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MOLLUSKS AS INDICATORS OF ENVIRONMENTAL QUALITY IN A TROPICAL ESTUARY

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ABSTRACT

This study aimed to evaluate the concentrations of total (Ct) and thermotolerant coliforms (CT) in *Anomalocardia brasiliana*, *Tagelus plebeius* and in water from the Pina Basin (state of Pernambuco, Brazil) as well as to identify which of these species is the best indicator of organic pollution. The Most Probable Number (MPN) of coliforms was determined following the standard procedures used in microbiology. Possible associations with environmental factors, including precipitation, water temperature, salinity, pH, dissolved oxygen and the biochemical oxygen demand, were then analyzed. The amounts of coliforms found in the mollusks were greater than in the water. The MPN for both Ct and CT varied from $< 3.0 \times 10$ to $\geq 2.4 \times 10^4$ g^{-1} in *A. brasiliana*, whereas for *T. plebeius*, the MPN ranged from 2.3×10^2 to $\geq 2.4 \times 10^4$ g^{-1} for Ct and 7.0×10 to $\geq 2.4 \times 10^4$ g^{-1} for CT. In the water, the MPN varied from 4.0×10 to $\geq 2.4 \times 10^4$ ml^{-1} for Ct and from 9.0 to $\geq 2.4 \times 10^4$ ml^{-1} for CT. Based on our results, we were able to demonstrate the influence of environmental parameters on the coliform concentrations in the bivalves and the water. The results for *A. brasiliana* were more consistent and indicated that this species was more resistant to pollution and, thus, can be used as a bioindicator of organic pollution in the area.

Key words: bioaccumulation, bioindicators, bivalves, coliforms, organic pollution

RESUMO

O objetivo desta pesquisa foi avaliar a concentração de coliformes totais (Ct) e coliformes termotolerantes (CT) em *Anomalocardia brasiliana*, *Tagelus plebeius* e na água da Bacia do Pina, Pernambuco, Brasil, e identificar, entre as espécies estudadas, a melhor indicadora de poluição orgânica. Foi determinado o Número Mais Provável (NMP) de coliformes e as análises seguiram os padrões usuais em microbiologia. Os resultados foram relacionados com fatores ambientais: índice pluviométrico, a temperatura da água, salinidade, pH, oxigênio dissolvido e demanda bioquímica de oxigênio. Os valores de coliformes encontrados foram maiores nos moluscos, do que na água. O NMP de Ct e CT variou de $< 3,0 \times 10$ a $\geq 2,4 \times 10^4$ g^{-1} no marisco, enquanto na unha-de-velho variaram de $2,3 \times 10^2$ a $\geq 2,4 \times 10^4$ g^{-1} para Ct e $7,0 \times 10$ a $\geq 2,4 \times 10^4$ g^{-1} para CT. O NMP de Ct na água variou de $4,0 \times 10$ a $\geq 2,4 \times 10^4$ mL^{-1} e 9,0 a $\geq 2,4 \times 10^4$ mL^{-1} para CT. Ficou demonstrada influência dos parâmetros ambientais sobre as concentrações de coliformes nos bivalves e na água. *A. brasiliana* mostrou-se mais constante e resistente e poderia ser utilizada como bioindicadora de poluição orgânica naquela área.

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Palavras-chave: bioacumulação, bioindicadores, bivalves, coliformes, poluição orgânica

INTRODUCTION

Estuaries are coastal environments of great importance, as these areas are almost always eutrophic and serve as natural shelters and breeding locales for marine, estuarine and fluvial organisms with ecological and economic value. Thus, studies in these areas are extremely relevant (FEITOSA;PASSAVANTE, 1990).

Most bivalve mollusks are collected from estuaries and feed on organic and inorganic matter present in the water using their gills. These organisms are sought out because they are flavorful and easy to collect and prepare (they are often eaten raw or only slightly cooked). However, their bioaccumulation capacity makes them one of the greatest public health risks among marine animals that live in environments contaminated by microorganisms. For this reason, these mollusks (especially oysters and mussels) are used worldwide as indicators of marine pollution (HENRIQUES et al., 2000).

Ecological bioindicators can be used to evaluate the condition of a given environment or to follow how its state evolves over time. They can serve as a warning of change in the environment and can be used to diagnose the cause of certain environmental problems. They also allow quantification of stress, the level of exposure to such stress or the degree of response to exposure (DALE; BEYELER, 2001).

Among the species of infaunal marine and estuarine mollusks, *Anomalocardia brasiliana* (Gmelin, 1791) and *Tagelus plebeius* (Lightfoot, 1786) are common bivalves along the entire Brazilian coast. They are important socioeconomically and are sold in varying amounts throughout the coastal region, especially in littoral communities, which use these species for subsistence. Depending on the region of the country, these species are referred to by different popular names in Brazilian Portuguese, such as “berbigão”, “papa-fumo”, “marisco pedra” and “unha-de-velho”(NARCHI 1972, RIOS, 1994).

The coliform group is the most commonly used to indicate contamination by bacteria. This group encompasses all gram-negative, non-sporulating, aerobic or facultative anaerobic bacteria, which ferment lactose by producing gas within 48 hours at 35°C. Nevertheless, a subgroup of the coliforms, the thermotolerant bacteria, are directly correlated with pollution by feces of warm-blooded animals. They can be detected due to their ability to ferment lactose at 44.5°C, which is associated with gas production (SOUZA et al., 1983).

The Pina Basin (located in the urban perimeter of the city of Recife, state of Pernambuco) is a highly productive area in terms of mollusk production and contains an important stock of edible species with great economic potential. The scarcity of data regarding sanitary aspects of these mollusks justifies the need for more in-depth studies on microorganism bioaccumulation to guarantee good quality for the seafood that is sold and consumed. Thus, this study aimed to determine i) the concentration of total (Ct) and thermotolerant coliforms (CT) in the species *A. brasiliana* and *T. plebeius* from the Pina Basin, Recife-PE, Brazil; ii) to correlate these data with abiotic parameters characterizing the water; and iii) to identify which of the two mollusk species is the best indicator of organic pollution by coliforms for the study area.

STUDY AREA

The Pina Basin estuary (08º04’03”S-34º52’16”W) is located on the coast of the state of Pernambuco in an area within the internal portion of the Port of Recife. The estuary results from a confluence between the Tejipió, Jiquiá, Jordão and Pina rivers and the southern stretch of the Capibaribe River (Fig. 1). It is a dynamic environment from a hydrographic standpoint (which is characteristic of estuarine ecosystems) and receives industrial and domestic discharges. Vessel traffic is also intense in the estuary, as it is used as a marina. It is 3.6 km long, has a total area of approximately 2.02 km^2, and its minimum width varies between 0.26 km and 0.86 km. The region has great socioeconomic importance, especially for the neighboring low-income population, which draws its sustenance from this area through daily collection of fish, mollusks and crustaceans (FEITOSA et al., 1999).

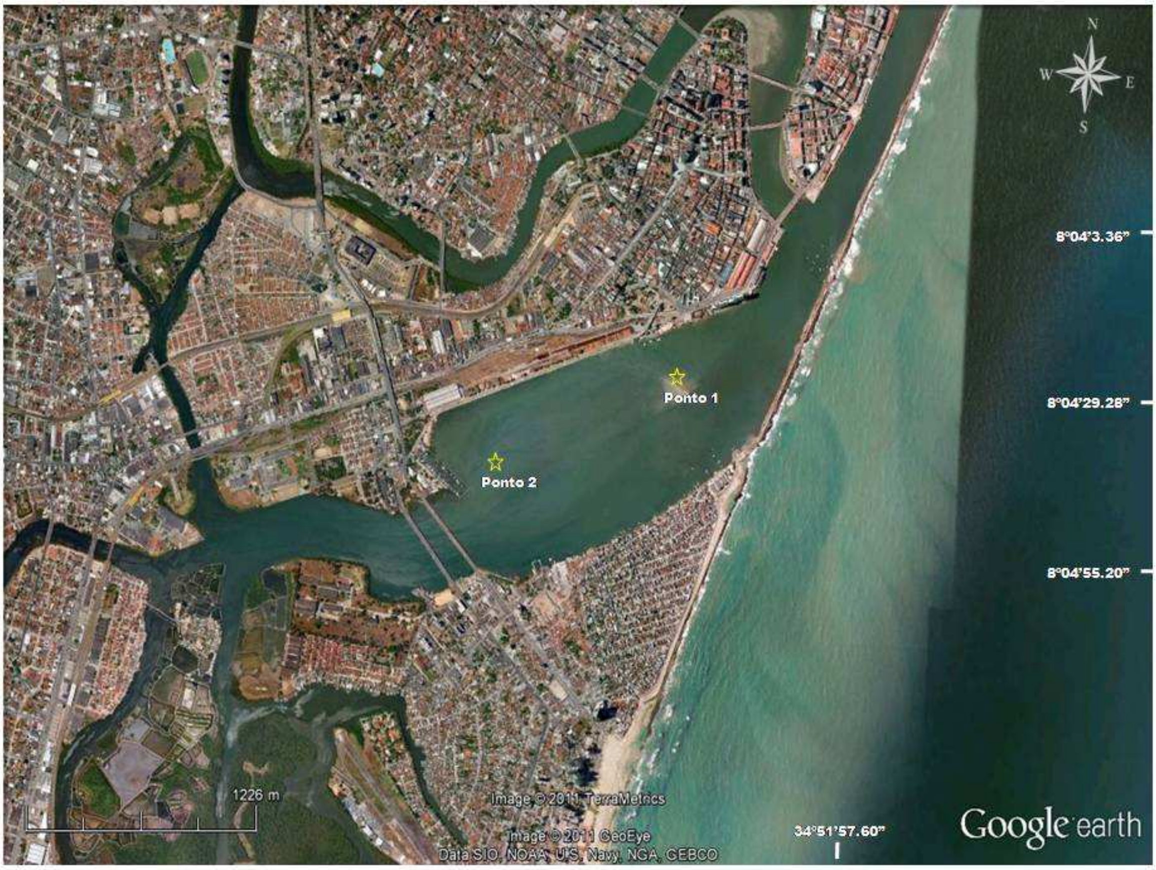

Figure 1 – Map of the study area; sampling points at the Pina Basin (Recife, Pernambuco, Brazil) are indicated (Font: Google earth).

MATERIALS AND METHODS

Bivalve and water samples

Mollusks were sampled every two weeks from June 2009 to May 2010 at two points on the Coroa do Passarinho in the Pina Basin (Fig. 1). During low tide, the bivalves *Anomalocardia brasiliana* (19.16mm) and *Tagelus plebeius* (35.00mm)were manually collected from a sandbar; each sample included approximately 80 medium-sized individuals, which were used to obtain a sufficient amount of inter-valve material to carry out bacteriological analyses (25 grams). The organisms were stored in plastic bags, and water collected from the area where they were extracted was stored in sterile 200 ml bottles. All of the materials were stored in isothermal boxes and transported to the Meat and Milk Inspection Laboratory (LICAL) of the Veterinary Medicine Department of the Federal Rural University of Pernambuco (UFRPE), where they were subjected to microbiological analyses.

At the laboratory, the bivalves were washed under running water and cleaned with a brush to remove material encrusted on their shells. The valves were then opened with a sterile knife, and the liquid within the valves and soft parts of the animals was aseptically transferred to a sterile grinder and homogenized for one minute; the portion necessary for the analysis was then removed. In the months when these bivalves were absent, no coliform data were obtained.

Most probable number (MPN) of total (Ct) and thermotolerant coliforms (CT)

For both the water and the bivalve samples, coliform counts were carried out according to the most probable number method (MPN), as described by Silva et al. (1997) and Brasil (2003).

Aliquots containing 25 g of each bivalve sample and 25 ml of the water samples were aseptically weighed and homogenized with 225 ml 0.1% sterile peptone water. Subsequent dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) of this initial dilution were prepared in tubes containing 9.0 ml of 0.1% peptone water. Then, the samples were inoculated in a series of three inverted Durham tubes containing Lauryl Sulfate Tryptose Broth (LST). The tubes were incubated at 35º±1ºC for 24-48 hours (presumptive evidence). One loopful was removed from the positive tubes (those that were turbid and producing gas) and transferred to inverted Durham tubes containing Brilliant Green Bile Lactose Broth (BGBL) and EC broth. The tubes containing BGBL were incubated at 35º±1ºC for 24-48 h, while the EC tubes were incubated for 24-48 h at 44.5º±1ºC in a stirring water bath.

In the BGBL and EC tubes, turbidity and gas production indicated the presence of total and thermotolerant coliforms, respectively ('s) (confirmatory evidence). Based on the combination of the number of positive tubes in each dilution series, the MPN table was consulted for three tubes to obtain the sample's coliform concentration in MPN/g and MPN/ml.

Environmental parameters

A common alcohol thermometer was used to determine the water temperature at the sampling locations *in situ*. Salinity was determined through the Morh-Knudsen method, while the Winkler method was used to record dissolved oxygen levels, both as described by Strickland and Parsons (1972). The biochemical oxygen demand (BOD) was verified using the technique described by Apha (1985). International Oceanographic Tables were used to verify the rate of oxygen saturation (UNESCO, 1986), and the pH was measured with a Hanna benchtop pH meter. Water analyses to determine physical-chemical parameters were carried out at the Laboratory of Chemical Oceanography of the Oceanography Department of the Federal University of Pernambuco.

The precipitation index was obtained from the Brazilian National Meteorology Institute (INMET), Curado station. The cumulative rainfall of the ten days prior to each collection was verified throughout the study.

Statistical analysis

To check for possible differences between sampling stations and seasons, an analysis of variance (ANOVA) was applied ($\alpha=0.05$). Tukey's parametric test was used for data with a normal distribution, whereas data without a normal distribution even after transformation [Log (10)] were analyzed using the nonparametric Kruskal-Wallis test. Additionally, correlation analyses were carried out between the environmental variables and the concentrations of total and thermotolerant coliforms detected in the bivalves based on Pearson's coefficient ($\alpha=0.05$) (CARDONHA et al., 2004; VIEIRA et al., 2007; RAMOS et al., 2010). All of the statistical calculations were performed using the BioEstat 5.0 software package (AYRES et al., 2007).

RESULTS

The environmental parameters recorded at both sampling points are shown in tables 1 and 2. The water temperature in the Pina Basin showed a small amplitude: a minimum of 26ºC at point 1 (January/2010) and a maximum of 32ºC at point 2 (October/2009). At point 2, the average temperature (29.13 ± 1.30ºC) was significantly higher than that recorded at point 1 (28.13 ± 1.22ºC) ($p < 0.01$), but significant differences between seasons were not recorded (Table 3).

Table 1 - Environmental variables at sampling point 1 in the Pina Basin estuary (Pernambuco, Brazil, June/2009-May/2010). DO: dissolved oxygen; BOD: biochemical oxygen demand; temp.: temperature.

Collection date	DO (ml l^{-1})	DO saturation (%)	BOD (mg l^{-1})	Salinity	pH	Water temp. (ºC)	Precipitation (mm)
08/Jun	4.93	100.41	5.40	15.77	7.76	29.00º	11.85
25/Jun	0.00	0.00	0.00	20.32	8.10	27.00º	11.42
06/Jul	3.39	63.25	0.00	11.34	7.68	25.50º	9.24
20/Jul	6.07	117.41	4.95	13.22	7.98	27.00º	6.96
04/Aug	3.42	69.23	1.21	17.98	7.99	28.00º	6.83
18/Aug	2.87	57.75	0.00	19.83	7.35	27.00º	5.83
01/Sep	3.86	79.59	0.74	24.33	7.71	27.00º	9.13
17/Sep	1.99	40.78	0.00	21.69	7.96	27.50º	7.27
05/Oct	7.10	155.36	9.12	29.09	7.45	29.00º	0.00
20/Oct	3.26	68.20	0.11	29.09	7.22	27.00º	1.31
03/Nov	3.22	69.7	0.37	28.76	7.88	28.50º	0.00
17/Nov	2.99	64.44	1.45	26.40	7.97	29.00º	1.30
01/Dec	4.40	95.24	0.00	24.22	7.31	30.00º	3.17
15/Dec	2.45	50.93	0.00	22.83	7.48	28.00º	0.00
12/Jan	1.60	32.19	0.00	23.19	7.14	26.00º	6.27
29/Jan	2.36	47.20	0.00	22.98	7.23	28.00º	5.63
03/Feb	3.66	80.79	0.00	29.41	7.71	29.50º	2.68
18/Feb	3.86	83.91	2.14	31.00	7.69	28.00°	3.50
01/Mar	2.55	57.05	3.20	31.81	7.59	29.50°	0.92
15/Mar	3.1	67.68	0.00	27.26	7.64	29.50°	0.22
12/Apr	0.88	18.53	0.00	22.18	7.66	29.00°	9.36
26/Apr	0.87	18.55	6.23	21.39	7.44	30.00°	9.72
10/May	1.47	31.08	3.04	26.20	7.49	28.00°	2.69
25/May	2.66	54.73	2.03	21.12	7.39	28.00°	2.48

Table 2 - Environmental variables at sampling point 2 in the Pina Basin estuary (Pernambuco, Brazil, June/2009-May/2010). DO: dissolved oxygen; BOD: biochemical oxygen demand; temp.: temperature.

Collection date	DO (ml l^{-1})	DO saturation (%)	BOD (mg l^{-1})	Salinity	pH	Water temp. (ºC)	Precipitation (mm)
08/Jun	5.16	102.38	6.12	11.49	7.74	29.00º	11.85
25/Jun	0.00	0.00	0.00	13.28	8.16	28.00º	11.42
06/Jul	3.96	78.57	0.00	14.51	7.90	28.00º	9.24
20/Jul	3.25	64.74	0.00	10.84	8.12	29.50º	6.96
04/Aug	2.86	57.08	0.63	15.47	7.55	28.00º	6.83
18/Aug	2.32	44.96	1.48	13.49	7.26	27.00º	5.83
01/Sep	2.53	50.50	0.00	15.34	7.60	28.00º	9.13
17/Sep	4.24	86.71	0.00	14.14	7.62	30.00º	7.27
05/Oct	11.43	253.44	14.33	22.48	7.19	32.00º	0.00
20/Oct	3.77	83.04	0.70	24.33	7.39	31.00º	1.31
03/Nov	3.73	79.36	2.84	22.79	7.72	29.50º	0.00
17/Nov	5.00	107.76	2.55	23.41	7.90	30.00º	1.30
01/Dec	4.51	92.80	0.00	20.72	7.20	28.00º	3.17
15/Dec	3.02	63.18	0.00	21.24	7.55	29.00º	0.00
12/Jan	1.35	26.78	0.00	17.52	7.20	27.00°	6.27
29/Jan	1.91	40.04	0.00	16.04	7.05	28.50°	5.63
03/Feb	3.55	76.18	0.00	25.40	7.97	29.00°	2.68
18/Feb	2.79	59.49	0.00	24.59	7.37	29.00°	3.50
01/Mar	3.19	70.11	4.73	27.26	7.54	30.00°	0.92
15/Mar	3.61	78.48	0.00	21.66	7.44	31.00°	0.22
12/Apr	1.29	26.60	0.00	17.11	7.60	29.50°	9.36
26/Apr	0.49	10.08	6.52	12.03	7.35	31.00°	9.72
10/May	1.94	40.67	2.48	21.39	7.46	29.00°	2.69
25/May	2.52	51.32	1.73	18.97	7.08	28.00°	2.48

The lowest concentration of dissolved oxygen detected in the study area was 0.00 ml l^{-1} at both sampling points (June/2009); the highest concentration recorded for this variable was of 11.43 ml l^{-1}, which was found at point 2 (October/2009). Some oscillations were observed in the saturation percentage: a minimum of 0.00% was detected at the two points, and maximum values of 155.4% and 253% were found at points 1 and 2, respectively. The average amount of dissolved oxygen at point 1 was 3.04 ± 1.60 ml l^{-1}; whereas at point 2, it was 3.27 ± 2.18 ml L^{-1}, without significant differences being detected between sampling points or seasons.

Table 3 - Results from analysis of variance considering sampling points and seasons. n.s.: not significant; P1: point 1; P2: point 2; D.S.: dry season; R.S.: rainy season; Ct: total coliforms; CT: thermotolerant coliforms; *: $p<0.05$; **: $p< 0.01$.

Variables	Sampling stations		Seasons	
	Variance	Test used	Variance	Test used
Dissolved oxygen	n.s.	Tukey	n.s.	Tukey
Biochemical oxygen demand	n.s.	Kruskal-Wallis	n.s.	Kruskal-Wallis
Salinity	E1>E2**	Kruskal-Wallis	D.S.>R.S.**	Tukey
pH	n.s.	Kruskal-Wallis	n.s.	Kruskal-Wallis
Water temperature	E2>E1**	Tukey	n.s.	Tukey
Precipitation	---	---	R.S.>D.S.**	Tukey
A. brasiliana Ct	n.s.	Tukey	n.s.	Tukey
T. plebeius Ct	n.s.	Tukey	R.S.>D.S.**	Kruskal-Wallis
A. brasiliana CT	n.s.	Tukey	R.S.>D.S.*	Tukey
T. plebeius CT	n.s.	Tukey	R.S.>D.S.**	Kruskal-Wallis
Water Ct	n.s.	Tukey	n.s.	Tukey
Water CT	n.s.	Tukey	n.s.	Tukey

The minimum BOD value observed was less than 1 mg l^{-1} at both sampling points, while the maximum value was 14.33 mg l^{-1} (point 2, October/2009). This average vales for this variable were 1.67 ± 2.48 mg l^{-1} and 1.84 ± 3.32 mg l^{-1} at points 1 and 2, respectively, without significant differences being found between study points or seasons.

Salinity varied between 10.8 (July/2009) and 31.8 (March/2010); the lowest values were recorded during the months when there was the greatest rainfall and freshwater intake from adjacent rivers. An average salinity of 23.9 ± 5.37 was determined at point 1, which was significantly higher than that recorded at point 2 (18.56 ± 4.89; $p < 0.01$). The differences between seasons were also significant ($p < 0.01$) (Table 3).

With respect to the hydrogen potential, the water was always alkaline; the minimum pH found was 7.05 at point 2 (January/2010), and the maximum was 8.16 at the same point (June/2009).

Rainfall ranged from 0.00 mm to 11.85 mm (average 4.91 ± 3.87 mm), and there was a significant difference between seasons ($p < 0.01$).

The results of the MPN analysis of Ct and CT in the bivalves *A. brasiliana* and *T. plebeius* for both sampling points in the Pina Basin are presented in tables 4 and 5.

The Ct concentrations in *A. brasiliana* varied from 3.0 x 10 MPN g^{-1} to 4.6 x 10^3 MPN g^{-1} at point 1. The highest rates were recorded during the second, third and sixteenth collections, which were carried out in June/2009, July/2009 and January/2010, respectively; the lowest MPN values for the Ct/g in the bivalves were found during the seventeenth and eighteenth collections (February/2010). The CT concentration varied from 3.0 x 10 g^{-1} to 4.6 x 10^3 MPN g^{-1}; the highest values were recorded during the second, third and sixteenth collection events (June/2009, July/2009 and January/2010, respectively), while the lowest values were observed during the seventeenth and eighteenth collections (February/2010). At point 2, the Ct levels varied from 3.0 x 10 MPN g^{-1} to 2.4 x 10^4 MPN g^{-1} for *A. brasiliana*. The lowest concentration was observed during the 23rd collection (May/2010), while the highest values were recorded during the first and fifth collection events (June and August/2009, respectively).

Tagelus plebeius exhibited higher Ct and CT concentrations, with 100% and 62.5% of the samples, respectively, showing concentrations higher than 10^2 MPN g^{-1} at point 1. The lowest MPNs of Ct were found in the samples collected during the ninth, nineteenth and twentieth collections (October/2009 and March/2010). The lowest CT levels were recorded in the samples obtained during the eighth collection (September/2009), while the highest were found during the first, third, fourth and fifth collections (between June and August/2009). At point 2, the values for Ct (83.33%) and CT (100%) varied from 2.3×10^2 to 1.1×10^4 MPN g^{-1}, and the lowest levels were found during the ninth, nineteenth, twentieth and 21st collections (October/2009, March/2010 and April/2010, respectively).

Table 4 - Most Probable Number (MPN) of total (Ct) and thermotolerant coliforms (CT) in the bivalves *Anomalocardia brasiliana* and *Tagelus plebeius* and the in the water at point 1 in the Pina Basin estuary, (Pernambuco, Brazil, June/2009-May/2010).

Collection date	Total coliforms			Thermotolerant coliforms		
	A. brasiliana (MPN g^{-1})	***T. plebeius*** (MPN g^{-1})	**Water** (MPN ml^{-1})	***A. brasiliana*** (MPN g^{-1})	***T. plebeius*** (MPN g^{-1})	**Water** (MPN mL^{-1})
08/Jun	2400	≥ 24000	75	2400	≥ 24000	9
25/Jun	4600	11000	240	4600	11000	23
06/Jul	4600	≥ 24000	≥ 2400	4600	≥ 24000	≥ 2400
20/Jul	1500	≥ 24000	≥ 2400	1500	≥ 24000	≥ 2400
04/Aug	2400	≥ 24000	≥ 2400	2400	≥ 24000	≥ 2400
18/Aug	930	11000	≥ 2400	430	11000	≥ 2400
01/Sep	2400	230	4600	2400	230	4600
17/Sep	2400	2400	2400	230	70	930
05/Oct	230	750	40	230	430	40
20/Oct	90	1500	90	90	1500	90
03/Nov	230	2400	90	90	2400	40
17/Nov	70	930	150	70	930	70
01/Dec	230	750	150	230	750	150
15/Dec	930	930	150	430	930	150
12/Jan	750	430	4600	750	430	930
29/Jan	4600	2400	4600	4600	2400	4600
03/Feb	<30	930	90	<30	430	40
18/Feb	<30	230	40	<30	230	40
01/Mar	430	930	150	430	930	150
15/Mar	40	2400	230	40	930	90
12/Apr	230	4600	2100	230	4600	2100
26/Apr	210	11000	11000	210	1500	11000
10/May	230	930	930	230	930	930
25/May	230	2400	4600	230	2400	930

The concentrations of thermotolerant coliforms in *A. brasiliana* ($p<0.01$) and *T. plebeius* ($p<0.0001$) were directly and significantly correlated with precipitation.

Table 5 - Most Probable Number (MPN) of total (Ct) and thermotolerant coliforms (CT) in the bivalves *Anomalocardia brasiliana* and *Tagelus plebeius* and in the water at point 2 in the Pina Basin estuary (Pernambuco, Brazil, June/2009-May/2010).

Collection date	Total coliforms			Thermotolerant coliforms		
	A. brasiliana	*T. plebeius*	Water	*A. brasiliana*	*T. plebeius*	Water
	(MPN g^{-1})		(MPN ml^{-1})	(MNP g^{-1})		(MPN ml^{-1})
08/Jun	≥ 24000	--	240	≥ 24000	--	240
25/Jun	2400	--	90	930	--	90
06/Jul	4600	--	1100	4600	--	1100
20/Jul	360	--	≥ 2400	360	--	≥ 2400
04/Aug	≥ 24000	--	≥ 2400	11000	--	≥ 2400
18/Aug	2400	--	≥ 2400	2400	--	≥ 2400
01/Sep	4600	--	2400	4600	--	2400
17/Sep	2400	11000	11000	110	4600	280
05/Oct	150	230	40	150	230	40
20/Oct	930	4600	230	430	2400	230
03/Nov	750	930	230	430	310	230
17/Nov	90	430	90	90	430	90
01/Dec	230	930	2400	230	930	2400
15/Dec	430	430	90	430	430	90
12/Jan	750	930	2400	750	930	2400
29/Jan	90	2400	4600	90	2400	4600
03/Feb	930	430	4600	930	430	4600
18/Feb	40	930	1500	40	930	1500
01/Mar	430	230	230	150	230	230
15/Mar	430	230	230	430	230	230
12/Apr	430	11000	11000	430	11000	11000
26/Apr	930	930	≥ 24000	930	930	≥ 24000
10/May	30	930	1500	30	930	750
25/May	--	4600	4600	--	930	210

Precipitation was also directly and significantly correlated with total coliforms in *A. brasiliana* ($p<0.01$) and *T. plebeius* ($p<0,0001$). Furthermore, in *T. plebeius*, the total coliforms ($p<0.05$) and thermotolerant coliforms ($p<0.01$) showed a significant inverse correlation with the water temperature (Table 6).

The Ct values from the water samples collected from points 1 and 2 varied from 4.0×10 to 1.1×10^4 MPN ml^{-1} and 4.0×10 to $\geq 2.4 \times 10^4$ MPN ml^{-1}, respectively, whereas the CT values varied from 9.0 to 1.1×10^4 MPN ml^{-1} and 4.0×10 to $\geq 2.4 \times 10^4$ MPN ml^{-1} (Tables 4 and 5).

Statistical analysis detected no significant differences in the concentrations of total and thermotolerant coliforms in the water between the two sampling points.

DISCUSSION

The higher concentrations of total and thermotolerant coliforms found in the water during the rainy season might have been due to the greater amount of organic matter from domestic sewage discharges carried by the rivers that flow into the area during this period. Similar results were found by Delgado-Gómez et al. (2008) when analyzing the water quality off the western coast of Havana (Cuba), as they found high concentrations of coliforms in the water during the months with the most rainfall. These authors reported that precipitation has a great influence on the density of microorganisms because it results in larger river volumes and more terrigenous runoff and promotes increases in the amounts of bacteria and contaminants that reach the coastal zone.

In most of the water samples analyzed in the present study, the total coliform levels were higher than the levels of thermotolerant coliforms. According to Goyal et al. (1977), this is not surprising, as total coliforms can originate from non-fecal sources such as plants and soil. These authors obtained results similar to those of this study and found a higher number of organisms in the winter; this was also verified by Faust et al. (1975) and Sayler et al. (1975).

Water temperature is an important parameter; nevertheless, in tropical areas, it is not a limiting factor. In the studied area, the observed temperatures were within what would be expected for an estuarine environment, and the pattern of seasonal and spatial variations was discrete.

According to Trabulsi et al. (2005), for each type of bacteria, there is an optimum temperature for nutrient absorption, which is closely related to the growth and development of a bacterial culture. Psychrophilic bacteria grow and absorb nutrients best at temperatures between 0º and 18ºC, whereas the optimum temperature for mesophiles is between 25º and 40ºC and that for thermophiles is between 50º and 80ºC. Thus, we can infer that the water temperature at Pina Basin during the period studied presented conditions favorable to the growth of mesophilic bacteria.

Salinity is an important environmental variable in estuaries that influences the spatial delimitation and distribution of organisms. At the Pina Basin, salinity varied according to precipitation and were higher during the dry season and at point 1. Feitosa et al. (1999), Nascimento et al. (2003) and Noriega et al. (2005) found similar values to those recorded by this study.

According to the classification of the Brazilian National Environmental Council (CONAMA, 2005), the waters of the Pina Basin can be classified as brackish. Hagler and Hagler (1988) reported that coliforms have little tolerance to seawater salinity; thus, the presence of coliforms in this kind of environment indicates recent and constant discharges of fecal material. It is also important to note the inversely proportional relationship between salinity and of coliform levels. Ferguson et al. (1996), Silva et al. (2003) and Vieira et al. (2008) verified an increase in coliform concentrations during the rainy season and other time points when salinity was low, while Goyal et al. (1977) found a negative correlation between total coliforms and salinity.

The characteristically alkaline pH observed during the study period agrees with the results reported by Feitosa et al. (1999) and Nascimento et al. (2003) for the same area. According to Trabulsi et al. (2005), coliforms develop within a wide pH range, from 4.4 to 9.0. Therefore, the variation in pH observed in this study might have favored coliform development.

The DO values in most of the samples considered to be brackish were below the level recommended by CONAMA Resolution nº 357 do CONAMA (CONAMA, 2005), which states that brackish waters where there is fishing activity or aquiculture related to intensive consumption must present DO values higher than 4 mg L^{-1}. With respect to BOD, the highest values corresponded to the highest rates of DO saturation (April /2010 was the exception).

Macêdo and Costa (1978) classified northeastern Brazil's estuarine zones according to oxygen saturation. Based on their system, we verified that at both sampling points, the Pina Basin estuary is a low saturation to polluted zone.

In Brazil, the CT concentrations in water are regulated by CONAMA Resolution n° 357/ 2005, which determines where bivalves can be collected or cultivated. The CT density (based on a minimum of 15 samples collected from the same location) must not surpass 43 MPN 100 ml^{-1}, and the 90^{th} percentile cannot be greater than 88 CT per 100 ml. According to the CT density, the water in the Pina Basin can be classified as Class I water (aquaculture, fishing activities and primary contact) and/or Class 2 (amateur fishing and secondary contact recreation), which shows that its quality goes against current legislation because of the high MPN ml^{-1} found for coliforms.

Machado et al. (2000) has suggested that the concentration of thermotolerant coliforms in the tissues and inter-valve liquid of mollusks is a more appropriate criterion for evaluating environmental quality than counting coliforms in water. According to Escobar Nieves (1988), the number of coliforms found in water is not a real measurement of environmental quality; instead, this author suggests that bivalves should be studied because water quality only predicts the probable pollution level at the moment of collection, whereas data from mollusks will indicate the level of bacteria bioaccumulated in their tissues (ZANETTE, 2006).

In a study involving the oyster *Crassostrea virginica* (Gmelin, 1791) in an estuary of the Gulf of Mexico, Burkhardt III and Calci (2000) found coliform concentrations that were four times higher in the mollusks than in the adjacent water. Pereira et al. (2006) studied oysters off the coast of Florianópolis (Santa Catarina state, Brazil) and found concentrations of CT that varied from <3 to >1.1 x 10^3 MPN g^{-1}. Kolm and Absher (2008) obtained values between 6.4 x 10^2 and 4.8 x 10^3 MPN g^{-1} in *Crassostrea rhizophorae* (Guilding, 1828) and *C. brasiliana* Lamarck from the estuarine complex of Paranaguá (state of Paraná, Brazil). The concentrations of total and thermotolerant coliforms in bivalves and the adjacent water found in this study followed a similar pattern; however, an inverse relationship was recorded in some samples, which might have been due to recent pollution discharges.

In a study involving oysters collected off the coast of the state of São Paulo, Sanchez et al. (1991) obtained CT levels that varied from 7.0 to 3.0 x 10^5 g^{-1}, i.e., higher than what was found in this study. In an analysis of bacteria originating from feces contaminating the oyster *C. rhizophorae* in the Cocó River estuary (state of Ceará, Brazil), the Ct and CT values found in the oyster's tissues varied from <1.8 g^{-1} to >1600 g^{-1} and <1.8 g^{-1} to 920 g^{-1}, respectively (Silva et al. 2003), which are all values lower than what was found in this study.

In a study investigating fecal contamination in *C. rhizophorae* at the Pacoti River estuary (state of Ceará, Brazil), Vieira et al. (2008) obtained MPN values for the oyster samples that varied between <1.8 and 3.5 x 10^3 g^{-1} for total coliforms and <1.8 and 2.8 x 10^3 g^{-1} for thermotolerant coliforms, i.e., values close to what was found in this study. Conversely, Vieira et al. (2007) detected MPN Ct values between <1.8 and 9200 g^{-1} and CT values that varied from <1.8 to 430 g^{-1} in *C. rhizophorae* in the Jaguaribe River estuary (Ceará, Brazil), which are values lower than those found in this study.

Tagelus plebeius exhibited a greater ability to bioaccumulate coliforms than *Anomalocardia brasiliana* at both sampling points. Huss (1997) and Bonadonna et al. (1990) showed that different mollusk species collected from the same bank under similar hygiene conditions can present different abilities in terms microorganism bioaccumulation, as this phenomenon depends on environmental conditions and the overall activities of a mollusk.

In general, the highest coliform concentrations occurred during the rainy season and decreased in hotter months. When comparing the variations in the levels of total and thermotolerant coliforms in the two species separately, we observed that in some samples, both concentrations followed a similar pattern. However, it must be noted that

T. plebeius was absent during the first seven collections, which can be explained by the greater influx of freshwater and sewage discharges through river effluents during this period. This species began to appear during the hotter months, when environmental conditions were favorable.

The bivalve *A. brasiliana* is considered to be quite resistant to both deficient levels of dissolved oxygen and to variations in salinity, which were conditions frequently observed in this study. As it is well adapted to conditions of hypoxia and even anoxia, this species is favored in locales exhibiting such conditions when compared to other species. *Anomalocardia brasiliana* was present during the entire study period. It was able to accumulate high concentrations of total and thermotolerant coliforms and was also tolerant to environmental pollution, which are characteristics that favor its use as a bioindicator of coliform levels in the study area.

With respect to environmental conditions, some authors (HAGLER and HAGLER, 1988; TRABULSI et al. 2005; VIEIRA et al., 2008) have recorded variations in coliform concentrations that were dependent on environmental factors. Similarly, the data obtained here show that variations in physical-chemical parameters influenced the coliform concentrations found in the bivalves and in the water analyzed from the studied environment.

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Table 6 – Values of Pearson’s correlation coefficient for the investigated environmental parameters and the total coliform concentrations in the bivalves *Anomalocardia brasiliana* and *Tagelus plebeius* and in the water of the Pina Basin estuary (Pernambuco, Brazil) during the period between June/2009 and May/2010. r_P: Pearson’s correlation coefficient; p: probability; AbCt: total coliforms in *A. brasiliana*; AbCT: thermotolerant coliforms in *A. brasiliana*; TpCt: total coliforms in *T. plebeius*; TpCt: thermotolerant coliforms in *T. plebeius*.

Environmental parameters	AbCt		AbCT		TpCt		TpCT		WaterCt		WaterCT	
	r_P	p	r_P	p	r_P	p	r_P	p	r_P	p	r_P	p
Dissolved oxygen	0.051	0.729	0.088	0.548	0.055	0.706	0.084	0.569	-0.227	0.119	-0.172	0.239
Biochemical oxygen demand	0.050	0.735	0.112	0.446	0.055	0.708	0.029	0.840	-0.257	0.076	-0.240	0.099
Salinity	-0.197	0.179	-0.193	0.186	-0.210	0.150	-0.196	0.180	-0.011	0.937	-0.003	0.979
pH	0.135	0.357	0.146	0.319	0.248	0.088	0.275	0.057	-0.179	0.223	-0.116	0.429
Water temperature	-0.145	0.323	-0.150	0.306	-0.248	0.088	-0.309	0.032	-0.444	0.001	-0.335	0.019
Precipitation	0.407	0.004	0.455	0.001	0.414	0.003	0.389	0.006	0.307	0.033	0.340	0.017

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