

MICROBIOLOGICAL MONITORING OF SEAWATER IN THE COASTAL ZONE OF PIAUÍ STATE, BRAZIL

Monitoramento microbiológico da água do mar na zona costeira do Estado do Piauí, Brasil

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ABSTRACT

The microbiological quality of beaches on the coast of Piauí State, Brazil was evaluated and the results compared with standards established by Brazilian legislation. No statistical correlation was found to exist between the rate of thermotolerant coliforms and rainfall indices. Low levels of thermotolerant coliforms were found in most samples so that most beaches can be ranked as excellent for bathing activities during the studied period.

Keywords: *sea water, microbiological surveillance, coastal zone, Piauí State.*

RESUMO

A qualidade microbiológica da água do mar de praias na costa do Piauí-Brasil foi avaliada e os resultados comparados com o padrão estabelecido pela legislação brasileira. Não foi observada correlação entre os níveis de coliformes termotolerante e índices pluviométricos. Níveis baixos de coliformes termotolerantes foram encontrados na maior parte das amostras categorizando as praias como excelentes para balneabilidade no período analisado.

Palavras-chaves: *água do mar, monitoramento microbiológico, zona costeira, Estado do Piauí.*

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INTRODUCTION

Water is a resource essential to life. All living organisms need water to survive and all metabolic processes occur with its direct or indirect action. In contrast, water is also an important vehicle for the transmission of a great number of diseases (Ponde, 2005).

Marine waters have been used for a variety of recreational purposes in different cultures. Doing physical activities or simply relaxing in marine waters promote great well-being. It is estimated that worldwide, local and foreign tourists spend US\$ 2 billion per day in coastal recreational environments (Shuval, 2003). Moreover, in recent years, several epidemiological studies have pointed out the relationship between bathing in marine waters and the occurrence of infectious diseases such as gastroenteritis, conjunctivitis and skin-, ear- and respiratory infections (Dwight *et al.*, 2005; Fleisher *et al.*, 2010). Data-based studies indicate that, each year, 120 million cases of gastrointestinal diseases and 50 million cases of severe respiratory illnesses in swimmers and sunbathers using polluted coastal waters occur in the world (Shuval, 2003).

The water quality in coastal and oceanic ecosystems allows a rational exploitation of living and non-living resources, ensuring the maintenance of the environment with the conservation of biodiversity and human health. Thus, the continuous monitoring of water quality of seas and oceans is not only an essential factor for public health, but also necessary to assess the environmental impact arising from anthropogenic activities on aquatic environments throughout time.

Piauí is the only state in Northeast Brazil whose capital is not located on the coast. Moreover, its coastline is the narrowest in the country, only 66-km wide, encompassing the counties of Parnaíba, Luis Correia and Cajueiro da Praia. Despite this apparent disadvantage, tourism in Piauí has grown in recent years owing particularly to the increased advertising of tours to the coast and the Parnaíba Delta, chosen in 2009 by the Brazilian Ministry of Tourism as one of the best destinations in Brazil (Brasil, 2009). Currently, this economic activity has been intensified through the wide dissemination of ecotourism in that state, (Brasil, 2011), although surveying of the quality condition of its beaches is not supposed to be a common practice and, hence, there is no data on the microbiological quality of the water off its coastline.

All things considered and given the trends in population growth and local development, this study was meant to evaluate, during one year, the microbiological quality of waters of three of the most visited beaches in the state: *Atalaia*, *Peito de Moça* and *Coqueiro* in Luis Correia county which has the largest stretch of coastline, about 46 kilometers, and 28,422 inhabitants (Brasil, 2010).

Therefore, two sampling points were determined on every beach, which are respectively, ATA1 (2°52'55"S, 41°38'09"W) and ATA2 (2°53'19"S, 41°37'37"W) in Atalaia Beach; PM1 (2°53'54"S, 41°36'38"W) and PM2 (2°53'55"S, 41°36'36"W) in Peito de Moça Beach; COQ1 (2°54'32"S, 41°45'10"W) and COQ2 (2°54'10"S, 41°34'17"W) in Coqueiro Beach (Figure 1), where seventy-two samples were collected early in the morning, on random days. The rainfall data was collected monthly using technical information from the meteorological station of the Institute of Meteorology (INMET). Thus, in the survey region, the period from December, 2010 through July, 2011 was considered as the rainy season, and the month of November, 2010 and the period from August, 2011 through October, 2011 as the dry season. Water samples were collected with sterile flasks at the 1 meter isobath- line and about 30 cm below the surface in the opposite direction of the waves, and kept on ice until laboratory analysis was started within 24 hours.

The quantification of total and thermotolerant coliform bacteria was made through the standard most probable number (MPN) method according to Clesceri *et al.* (2005). Coliforms were enumerated using lactose broth for the presumptive phase. In the confirmatory phase, brilliant green bile 2% broth was incubated at $35.5 \pm 0.5^\circ\text{C}$ for the quantification of total coliforms, and EC broth was incubated at $44.5 \pm 0.5^\circ\text{C}$ for thermotolerant coliforms. The software *GraphPad Prism version 5* was used to perform statistical analyses of the values obtained during the dry and rainy seasons.

Values of MPN per 100 mL for total and thermotolerant coliforms in monthly water samples, and rainfall data, are laid out in Table I, whence it can be seen that the higher counts of thermotolerant coliforms were 300 MPN per 100 mL and 900 MPN per 100 mL in the dry and rainy seasons, respectively. The correspondent values of dispersion are as follows for standard deviation and standard error: 144.50 per 100 mL and 20.85 per 100 mL (rainy season), and 72.85 per 100 mL and 14.87 per 100 mL (dry season). In addition, the 95% confidence intervals for thermotolerant coliforms had values of 14.31 -

96.06 per 100 mL in rainy season and 5.10 - 63.39 per 100 mL in dry season. Considering the seasonality of the results of the three beaches examined, we observed that the mean of rainy season of the data set is higher than in the dry season for both total and thermotolerant coliforms. However, there was no statistically significant difference between them ($p = 0.8233$, Wilcoxon-Mann Whitney's U-test).



Figure 1 - A: Map showing the location of Piauí State and Luis Correia; B: Satellite images of coastline showing Luis Correia, Parnaíba Delta and the sampling points (source: Google Maps, 2012).

Table I - Most Probable Number (MPN) of total and thermotolerant coliform per 100 mL of water sampled at Luis Correia Beach, Piauí State.

Month/year	Rainfall (mm)	Counts in MNP per 100 ml											
		ATA 1		ATA2		PM1		PM2		COQ 1		COQ 2	
		Total coli-form	Fecal coli-form	Total coli-form	Fecal coli-form	Total coli-form	Fecal coli-form	Total coli-form	Fecal coli-form	Total coli-form	Fecal coli-form	Total coli-form	Fecal coli-form
November, 2010	3.5	2	2	4	4	4	4	4	<2	11	9	30	4
December, 2010*	92.6	<2	<2	2	2	300	4	<2	<2	2	<2	2	2
January, 2011*	82.8	11	4	140	110	23	8	14	6	27	22	350	8
February, 2011*	225.6	130	80	13	8	30	13	50	17	4	2	240	220
March, 2011*	194.1	80	50	70	50	130	30	80	14	26	9	280	4
April, 2011*	264.7	14	4	34	8	22	4	22	9	70	70	14	8
May, 2011*	74.5	900	21	240	7	140	30	170	13	300	280	900	900
June, 2011*	63.7	17	<2	7	4	1600	280	23	23	80	2	900	280
July, 2011*	60.9	23	23	11	2	6	4	2	<2	12	2	6	2
August, 2011	5.3	50	50	2	2	350	300	9	4	26	4	20	13
September, 2011	-	30	23	140	34	900	220	280	80	17	2	17	4
October, 2011*	44.4	33	14	33	9	26	22	14	6	11	8	4	2

Convention: * rainy months.

It is noticeable that these seasonal standard deviation values are high because of dispersion of data about the mean, as shown by a few outliers which stand out in the statistics summary of each season's group (Figure 2).

The Brazilian legislation enacted through the National Council for the Environment's Resolution N°. 724 of November, 11th, 2000 (Brasil, 2000) classifies as excellent, recreational swimming waters presenting a maximum of 250 thermotolerant

coliforms per 100 mL in 80% or more of the samples taken at the same location, once a week, during the five previous weeks. Monthly samples were collected in each point and, according to the pollution-related management, the results of this study indicate that the surveyed beaches may be correctly classified as excellent for bathing, since 93% of the samples presented values below the maximum recommended target.

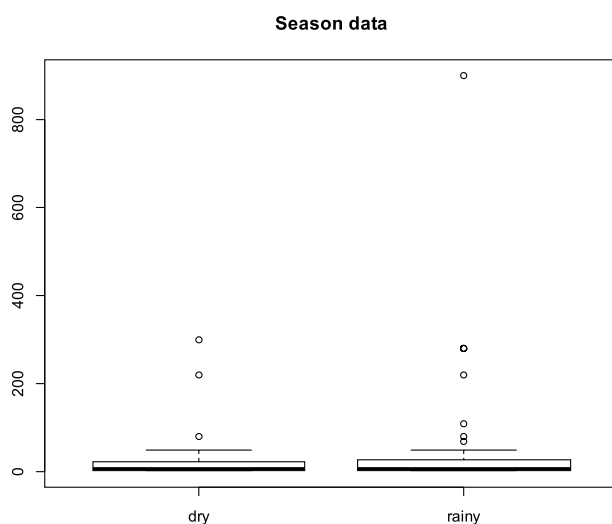


Figure 2 - Boxplots of the counts of Most Probable Number (MPN) of thermotolerant coliforms per 100 mL, in water samples from Luis Correia, Piauí State, during the dry and rainy seasons.

Time-space variations should be reckoned with when doing beach-monitoring since rainfall is an important cause of interference in the seawater quality as it carries sewage, garbage and other debris onto the coastal region through stormwater galleries, streams and drainage channels, thereby producing considerable increase in the bacteria density (CETESB, 2004). But sunlight and ultra-violet (UV) light are known to inactivate microbes in water, and the solar insolation level is among one of the most potent abiotic factors on the inactivation or death of bacteria such as thermotolerant coliforms and enterococci (Fujioka *et al*, 1981; Sinton *et al*, 2002; Noble *et al*, 2003; Whitman *et al* 2004; Abdelzaher *et al*, 2010; Zhu *et al*, 2011). The coast of Piauí has temperatures ranging from 25°C to 37°C, with a hot tropical climate presenting high UV index during most of the year. These environmental characteristics may contribute to the low counts of coliforms in all months of the year, even during the rainy season.

Quantification of enterococci in marine waters is suggested due the tolerance in hypertonic

environments, but there have been found to be consistent relationships between enterococci and thermotolerant coliform, *E. coli* and *Clostridium perfringens*, implying that environmental conditions affect their survival. Some recent studies have shown no statistically significant correlation between bacterial indicators (thermotolerant coliform and *Enterococcus faecalis*) and enteric viruses, which are supposed to be the most reliable for environmental monitoring due to its prolonged persistence in aquatic environments (*e.g.*, Ngazoa, Fliss and Jean, 2008; He *et al* 2012; Kishida *et al.*, 2012; Yang *et al.*, 2012).

In most coastal Brazilian capital cities sewerages are connected to ocean disposal of their effluents so as to lower chances of costal water pollution (Vieira, 2000), despite the power of stormwaters to this effect (Lourenço *et al.*, 2006; Vieira *et al.*, 2011). In Luis Correia county, only the *Atalaia* Beach is urbanized with a few medium-sized buildings and a sidewalk. *Peito de Moça* and *Coqueiro*, like the most other beaches in Piauí State, are not urbanized and boast only a few buildings. The sewer system used along the state's coast is septic tank, therefore, without direct discharge into the ocean what probably accounts for the low thermotolerant coliforms counts in most water samples throughout the year.

Summing up, the low thermotolerant coliforms counts in this period ranks the coast of Piauí as excellent as far as microbiological quality is concerned according to the standards adopted by CONAMA. However, some factors may influence these results such as the inactivating sunlight on coliforms and the fact that the coast is sparsely populated. The complexity of beach systems needs more innovative and comprehensive approaches to assess water quality in order to protect the bathers' health (Abdelzaher *et al*, 2011) and to help enhancing a fast-growing growing economy with the aid of a tourism industry, on-coming installations of a marine harbor, export processing zone and other projects. The continuous monitoring of the quality of these environments is crucial for the conservation of local biodiversity and human health.

Acknowledgements - We would like to thank FAPEPI - Piauí State's Foundation for Research Support and CNPq - National Council for the Scientific and Tecnological Development, for their financial support, and also UFPI - Federal University of Piauí for the scholarship PIBIC 2010-2011 awarded to Erik Vinicius de Sousa Reis.

REFERENCES

- Abdelzaher, A.M.; Wright, M.E.; Ortega, C.; Hasan, A.R.; Shibata, T.; Solo-Gabriele, H.M.; Kish, J.; Withum, K.; He, G.; Elmir, S.M.; Bonilla, J.A.; Bonilla, T.D.; Palmer, C.J.; Scott, T.M.; Lukasik, J.; Harwood, V.J.; McQuaig, S.; Sinigalliano, C.D.; Gidley, M.L.; Wanless, D.; Plano, L.R.; Garza, A.C.; Zhu, X.; Stewart, J.R.; Dickerson, J.W.; Yampara-Iquise, H.; Carson, C.; Fleisher, J.M. & Fleming, L.E. Daily measures of microbes and human health at a non-point source marine beach. *J. Water Health*, v.9, n.3, p.443-457, 2011.
- Abdelzaher, A.M.; Wright, M.E.; Ortega, C.; Solo-Gabriele, H.M.; Miller, G.; Elmir, S.; Newman, X.; Shih, P.; Bonilla, J.A.; Bonilla, T.D.; Palmer, C.J.; Scott, T.; Lukasik, J.; Harwood, V.J.; McQuaig, S.; Sinigalliano, C.; Gidley, M.; Plano, L.R.; Zhu, X.; Wang, J.D. & Fleming, L.E. Presence of pathogens and indicator microbes at a non-point source subtropical recreational marine beach. *Appl. Environ. Microbiol.*, v.76, n.3, p.724-732, 2010.
- BRASIL. *Jornalistas italianos conhecem rota das emoções*. Ministério do Turismo, 2011 Available at: http://www.turismo.gov.br/turismo/noticias/todas_noticias/20110926-1.html. Accessed 27 December 2012.
- BRASIL. *Censo Populacional 2010*. Instituto Brasileiro de Geografia e Estatística. Available at: <http://www.ibge.gov.br/home/estatistica/populacao/censo2010/>. Accessed 26 December 2012. 2010.
- BRASIL. *Vencedores do Troféu Roteiros do Brasil*. Ministério do Turismo. Available at: http://www.turismo.gov.br/turismo/noticias/todas_noticias/200906176.html. Accessed 27 December 2012. 2009.
- BRASIL. *Resolução nº 274, de 29 de novembro de 2000*. Conselho Nacional do Meio Ambiente, Brasília, 2000.
- CETESB. *Relatório de qualidade das águas interiores do Estado de São Paulo - 2003*. Secretaria do Meio Ambiente, São Paulo, 2004.
- Clesceri, L.S.; Greenberg, A.S. & Eaton, A.D. *Standard methods for the examination of water and wastewater*. APHA, 1220 p., Washington, D.C., 2005.
- Dwight, R.H.; Fernandez, L.M.; Baker, D.B.; Semenza, J.C. & Olson, B.H. Estimating the economic burden from illnesses associated with recreational water pollution - a case study in Orange county, California. *J. Environ. Manag.*, v.76, n.2, p.95-103, 2005.
- Fleisher, J.M.; Fleming, L.E.; Solo-Gabriele, H.M.; Kish, J.K.; Sinigalliano, C.D.; Plano, L.; Elmir, S.M.; Wang, J.D.; Withum, K.; Shibata, T.; Gidley, M.L.; Abdelzaher, A.; He, G.; Ortega, C.; Zhu, X.; Wright, M.; Hollenbeck, J. & Backer, L.C. The beaches study: health effects and exposures from non-point source microbial contaminants in subtropical recreational marine waters. *Int J Epidemiol.*, v.39, n.5, p.1291-1298, 2010.
- Fujioka, R.S.; Hashimoto, H.H.; Siwak, E.B. & Young, R.H. Effect of sunlight on survival of indicator bacteria in seawater. *Appl. Environ. Microbiol.*, v.41, n.3, p.690-696, 1981.
- He, X.; Wei, Y.; Cheng, L.; Zhang, D. & Wang, Z. Molecular detection of three gastroenteritis viruses in urban surface waters in Beijing and correlation with levels of fecal indicator bacteria. *Environ Monit Assess.*, v.184, n.9, p.5563-5570, 2012.
- Kishida, N.; Morita, H.; Haramoto, E.; Asami, M. & Akiba, M. One-year weekly survey of noroviruses and enteric adenoviruses in the Tone River water in Tokyo Metropolitan Area, Japan. *Water Res.*, v.46, n.9, p.2905-2910, 2012.
- Lourenço, E.M.L.; Vieira, G.H.F.; Festivo, M.L.; Rodrigues, D.P. & Vieira, R.H.S.F. Balneabilidade das praias do Odus e das Barreiras (Camocim, Ceará). *Bol. Téc. Cient. CEPNOR*, Belém, v.6, n.1, p.19-32, 2006.
- Ngazoa, E.S.; Fliss, I. & Jean, J. Quantitative study of persistence of human norovirus genome in water using TaqMan real-time RT-PCR. *J. Appl. Microbiol.*, v.104, n.3, p.707-15, 2008.
- Noble, R.T.; Moore, D.F.; Leecaster, M.K.; McGee, C.D. & Weisberg, S.B. Comparison of total coliform, fecal coliform, and enterococcus bacterial indicator response for ocean recreational water quality testing. *Water Res.*, v.37, n.7, p.1637-1643, 2003.
- Pond, K. *Water recreation and disease: plausibility of associated infections: acute effects, sequelae and mortality*. IWA Publishing, London, 2005.
- Shuval, H. Estimating the global burden of thalassogenic diseases: human infectious diseases caused by wastewater pollution of the marine environment. *J. Water Health*, v.1, n.2, p.53-64, 2003.
- Sinton, L.W.; Hall, C.H.; Lynch, P.A. & Davies-Colley, R.J. Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. *Appl. Environ. Microbiol.*, v.68, n.3, p.1122-1131, 2002.

Vieira, R.H.S.F. Poluição microbiológica de algumas praias brasileiras. *Arq. Ciên. Mar*, Fortaleza, v.33, p.77-84, 2000.

Vieira, R.H.S.F.; Menezes, F.G.R.; Costa, R.A.; Marins, R.V.; Abreu, I.M.; Fonteles-Filho, A.A. & Sousa, O.V. Storm drains as a source of fecal-derived pollution to Fortaleza city's coastal zone. *Arq. Ciên. Mar*, Fortaleza, v.44, n.2, p.5-12, 2011.

Whitman, R.L.; Nevers, M.B.; Korinek, G.C. & Byappanahalli, M.N. Solar and temporal effects on *Escherichia coli* concentration at a Lake Michigan

swimming beach. *Appl. Environ. Microbiol.*, v.70, n.7, p.4276-4285, 2004.

Yang, N.; Qi, H.; Wong, M. M.; Wu, R.S. & Kong, R.Y. Prevalence and diversity of norovirus genogroups I and II in Hong Kong marine waters and detection by real-time PCR. *Mar Poll. Bull.*, v.64, n.1, p.164-168, 2012.

Zhu, X.; Wang, J.D.; Solo-Gabriele H.M. & Fleming, L.E. A water quality modeling study of non-point sources at recreational marine beaches. *Water Res.*, v.45, n.9, p.2985-2995, 2011.