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Comparison between the efficiency of two bioindicators for determining surface water quality in an urban environment

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ABSTRACT. This study evaluated the efficiency of two bioindicators, fecal coliforms and ecotoxicity tests, set out in CONAMA Resolution 274/00 and CONAMA Resolution 357/05, in assessment of water quality. For this study, Lake Paranoá, Federal District of Brazil, was chosen, since it is a water body directly contaminated by effluents from a sewage treatment plant. Four sampling points were chosen in accordance with the map of recreational water quality published weekly by CAESB/DF, after analysis of fecal coliforms. Samples from these points were collected for 6 months and tested on *Danio rerio* fish (acute toxicity) and on the microcrustacean *Ceriodaphnia dubia* (acute and chronic toxicity), besides measuring chemical and physico-chemical parameters. The data obtained show great consistency between the observed biological parameters, suggesting that in this urban aquatic environment, under great anthropogenic pressure, the fecal coliform bioindicator seems to be more restrictive and enough to evaluate the safety of surface water.

Keywords: water quality, ecotoxicity tests, fecal coliforms, Paranoá lake, recreational water, safety.

RESUMO. Comparação entre a eficiência de dois bioindicadores para a determinação da qualidade de águas superficiais em um ambiente urbano. Este trabalho comparou a eficiência dos bioindicadores coliformes fecais ou termotolerantes e ensaios de ecotoxicidade, propostos pelas Resoluções Conama 274/00 e Conama 357/05, na avaliação da qualidade de água. Para a realização desse estudo foi escolhido o Lago Paranoá, Brasília, Distrito Federal, por ser um corpo hídrico impactado diretamente pelo lançamento de efluentes de uma estação de tratamento de esgotos. Foram definidos quatro pontos de acordo com o mapa de balneabilidade, publicado semanalmente pela Caesb/DF, em função do teor de coliformes termotolerantes. Amostras desses pontos colhidas durante seis meses foram testadas com o peixe *Danio rerio* (toxicidade aguda) e o com o microcrustáceo *Ceriodaphnia dubia* (toxicidade aguda e crônica), além da determinação de parâmetros químicos e físico-químicos. Os resultados obtidos mostraram grande equivalência entre os parâmetros biológicos observados, sugerindo que nesse ambiente sob grande influência antrópica, o bioindicador coliformes termotolerantes foi mais restritivo e suficiente para avaliar a segurança das águas superficiais.

Palavras-chave: qualidade da água, testes ecotoxicológicos, coliformes termotolerantes, lago Paranoá, balneabilidade, segurança.

Introduction

The evaluation of water quality has been adopted as one of the main criteria in establishing uses for any aquatic environment. These standards aim to maintain, above all, the human health and safety and the aquatic species conservation.

The major problem lies in the fact that human occupation in nature has been conditioned by the need to be near water. This has meant that the same water which meets humankind's many needs (consumption, energy production, irrigation etc) is also the place where populations get rid of waste, directly or indirectly. The quality of our planet's water has, therefore, undergone such intense alterations that, in many cases, there are high levels of environmental degradation that have led to numerous adverse effects and the potential extinction of aquatic species, which do not succeed in recovering their losses in the altered environment (SEITZ; POULAKIS, 2006; HEWITT et al., 2006; DOMÍNGUEZ-DOMÍNGUEZ et al., 2007). In this context, the use of biological indicators to evaluate water quality has become an important tool, not only concerning to protecting human health but also preserving and maintaining life in the environment (VAN DER SHALIE et al., 1999; MARTINS et al., 2007).

Due to a historical concern with microbiological quality standards, mainly in order to ensure public health, bacteria from the coliform group have for many years been used as a water quality indicator. Since its first use in 1904, the microbiological assay to determine coliforms has been the main tool to establish the relationship between water contamination and the presence of fecal material. However, this type of assay still raises many questions, especially when bacteria of non-fecal origin are the main inhabitants of the environment (DOYLE; ERICKSON, 2006). Furthermore, there is still the possibility that bacteria found in the environment (introduced or otherwise) may not survive the presence of certain chemicals and, in such cases, their absence may not be evidence of water quality but rather of chemical contamination. This is not a recent concern, and Willingham and Anderson (1966, 1967) have already discussed the issue, even raising the possible use of microcrustaceans to evaluate the presence of toxic substances in water. Several authors deal with the use of bioindicators (CICOTELLI et al., 1998; YEOM et al., 2007), as well as ecotoxicity assays (LATIF; LICEK, 2004; GIROTTI et al., 2008; PALMA et al., 2009), to evaluate contamination of aquatic environments. In Brazil, researches with bioindicators have been performed mainly with benthic macroinvertebrates (MARQUES et al., 1999; TUPINAMBÁS et al., 2007), but the use of these organisms has not been accepted as a criterion by Brazilian regulations.

Among the criteria and federal regulations to establish water quality within environmental standards, National Environmental Council (CONAMA) Resolution no. 274/00 (BRASIL, 2001) and CONAMA Resolution no. 357/05 (BRASIL, 2005) stand out.

From the viewpoint of environmental health and safety, and focusing on recreational use/bathing in waterbodies, CONAMA Resolution no. 274/00 points to the quantification of total and fecal coliforms present for evaluating water quality. Recently, taking a more ecological line, CONAMA Resolution no. 357/05, published on 17th March 2005, brings the then applicable CONAMA 20/86 up to date and incorporates other norms, among them CONAMA Resolution no. 274/00. This Resolution, as well as including previous parameters, adds the measurement of ecotoxicological effects and thus establishes the maintenance of aquatic life as a criterion for classifying the quality of waterbodies.

This legislative innovation is significant, since a large quantity of compounds may reach aquatic environments and chemical analysis may not always be effective in their detection, as in general it does not involve evaluation of the effects of diluted chemicals on living organisms. This gap has been closed by biological methods of measurement, such as ecotoxicological tests, which serve the purpose of finding out if, and in what abundance, isolated or mixed chemicals are toxic and how their effects are expressed (BUIKEMA JR. et al., 1982; CHAPMAN, 1995). Since biological systems react to concentrations of chemicals well below the detection limits by chemical analyses, this allows a safer evaluation of the toxic potential of substances or contaminated environments. In addition, bioassays involve quick and low-cost procedures, in comparison with classic chemical analyses (KNIE; LOPES, 2004).

In this context, the current study aims to compare the fecal coliforms, generated in the framework of CONAMA Resolution no. 274/00, carried out by the Federal District's Environmental Sanitation Company (CAESB), and the ecotoxicity tests, proposed by CONAMA Resolution no. 357/05, using the fish *Danio rerio* and the microcrustacean *Ceriodaphnia dubia*. The final objective is to determine the efficiency of these bioindicators in diagnosing contamination of this aquatic environment in Brazil's Federal District.

Material and methods

In order to carry out the proposed comparison, four water sampling points were chosen in Paranoá Lake, Brasília (Federal District of Brazil), in accordance with the map of recreational water use created by CAESB, and denominated thus: P1 (15°51.074' South and 47°55.929' West) at the bridge which links the Asa Sul district to the airport, defined as Riacho Fundo – Paraná River basin – localized upstream from effluent flowing into the lake from Sewage Treatment Plant ETE Sul; it should be highlighted that CAESB does not monitor this locality; P2 (15°50.321' South and 47°53.968' West) at the Garças Bridge, downstream from effluent discharged by Sewage Treatment Plant ETE Sul; P3 (15°49.994' South and 47°53.433' West) at the Nipo-Brasileiro Club; and P4 (15°49.479' South and 47°52.463' West) at Pontão do Lago Sul, in front of the Mormaii Surf Bar, considered to be a region with high-quality water. Figure 1 presents the water sampling points. Water samplings were conducted on a monthly basis from October 2007 to May 2008, except in April 2008, when the sampling was not carried out due to operational issues.

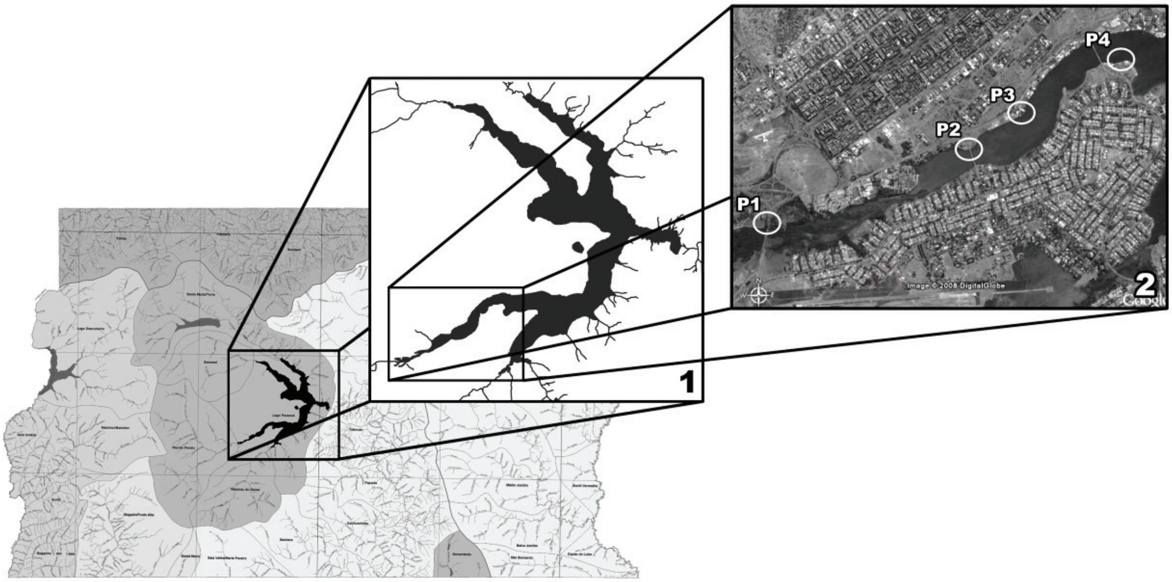

Figure 1. Map of the Federal District, highlighting Lake Paranoá (1) and a further close-up of the extreme south of the Lake, defining the sampling points (2), adapted from Google Earth.

From October 2007 through January 2008 ecotoxicity assays were carried out with a fish species (*Danio rerio*). The assays followed the standard protocol set out by NBR 15088 of the Brazilian Association for Technical Standardization – ABNT (ABNT, 2004). For tests of acute toxicity 3,000 milliliter beakers were used for each sampling point and, in each of these, four adult fish were exposed for 48 hours (static system), after which the number of dead organisms was recorded.

Because of their greater resistance and lower susceptibility to acute effects, the fish were replaced by the microcrustacean *Ceriodaphnia dubia*, during the months of February, March and May 2008, and the endpoint observed in the assay became chronic toxicity. This assay followed protocols established in NBR 13373 of the Brazilian Association for Technical Standardization (ABNT, 2005). Ten organisms were exposed in each sample, one in each 30 milliliter beaker containing 15 mL of lake water. The negative control group was exposed to synthetic soft water prepared in the Laboratory of Ecotoxicology at Embrapa Cerrados. Each beaker contained a test organism with less than 24 hours old, distributed randomly; the beakers were kept in an incubator at a temperature of 25°C and photoperiod of 12h light/dark, for seven days, renewing the test solution and food source each day. At each change of test solution, the number of living adult organisms and the number of offspring from each exposed female was recorded. To evaluate the sensitivity of the organisms, the acute toxicity assay was carried out each month with sodium chloride as the reference substance.

Analysis of the results was established by comparing survival rates of the organisms in each sample with those from the control group. This same comparison was also carried out for the average number of juvenile organisms produced, using statistical methods to establish significant differences. Without statistical difference the result was expressed as "Non-Toxic" (NT), while a result with a significant statistical difference was expressed as "toxic". When mortality occurred within 48h, with significant difference, the result was expressed as "Acute Toxicity" (AT), and when there was a significant difference between the number of offspring in the control group and the number of offspring in the test groups, the result was expressed as "Chronic Toxicity" (CT). To calculate acute toxicity, the Fischer test was employed and to determine chronic toxicity the ANOVA test was used, followed by the Dunnett test (DUNNETT, 1955).

For each sample of water collected, the following parameters were measured: temperature, dissolved oxygen, conductivity and pH, using the HACH SensION 156 Multi-Parameter Meter. Water hardness was taken by titulometry with EDTA (ABNT, 1992), and turbidity was measured with the help of a portable HANNA HI 93703 turbidimeter. In addition, the presence of metallic chemical elements such as aluminum, arsenium, cadmium, cobalt, copper, iron, manganese, nickel, lead and zinc was checked, using an optical emission spectrometer with Inductively Coupled Plasma-Emission Automated System (ICP EAS); nutrients such as fluoride, chloride, nitrite, nitrate, bromide, phosphate, sulfate and ammonia were measured using ionic chromatography.

Results

From October 2007 to January 2008 no mortality was observed in the tests with *Danio rerio* fish exposed to samples from the four sampling points. The results of assays with *Ceriodaphnia dubia* showed that in February 2008 chronic toxicity was observed with water samples from points 2 and 3; in March 2008 water from point 2 showed acute toxicity, and the other sampling points showed an absence of toxicity during the three months (February, March and May) when we accomplished analysis with this microcrustacean.

During the sampling period, physical and chemical parameters showed no remarkable changes (Table 1). Turbidity decreased as depth and dilution increased, as did water hardness, while chloride and sodium increased at point 2, and became more diluted downstream.

In the analysis of metals, only the cadmium was observed every month and at every point, being detected in sufficient quantity to provide the water a classification of 3 or 4. According to CONAMA Resolution no. 357, this would mean a satisfactory and unsuitable level, respectively, thus disagreeing with the classification given to Paranoá Lake, which stands at 2.

All the assays with positive control (sodium chloride) showed the organism's susceptibility. In the four assays carried out, the values obtained for EC_{50} immobilization were within variability limits (average ± 2 standard deviation) considered acceptable by the current protocols (Table 2).

Discussion

Comparing CONAMA Resolutions nos. 357 and 274, in terms of fecal coliforms, we can verify that the classifications are equivalent and that this is the common point between the two directives and the ecotoxicological effects (Table 3).

On the recreational water quality map published weekly by CAESB the water quality of the lake can be seen in terms of microbiological parameters. However, only three of the four sampling points from our study appear among the monitored sites, because point 1, upstream from sewage treatment plant ETE Sul, does not appear in the company's analyses.

Table 4 allows us to compare the results published by CAESB and those from the ecotoxicological tests carried out with the microcrustacean *C. dubia*. As seen in Table 4, the levels found in tests with *C. dubia* were equivalent to the quantification of coliforms presented by CAESB.

Comparing CONAMA Resolutions nos. 357 and 274 in terms of classification of the water body quality, all the sampling points received the same classification, including point 2, in March, where acute toxicity was observed. As regards the water at point 1, unmonitored by CAESB, the content of fecal coliforms was determined in March at the Laboratory of Ecotoxicology from Embrapa Cerrados. During this same period, although no toxicity was observed for *C. dubia* or for *D. rerio*, a high concentration of thermotolerant coliforms was found.

Table 1. Physical and chemical parameters and ions evaluated from October 2007 to May 2008, at selected points in Lake Paranoá (Brasília, Distrito Federal).

	P1				P2				P3				P4			
Parameter	Av	Max.	Min.	SD	Av	Max.	Min.	SD	Av	Max.	Min.	SD	Av	Max.	Min.	SD
Temperature °C	23.7	27.7	19.1	3.3	26.6	27.6	24.9	1.3	27.1	28.3	25.0	1.2	27.1	31.9	24.2	2.5
pH	6.4	7.1	5.9	0.3	6.6	7.2	5.9	0.4	6.7	7.2	6.0	0.4	6.8	7.3	6.0	0.5
Turbidity UNT	98.5	228	3.7	80.2	34.9	111	5.2	40.5	9.0	16.1	3.6	4.5	4.1	8.2	0.8	3.3
OD mg L^{-1}	5.8	7.6	4.5	0.9	5.4	8.2	4.0	1.3	5.4	6.3	4.4	0.6	5.6	7.8	3.7	1.3
(*) Cond. µS cm^{-1}	107	127	85	16.8	121	129	111	7.7	118	130	112	7.9	106	110	103	2.9
(**) Hardness mg L^{-1}	38.0	50.5	20.5	11.5	21.2	25.0	18.0	2.7	19.2	21.5	18.0	1.4	15.1	18.5	13.0	1.9
Ca^+ mg L^{-1}	11.5	14.1	8.7	1.8	9.1	10.0	8.5	0.5	9.0	10.0	8.2	0.7	8.2	9.9	7.4	0.9
Na^+ mg L^{-1}	5.6	6.8	4.3	1.1	12.4	20.3	9.5	3.8	11.1	16.0	9.0	2.4	9.9	12.8	8.9	1.5
K^+ mg L^{-1}	1.1	1.7	0.7	0.6	2.3	2.7	2.1	0.2	2.3	3.4	1.8	0.5	1.6	2.0	1.8	0.8
Mg^+ mg L^{-1}	0.8	1.1	0.8	0.4	0.8	1.0	0.9	0.4	0.8	1.0	0.9	0.4	0.7	0.9	0.7	0.3
F^- mg L^{-1}	0.09	0.3	0.03	0.09	0.1	0.3	0.1	0.05	0.1	0.2	0.01	0.07	0.1	0.1	0.08	0.05
NO_3^- mg L^{-1}	3.2	4.8	2.4	1.0	2.2	2.7	1.7	0.5	2.2	2.8	1.9	0.4	2.0	3.0	1.6	0.4
SO_4^- mg L^{-1}	8.3	23.1	1.9	7.0	12.1	23.6	5.1	5.8	12.9	17.7	9.8	3.6	9.9	15.8	6.1	2.9
NH_4^+ mg L^{-1}	0.4	1.6	0.1	0.6	1.3	4.9	0.1	1.7	1.0	2.6	0.4	0.8	0.6	1.5	0.8	0.5

(Av = average; Max. = Maximum; Min. = Minimum; SD = standard deviation) / (*) Cond. = Conductivity / (**) Hardness mg L^{-1} of $CaCO_3$.

Table 2. Acute toxicity, with 95% confidence intervals of the reference substance sodium chloride (g L^{-1}) for the microcrustacean *C. dubia*, after 48 hours' exposure.

Month	48-h EC_{50} (g L^{-1})
February	1.11 (0.96-1.29)
March	1.33 (1.10-1.63)
April	1.15 (0.97-1.36)
May	0.96 (0.79-1.18)
Average ± Standard Deviation	1.14 ± 0.15

Table 3. Comparison between norms in CONAMA no. 274/00 and no. 357/05 with regard to presence of microorganisms from the coliform group in the water.

Microorganisms	CONAMA no. 274/00	CONAMA no. 357/05
Maximum 250 fecal coliforms (thermotolerant), or 200 *Escherichia coli* or 25 enterococci per 100 mL	Excellent	Class 1
Maximum 500 fecal coliforms (thermotolerant), or 400 *Escherichia coli* or 50 enterococci per 100 mL	Very Good	Class 2
Maximum 1000 fecal coliforms (thermotolerant), or 800 *Escherichia coli* or 100 enterococci per 100 mL	Satisfactory	Class 3
Maximum 2500 fecal coliforms (thermotolerant), or 2000 *Escherichia coli* or 400 enterococci per 100 mL	Unsuitable	Class 4

Table 4. Comparison between the results obtained with fecal coliforms published by CAESB-DF and the results obtained in bioassays with the microcrustacean *Ceriodaphnia dubia*.

	February		March		May	
	C. dubia	Coliforms	*C. dubia*	Coliforms	*C. dubia*	Coliforms
P 1	Non-Toxic	*	Non-Toxic	*	Non-Toxic	*
P 2	Chronic Toxicity	Unsuitable	Acute Toxicity	Unsuitable	Non-Toxic	Unsuitable
P 3	Chronic Toxicity	Unsuitable	Non-Toxic	Very Good	Non-Toxic	Very Good
P 4	Non-Toxic	Unsuitable	Non-Toxic	Excellent	Non-Toxic	Excellent

* Point not monitored by CAESB.

This comparison shows that although a high number of fecal coliforms may be detected at some points, this factor is not significant for the observation of toxicity to aquatic species, bearing in mind that the presence of chemicals in the water is the main factor for the occurrence of toxic effect. This toxic effect was observed at Point 2 (downstream from the discharge of effluent from the sewage treatment plant), but only during the months of February and March 2008, when there may have been a different level of discharge of chemicals compounds.

Takenaka et al. (2006) also observed an absence of effects from reservoir water upon the microcrustacean *Ceriodaphnia silvestrii*. Knie and Lopes (2004) stress that biotests have the function of determining the effect of physical and chemical influences that may not be detected by chemical analyses on test organisms under specific experimental conditions. In this context, it should be noted that in the Report on the Sanitary Wastewater System of the Federal District – SIESG - (CAESB, 2006) it is stated that of all the sewage produced in the city ('Plano Piloto') of Brasilia, only 0.09% came from industry. Therefore, almost all the sewage in the city that passes through the ETE Sul and Norte treatment plants is of domestic origin.

Other studies have shown an increase in populations of some planktonic crustaceans in places where the domestic effluent is released into aquatic environments (THOUVENOT et al., 1999; NANDINI et al., 2004). This makes it easier to understand the absence of effects upon microcrustaceans in most of our assays, and supports the greater reliability of coliform quantification as an indicator of water quality in Paranoá Lake. It is worth noting that although Moreira and Boaventura (2003) have reported significant concentrations of metals in the sediment from the sampling points, these elements seem not to be soluble in surface water, which would explain their non-detection in chemical analyses, as well as the lack of toxicity for microcrustaceans.

Although this study has shown the coliform indicator to be more restrictive, we should keep in mind that effluents are extremely variable in their composition and that complete monitoring should continue, regarding the constant changes generally found in sewage composition. Recently, Berto et al. (2009) presented the importance of a whole range of tests, including fecal coliforms quantification and ecotoxicity, for monitoring the contamination from hospital effluents, composed by pathogenic microorganisms and potentially dangerous chemicals substances.

Conclusion

In the comparison of two water quality bioindicators, the data of this study evidenced that, in the months where a toxic effect was observed for the microcrustacean *Ceriodaphnia dubia*, these levels were equivalent to the quantification of coliforms presented by CAESB, including when acute toxicity was the observed effect. The result obtained, therefore, suggests that in this environment, under urban influence and impacted by effluents from a sewage treatment plant; the index of fecal coliforms seems to be a satisfactory indicator to evaluate the levels of contamination in surface water of Paranoá Lake. Thus, we may observe that the measurements carried out by CAESB are suitable for the proposed use, from the point of view of surface water quality in the lake.

There are still doubts about places without influence of this type of discharge, for example in rural areas, where discharge of organic matter is low, but where chemical contamination may occur.

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