Evaluation of the microbiological and physicochemical quality of seawater from the coast of Pernambuco, Brazil

Avaliação da qualidade microbiológica e físico-química de água do mar do litoral de Pernambuco, Brasil

Thiago Marques da Silva
Graduation in Chemical Engineering, Catholic University of Pernambuco

Fernando Buarque Ramalho dos Santos
Graduation in Chemical Engineering, Catholic University of Pernambuco

João Victor Mendes da Silva
Graduation in Chemical Engineering, Catholic University of Pernambuco

Carlos Eduardo Anjos da Silva
Graduation in Chemical Engineering, Catholic University of Pernambuco

Pammello Pablo Martins da Silva Barros
Chemistry Technician; Catholic University of Pernambuco

Ákylla Fernanda Souza Silva
PhD in Biotechnology, Rede Nordeste de Biotecnologia (RENORBIO), Universidade Federal Rural de Pernambuco

Rafael de Souza Mendonça
PhD in Biotechnology, Rede Nordeste de Biotecnologia (RENORBIO), Universidade Federal Rural de Pernambuco

Sérgio Carvalho de Paiva
Professor of Chemical Engineering, ICAM-Tech School; PPG-DPA, NPCIAMB, Catholic University of Pernambuco

Galba Maria de Campos-Takaki
Professor of Chemical Engineering, ICAM-Tech School; PPG-DPA, NPCIAMB, Catholic University of Pernambuco
E-mail: galba.takaki@unicap.br

Keywords: Seawater, Coast of Pernambuco, Sea pollution.

1 INTRODUCTION

Seawater pollution has increasingly become a problem of global concern. There are recurring problems with the constant improper disposal of garbage and the coastal discharge of untreated domestic sewage, as well as urban stormwater runoff and river discharges rich in animal waste, which contribute to the contamination of water and sand

on beaches intended for urban recreation. (ADENIJI; SIBANDA; OKOH, 2019; ODONKOR; AMPOFO, 2013).

The coast of Pernambuco is recognized as one of the most important tourist routes in the country, known for its beautiful beaches and numerous reefs, which serve as a home for marine fauna. However, with the expansion of urban centers, industries, and livestock and agricultural activities, a greater rise in the rate of contamination of the seas has been observed. Recreational areas of the state coast have been monitored from the microbiological and physicochemical point of view, being constantly reported with high densities of microorganisms indicators of fecal contamination. (BARBOSA et al., 2022; DOS SANTOS et al., 2021) which could cause health problems such as gastrointestinal, skin, eye and ear infections among bathers who frequent these beaches (SABINO et al., 2014).

The microbiological quality of water and food can be established, using, as a parameter, microorganisms indicating fecal contamination, such as the Coliform group, having as main representative the bacterium *Escherichia coli*, which evaluates its hygienic conditions evidencing its relationship with the history of the sample. (MENDONÇA et al., 2017; SILVA et al., 2016).. The coliform group is currently considered the group of indicator organisms of choice for determining the microbiological quality of seawater, since, under extreme environmental conditions, such as high temperature and salt concentration, these microorganisms are able to mimic the fate and spread of numerous of the most persistent environmentally pathogens. (ADENIJI; SIBANDA; OKOH, 2019).

Thus, it is in the interest of public health, monitoring and assessment of beach quality based on microbiological and physicochemical parameters are considered a vital part of coastal management programs as recreational activities increase (DE SANTANA et al., 2022). In this sense, the present study aimed to evaluate the microbiological and physicochemical quality of seawater from the coast of Pernambuco.

2 OBJECTIVE

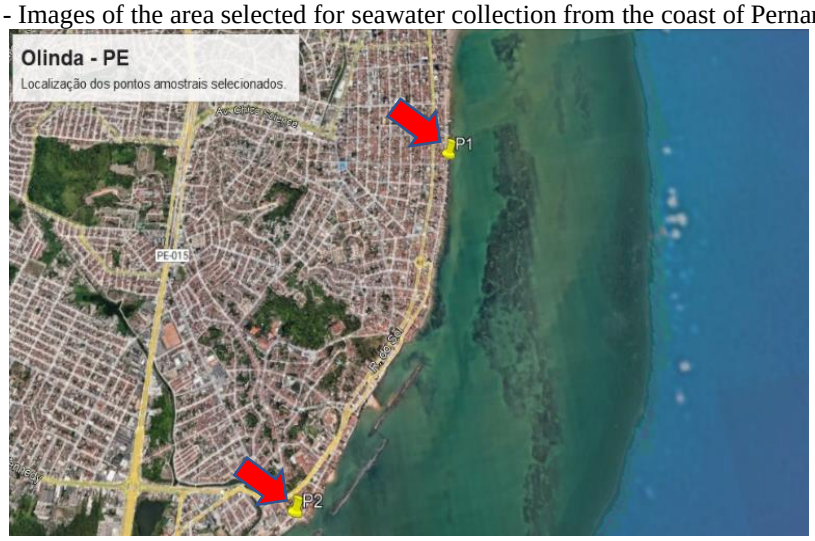
To evaluate the microbiological and physicochemical quality of seawater from the coast of Pernambuco.

3 METHODOLOGY

3.1 LOCATION AND CHARACTERIZATION OF THE SAMPLING AREA

The present study had a laboratory experimental design. The research universe was composed of seawater samples from the coast of Pernambuco. The collections were carried out from April to May 2023, and strategic points were selected along the coastal strip of the state, using the Google Earth tool in an attempt to establish equidistant points in order to obtain the maximum standard in the collections. Thus, the collection points were selected, considering that they are tourist points of greater frequency in Olinda and that, in turn, may present a greater risk to health due to the incorrect discharge of effluents (Figure 1). The coordinates and characteristics of the two collection points are described in Table 1.

Figure 1 - Images of the area selected for seawater collection from the coast of Pernambuco.



Source: Google Earth (2023).

Table 1. Characteristics of the selected sampling points.

Poins	Latitude	Longitude	Relative Humidity (%)	Temperature (ºC)
P1	8°01'20.3"S	34°51'07.5"W	78,00	28,0
P2	8°00'29.2"S	34°50'27.9"W	78,00	28,0

Source: Authors (2023).

3.2 SAMPLE COLLECTION

Water samples for the studies were collected according to the methodology described by Parron et al, (2011). 1.5L of surface water was collected using 2 sterile 500 mL polypropylene plastic collection bottles. The collection was carried out by removing the lid, followed by inversion of the lower base with the mouth down, keeping it in this

position, making the dive of the bottle at a depth of 15 cm. With the bottle submerged, it was turned horizontally in the opposite direction to the current. After collection, the flask was sealed, dried and identified and packed in an isothermal box, keeping the temperature at 4°C. It was then transported to the Laboratory of the Center for Research in Environmental Sciences and Biotechnology - NPCIAMB of the Catholic University of Pernambuco, where the physical-chemical and microbiological analyzes were immediately performed and then stored at a temperature of 4°C.

3.3 MICROBIOLOGICAL ANALYSIS

The microbiological analysis was performed using the Multiple Tube Technique, a method recommended by the Standard Methods for the Examination of Water and Wasterwater (APHA, 2012)This method is divided into three successive phases: presumptive, confirmatory and complementary, thus allowing the quantification by "Most Probable Number" (MPN) of coliform bacteria. In the presumptive phase, the samples were homogenized and transferred to 5 test tubes containing Durhan's broth in double lactosate (CD) at the bottom. Then, 1mL of the sample from each tube was inoculated with simple lactosate broth (CS). 1mL of the samples was transferred to a dilution tube containing 9mL of sterile water, of this dilution 1mL was inoculated into each tube (5) of simple lactosate broth (CS1). All tubes were incubated at 37°C for 24/48 hours. In the confirmatory phase, samples from the positive presumptive tubes were transferred to tubes containing brilliant green broth. All tubes were incubated at 37°C for 24/48 hours, and then the tubes that had total coliform growth were identified by gas production in Durhan tubes.

The complementary phase was carried out to identify fecal *coliforms* by transferring the presumptive tubes to tubes containing *Escherichia coli* (E.C) broth, which were incubated at 44.5°C in a water bath for 24 hours. A confirmatory assay was also carried out in solidified medium by inoculation into Petri dishes containing specific differential medium for coliforms (Eosin Methylene Blue - EMB; Endo, Mac Conkey or others) incubated at 35^{o} C for 24-48 hours.

3.4 ANALYSIS OF PHYSICO-CHEMICAL PARAMETERS

The physicochemical parameters analyzed were: pH, using a pH meter model (TECNAL - TEC - 7); conductivity, using a conductivity meter model (mCA 150); turbidity, using a turbidity meter model (ALFAKIT); apparent color, determined using

visual colorimeter (DLNH-100).

For the determination of total solids (TS), the methodology described by the MAPA work tool was used.

Ministry of Agriculture, Livestock and Supply (2014), calculated using the following equation:

Equation 1.

Where:

- **ST** = Total solids in mg/L
- **MS** = Mass of the sample dried at 105°C, in mg
- **MR** = Mass of the porcelain capsule in mg
- **VA** = Volume of the sample in mL

The Mohr method was used to determine the chloride content, while sulfates were determined by turbidimetry. Total hardness was determined by titration with EDTA (complexometry). The Kjeldahl method was used to determine total nitrogen, while sodium and potassium were determined by photometry. Total iron content was determined by UV-Vis spectrophotometry.

The determination of Biochemical Oxygen Demand (BOD) was carried out by the dilution and incubation method at 20ºC.

- 5 days, Chemical Oxygen Demand (COD) was determined by the dichromate digestion method in acid medium and titration with ammoniacal ferrous sulfate and Dissolved Oxygen (DO) was determined by the Winkler method.

4 DEVELOPMENT

4.1 MICROBIOLOGICAL ANALYSIS

The classification of beaches is based on what is established by Resolution CONAMA Nº 357/2005which defines water quality standards for bathing. The classification criterion is based on thermotolerant coliform concentrations. Saline waters intended for primary set recreation are classified as: proper (when 80% or more of the samples obtained present a maximum of 1000 thermotolerant coliforms per 100 mL of sample) and improper (when the criteria for proper waters are not met or present more than 2500 thermotolerant coliforms in the last sampling).

According to Table 2, 5 positive tubes identified with the presence of gas can be observed, confirming the presence of total coliforms in the water at the points selected for the analysis. In a study conducted by (SOUZA; SILVA, 2015) on Barra Grande beach, Salvador-BA, high concentrations of total coliforms were found in high season periods, corroborating this study.

Table 2. Results per sample of the presumptive tests

Points	**Inoculated points**	**Positive tubes Double broth**		**Positive tubes Simple broth**		**Positive tubes Simple broth 1**		**Table of contents (MPN/100 ml)**
		Nº	**%**	**Nº**	**%**	**Nº**	**%**	
P1	5	5	100	5	100	2	40	500
P2	5	5	100	5	100	3	60	900

Source: Authors (2023).

In the EC medium, all tubes showed gas production, being positive for fecal coliforms as shown in Table 3. According to CONAMA Resolution No. 274/2000 and CONAMA Resolution No. 357/2005, the analyzed areas (P1 and P2) have high levels of total and fecal *coliforms* and *E. coli*, presenting themselves as unsuitable for bathing. The bacteria were identified as: *Escherichia coli* variety I (Group 1), *Enterobacter aerogenes* variety II (Group 2) and *Enterobacter aerogenes* variety I (Group 3).

Table 3. Confirmatory test result per sample

Points	**Inoculated points**	**Positive tubes Double broth *E. coli***		**Positive tubes Simple broth *E. coli***		**Positive tubes Simple broth 1 *E. coli***		**Table of contents (MPN/100 ml)**	**Track (MPN/100 ml)**
		Nº	**%**	**Nº**	**%**	**Nº**	**%**		
P1	5	4	80	2	40	1	20	26	<12 a > 65
P2	5	5	100	5	100	-	0	240	< 100 a > 940

Source: Authors (2023).

Table 4: Results of bacteria identification in each water sample.

Points	INDOL	METHYL RED	VOGES PROSKAUER	CITRATE	ISOLATED BACTERIA
P1	– – +	+ – +	– + +	– + +	*Escherichia coli* -Variety II *Enterobacter aerogenes* - Variety I *Proteus vulgaris*
P2	– – + –	– + + +	+ + + +	– + + –	*Enterobacter aerogenes* -Variety I *Proteus mirabilis* *Proteus vulgaris* *Klebsiella pneumoniae*

Source: Authors (2023).

The presence of total and fecal *coliforms* and *Escherichia coli* in the marine environment may be due to microbial contamination of fecal origin, so it is indicative of inadequate sanitary conditions. (Destro (2020) and KKochinski (2020) concluded in their study that the presence of animals, sanitary sewage dumping, change of season, and many bathers contribute to the survival and dispersion of pathogenic microorganisms in saline waters.

The physicochemical analyses showed that only the total nitrogen (N_2) and nitrite (NO_2^-) nitrate (NO_3^-) and ammonium (NH_4^+) variables were within the parameters specified by the legislation at the collection points analyzed. When analyzing the microbiological parameters it was observed that in the quantification of total and fecal coliforms in all points were with indexes above the quality control. In the confirmatory test for *Escherichia coli* 100% of the samples showed values above the tolerable limits of contamination. In this context, the areas analyzed in beaches of the coast of Pernambuco present unsatisfactory physicochemical and microbiological quality, not meeting the quality standards established by CONAMA Resolution No. 274/2000 and CONAMA Resolution No. 357/2005.

4.2 PHYSICO-CHEMICAL PARAMETERS

Table 05 presents the results of the physicochemical analyses related to the analyzed points that were interpreted and compared with the quality standard according to the Resolution of the National Council of Environment (CONAMA) No. 357/2005, related to saline waters class 1, 2 and 3.

The electrical conductivity of water is an important indicator of possible pollutant sources, however, it does not discriminate which ions are present in the water (VON SPERLING, 2007). Electrical conductivity above 0.100 mS/cm, according to CETESB, indicates corrosive characteristics of water and environmental impacts by anthropic actions. In this study, according to Table 2, it can be observed that point P2 presented high pH (8.02) and point P1 electrical conductivity with 66.8 mS, which can be justified by the dilution of river waters to domestic sewage discharged into the sea.

Table 5. Physico-chemical parameters of the selected sampling points.

Parameters (Unit)	**Point P1**	**Point P2**
pH	7,97	8,02
Electrical Conductivity (µS/cm)	66.800,00	66.220,00
Apparent color (mg/L Pt-Co)	1,50	-
Turbidity (mg/L SiO_2)	0,00	0,00
Temperature (^{o} C)	28,00	28,00
Chlorides (mg/L Cl^-)	8.600,00	8.350,00
Total hardness (mg/L CaCO3)	2.800,00	3.010,00
Sodium (mg/L Na^+)	11.397,74	11.172,22
Potassium (mg/L K^+)	446,50	364,08
Total solids (mg/L)	37,46	29,87
Dissolved oxygen (mg/L O_2)	3,20	5,60
COD (mg/L O2)	172,20	114,02
BOD (mg/L O2)	13,33	16,85

Source: Authors (2023).

According to the Resolution of the National Environmental Council (CONAMA) No. 357/2005, the pH of saline waters should be in the range of 6.5 to 8.5, with no natural variations greater than 0.2 units. In this work, the two points were below the specifications for saline waters class 1,2 and 3.

According to the data obtained through the analyzes, it is noted from Table 5 that the dissolved solids values are high due to the high levels of sodium chloride because it is saline water. The results obtained indicate high values of volatile solids at points P1 and P2, indicating the presence of organic matter from the mixture of domestic sewage and river water, making the study area unsuitable for use in primary and secondary contact, according to CONAMA resolution 357/2005. The disposal of industrial and

domestic waste in saline waters becomes a danger to human and animal health, in addition to contaminating the waters favors the appearance of waterborne diseases. (BARBOSA et al., 2022).

There are no maximum or minimum values stipulated in the legislation for the parameters of chlorides, acidity, alkalinity, total hardness, calcium, magnesium, sulfates, sodium and potassium. However, the samples showed high values of chlorides (Cl^-), sulphates (SO^{2-}_4) and sodium (Na^+) as shown in Table 5. According to De SSouza et al. (2018)the presence of chlorides, phosphorus, nitrites and nitrates are factors indicative of eutrophication of this ecosystem, so there is greater production of organic matter than its consumption and decomposition. They can come from natural sources such as rock composition and carried by rainwater runoff, particulate matter present in the atmosphere, as well as from artificial sources such as domestic sewage, sand removal.

The values found for dissolved oxygen (DO) for points P1 and P2 were 3.2 and 5.6 mg/L respectively, being in accordance with CONAMA resolution No. 357/2005 for saline waters that should not be less than 6.0 mg/L. The results of the chemical oxygen demand (COD) and biochemical oxygen demand (BOD) portray a large amount of organic matter at points P1 and P2, thus confirming the presence of domestic sewage discharges in the study area due to river interference and the proximity of bars and restaurants, in addition, the CPRH (State Environment Agency) identified in March 2022 the poor sanitation at these points, whose untreated sewage is clandestinely discharged into the sea.

5 FINAL CONSIDERATIONS

Pollution of recreational waters, either by fecal or industrial waste, is a major public health hazard. In the present study, the levels of the physicochemical quality parameters of the samples from Olinda Beach did not comply with the standards established for recreational water. Likewise, the microbiological quality of the water samples suggests possible health threats if ingested, since they showed high levels of contamination by the Coliform group and presence of opportunistic pathogenic bacteria. Thus, the results of this study indicate that a ban on these leisure areas is necessary and that further research should be carried out in order to monitor the quality of these waters as well as their health safety.

ACKNOWLEDGMENTS

The authors wish to thank FACEPE (Foundation for the Support of Science and Technology of Pernambuco) for the grant No. IBPG-1775-2.00/21 to Ákylla Fernanda Souza Silva; CAPES for the grant to Rafael de Souza Mendonça and CNPq (National Council for Scientific and Technological Development) for the grant No. 312241/2022-4 to Galba M. Campos-Takaki and the Catholic University of Pernambuco for the structuring and provision of academic and laboratory spaces.

REFERENCES

ADENIJI, O. O.; SIBANDA, T.; OKOH, A. I. Recreational water quality status of the Kidd's Beach as determined by its physicochemical and bacteriological quality parameters. Heliyon, v. 5, n. 6, p. e01893, 1 jun. 2019.

APHA, A. WEF (2012) Standard Methods for examination of water and wastewater. Washington: American Public Health Association. Washington: ISBN 978-087553-013-0, 2012.

BARBOSA, A. C. et al. Caracterização físico-química e microbiológica da água do mar de praias do litoral de Pernambuco. Research, Society and Development, v. 11, n. 15, p. 20111536240–20111536240, 8 nov. 2022.

CONAMA. Resolução CONAMA Nº 357 DE 17/03/2005. , 2005. Disponível em: <http://conama.mma.gov.br/?option=com_sisconama&task=arquivo.download&id=450>. Acesso em: 30 maio. 2023

DE SANTANA, V. V. et al. Práticas turísticas nos ambientes recifais de Porto de Galinhas, Pernambuco: gestão, manejo e estrutura de governança. Guaju, v. 8, 2022.

DE SOUZA, N. M. et al. Spatial and temporal evaluation of the water quality index and trophic state index of the Curuçá River, Maranhão, Brazil. Ciência e Natura, p. 58–70, 2018.

DESTRO, J. O. et al. Qualidade microbiológica das areias de praia do Litoral Norte gaúcho. Scientia Prima, v. 6, n. 1, p. 48–58, 2020.

DOS SANTOS, A. R. et al. Identificação e caracterização de bactérias potencialmente patogênicas isoladas de duas praias do litoral norte de Pernambuco. Research, Society and Development, v. 10, n. 11, p. e184101119402–e184101119402, 2021.

KOCHINSKI, T.; BARBOSA, P.; ROMANELLO, L. Análise microbiológica de bactérias patogênicas em areias de praças públicas no município de União da Vitória-Paraná. LUMINÁRIA, 2020.

MENDONÇA, M. H. M. et al. Análise bacteriológica da água de consumo comercializada por caminhões-pipa. Revista Ambiente & Água, v. 12, n. 3, p. 468–475, 2017.

ODONKOR, S. T.; AMPOFO, J. K. Escherichia coli as an indicator of bacteriological quality of water: an overview. Microbiology research, v. 4, n. 1, p. e2, 2013.

PARRON, L. M.; MUNIZ, H. DE F.; PEREIRA, C. M. Manual de procedimentos de amostragem e análise físico-química de água. 2011.

SABINO, R. et al. Routine screening of harmful microorganisms in beach sands: Implications to public health. Science of the Total Environment, v. 472, p. 1062–1069, 2014.

SILVA, Á. F. S. et al. Análise bacteriológica das águas de irrigação de horticulturas. Revista Ambiente & Água, v. 11, n. 2, p. 428–438, 2016.

SOUZA, J. L. DE; SILVA, I. R. Avaliação da qualidade ambiental das praias da ilha de Itaparica, Baía de Todos os Santos, Bahia. Sociedade & Natureza, v. 27, p. 469–483, 2015.

VON SPERLING, M. Wastewater characteristics, treatment and disposal. [s.l.] IWA publishing, 2007.