and according to the results found, it is possible to conclude that, with regard to robustness, it could be verified when using the same sample collected, at different concentrations evaluated, being the lowest than 10%, and the highest concentration of 90%, so that the toxic effect was more clearly evaluated in relation to the corresponding concentration, as seen in the concentrations of 10%, 25% and 50% referring to point 2 in the second collection, where observed the toxic effect based on the mortality rate, according to the LD50.

Regarding physicochemical parameters, only pH, dissolved oxygen, salinity and fecal coliforms were correlated with the values delimited by CONAMA Resolution 357/2005, since the other parameters analyzed are not included in this Resolution.

The correlation between the acquired physicochemical parameters and the data obtained in the ecotoxicological tests proved to be coherent, since as it could be seen in the analysis of the data presented, in the second collection, high coliform values were verified, the main determinant parameter for the analysis of bathing-ability.

Only in samples of the second collection were verified toxic effects in the evaluation of the LD50, and it was also the one that presented the highest values for certain physical and chemical parameters in this study, being this result possibly influenced by occurrence of rain in the second collection period, since the presence of rain leads to a greater spread of pollutants and bacteria that are transported to the receiving water body through rainwater galleries, streams and drainage canals. Thus, the occurrence of rain is one of the main causes of interference in the water quality of the beaches.

The recommendations for future studies are an evaluation of long-term toxicity, using chronic tests, because the chronic effect is reflected by the response to a stimulus that continues for a long time, usually for periods that may encompass part or the whole life cycle of organisms, besides the verification of toxicity also in sediments, because the water body is frequently modified by the natural action of the currents, and a longer sampling period, with more collection points, so that coefficients of variation of the samples in different dilutions could be obtained. It is hoped that the results of ecotoxicological tests can be used as subsidies for decision makers in relation to public policies of the environment, thus influencing in contexts related to greater control of the launching of pollutants in water bodies and effective supervision by environmental agencies.

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