Microbiological characterization of pathogenic bacteria and physicochemical in sea waters of Olinda – PE

Caracterização de microbiológica de bactérias patogênicas e físico-química em águas do mar de Olinda - PE

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ABSTRACT
Beaches around the world provide recreational opportunities for hundreds of millions of people and serve as important components of coastal economies. Monitoring the water quality of beaches is often carried out for their microbiological quality and physicochemical parameters in order to detect the presence of indicators of human contamination by sewage in order to prevent public health outbreaks associated with contact with water (ADENIJI; SIBANDA; OKOH, 2019; BARBOSA et al., 2022).

Keywords: Microbiological, Physicochemical, Water.

RESUMO
As praias em todo o mundo oferecem oportunidades de lazer para centenas de milhões de pessoas e servem como componentes importantes das economias costeiras. O monitoramento da qualidade da água das praias é frequentemente realizado quanto à sua qualidade microbiológica e parâmetros físico-químicos, a fim de detectar a presença de indicadores de contaminação humana por esgoto, a fim de prevenir surtos de saúde pública associados ao contato com a água (ADENIJI; SIBANDA; OKOH, 2019; BARBOSA et al., 2022).

Palavras-chave: Microbiológica, Físico-química, Água.

1 INTRODUCTION

Beaches around the world provide recreational opportunities for hundreds of millions of people and serve as important components of coastal economies. Monitoring the water quality of beaches is often carried out for their microbiological quality and physicochemical parameters in order to detect the presence of indicators of human contamination by sewage in order to prevent public health outbreaks associated with contact with water (ADENIJI; SIBANDA; OKOH, 2019; BARBOSA et al., 2022).

However, growing evidence suggests that beach water may present microorganisms with great pathogenic potential and in high concentrations, becoming harmful to human health. Currently, the standards for monitoring, sampling, analysis or management of the water quality of beaches are carried out on the basis of the Resolutions CONAMA No 357/2005 and CONAMA N° 274/2000, 2000). The public health threat associated with populations of microorganisms through direct and indirect contact is unknown, since little research has been conducted related to health outcomes associated with beach water quality.

Microorganisms that indicate fecal contamination, such as the Coliform group, whose main representative is the bacterium *Escherichia coli*, are used in the evaluation of the microbiological quality of the water and evidence its hygienic conditions and 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 the determination of 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, the present study aimed to evaluate the microbiological and physicochemical quality of seawater from the coast of Pernambuco, since it is in the interest of public health, the monitoring and evaluation of the quality of beaches, being considered a vital part of coastal management programs, as recreational activities increase (DE SANTANA et al., 2022).

2 GOAL

To evaluate and confirm the presence of pathogenic bacteria and altered levels of physicochemical components in seawater from the coast of Olinda – PE.

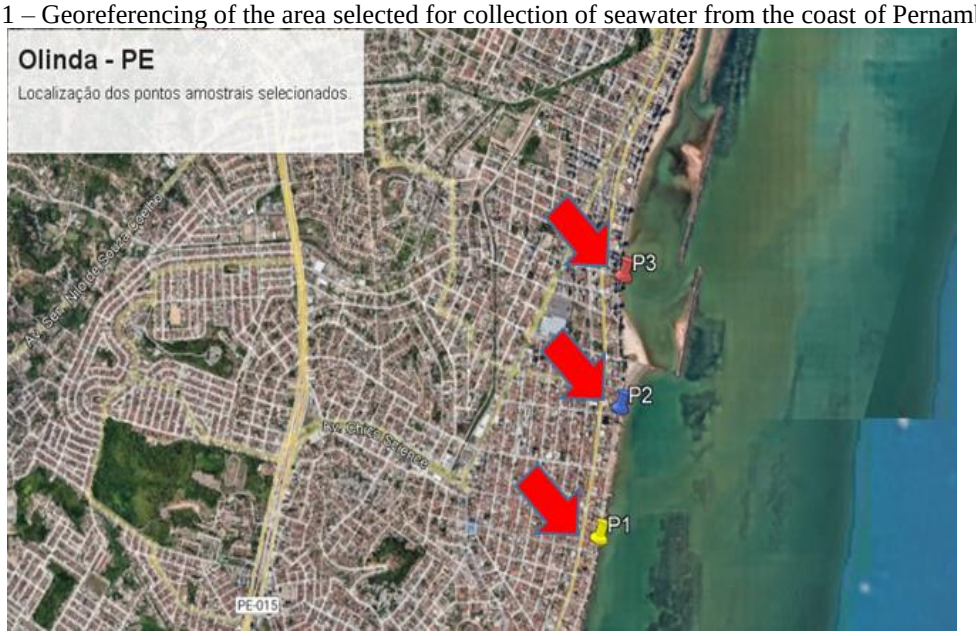
3 METHODOLOGY

3.1 LOCATION AND CHARACTERIZATION OF THE SAMPLING AREA

The coast of Pernambuco is 187 km long and covers 21 municipalities, which concentrate 56% of the state's population. However, in the coastal strip there are only 13 municipalities of Pernambuco, which are segmented into North, Metropolitan Core and South (DE AMORIM et al., 2023). The coast of Pernambuco, known for its beautiful beaches and its numerous reefs, which serve as a home for marine fauna, is the destination point of many tourist itineraries, but the diversity of this ecosystem enters crisis due to pollutants released directly into the water. The area covered by seas and oceans is significantly larger than that of land, covering more than 71% of the planet's surface. In this sense, it is understood the importance of marine conservation not only for life concentrated in marine waters, but also for all organisms associated with salt water (DOS SANTOS; DE SOUZA ABESSA, 2021).

The study areas have a humid tropical climate, with an average annual temperature of 25º to 30ºC. The region is characterized by a dry period (September to February), and a rainy period (March to August) (PEREIRA et al., 2017). For the collection of samples, some points along the coastal strip of the coast of Pernambuco were selected, using the Google Earth tool, to establish equidistant points, in order to obtain the maximum standard in the collections. The collections were carried out on the beach of Olinda considering a high influx of the public and because it presented a greater potential risk to health, due to the incorrect dumping of sanitary sewage (Figure 1).

Figure 1 – Georeferencing of the area selected for collection of seawater from the coast of Pernambuco.



Source: Google Earth (2023).

Table 1 - Characteristics of the selected sampling points.

Stitches	Latitude	Longitude	Relative Humidity (%)	nperature (ºC)	Rainfall (mm)
P1	8º12’21.7”S	34º55'02.0"W	85,0	28,0	5,9
P2	8º11’48.3”S	34º55'02.6"W	85,0	27,5	5,9
P3	8º16’15.6”S	34º56'44.0"W	85,0	28,0	4,9

Source: Authors.

3.2 COLLECTION OF SAMPLES

The water samples intended for the studies were collected according to the methodology described by Parron et al (2011). A total of 1.5 L of surface water was collected using 2 500 mL sterile polypropylene plastic collecting bottles. The collection was performed with the removal of the cap, followed by inversion of the lower base with the mouth down, keeping in this position, performing the dive of the vial at a depth of 15 cm. With the flask submerged, it turned horizontally in the opposite direction to the current. After collection, the weak ones were sealed, dried and identified and packed in an isothermal box, keeping at 4°C. Then, it was transported to the Laboratory of the Center for Research in Environmental Sciences and Biotechnology - NPCIAMB of the Catholic University of Pernambuco, where immediately performed the physical-chemical and microbiological analyzes and later, the collected material was stored at a temperature of 4°C.

3.3 MICROBIOLOGICAL ANALYSIS

For the microbiological analysis of the samples, the method of multiple fermentation tubes divided

into three successive phases was used: presumptive, confirmatory and complementary, thus allowing the quantification by "most probable number" (MPN) of coliform organisms.

In the presumptive phase, homogenization and transfer of the samples to 5 test tubes contained, in the background, all Durhan in double lactosed broth. Then, 1mL of the sample of each was inoculated with simple lactated broth (CSI). The transfer of 1mL of the samples was performed to a dilution tube containing 9mL of sterile water, of this dilution, 1mL was inoculated in each tube (5) of remaining simple lactated broth. All tubes were incubated at 35ºC for 24/48 hours.

In the confirmatory phase, samples were transferred from the positive presumptive tubes to tubes containing bright green broth. All tubes were incubated at 35ºC for 24/48 hours, after which the tubes that had total coliform growth were identified through the production of gas in Durhan tubes.

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

3.3.1 Microbiological analysis

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3.4 ANALYSIS

The analyses were performed according to the techniques recommended by the American Public Health Association (APHA, 2012). In this work, microbiological analyses and physicochemical parameters were performed.

3.5 ANALYSIS OF PHYSICO-CHEMICAL PARAMETERS

The physicochemical parameters analyzed were: pH, using a model pH meter (TECNAL – TEC – 7); conductivity, using a model conductivity meter (mCA 150); turbidity, using a model turbidimeter (ALFAKIT); apparent color, determined using visual colorimeter (DLNH-100).

To determine the total solids (TS), the methodology described by the ABPM working instrument was used

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

Equation 1.

$$ST = \frac{(MS - MR)x1000}{Va}$$

Where:

TS = Total solids in mg/L

DM = 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

To determine the chloride content, the Mohr method was used, while the 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. The total iron content was determined by UV-Vis spectrophotometry.

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

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

4 DEVELOPMENT

4.1 MICROBIOLOGICAL ANALYSIS

The results of the present study showed that the vast majority of beach water samples collected at

the three points (P1, P2 and P3) of the Pernambuco coast presented high counts of total coliforms, thermotolerant coliforms, thus being in disagreement with the bathing standards established in CONAMA Resolution No. 274/2000 and CONAMA Resolution No. 357/2005. Table 2 shows the results of the presumptive tests related to the determinations of the NMP (Most Probable Number), where it is possible to observe 5 positive tubes identified with the presence of gas, confirming the presence of total coliforms in the water at the points selected for the analysis. In a study conducted by (SOUZA; Smith, 2015), on the beach of Barra Grande, Salvador-BA, in the periods of high season were found high concentrations of total coliforms, corroborating this study.

Table 1. Results per sample of Presumptive Tests

Stitches	Inoculated stitches	Positive tubes Double broth		Positive tubes Simple broth		Positive tubes Simple broth 1		Index (NMP/100 ml)
		No	%	No	%	No	%	
P1	5	5	100	5	100	5	100	>1600
P2	5	5	100	5	100	5	100	>1600
P3	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. Regarding the identification of the isolated bacteria (Table 4), the following were found in the collected samples: *Escherichia coli* variety I (Group 1), Enterobacter aerogenes variety II (Group 2) and *Enterobacter aerogenes* variety I (Group 3).

Table 2. Confirmatory test result per sample

Stitches	Inoculated stitches	Double broth poitive tubes *E. coli*		Poitive tubes Simple broth *E. coli*		Poitive tubes Simple broth 1 *E. coli*		Index (NMP/100 ml)	Belt (NMP/100 ml)
		No	%	No	%	No	%		
P1	5	5	100	4	80	2	40	220	<100 a > 580
P2	5	5	100	4	80	3	60	280	< 120 a > 690
P3	5	5	100	5	100	1	20	300	< 100 a > 1300

Source: Authors (2023).

Table 4. Test result identifying the bacteria in each sample

Stitches	INDOLE	METHYL RED	VOGES PROSKAUER	CITRATE	ISOLATED BACTERIA
P1	+ – – + +	+ – + – +	+ – – – +	+ + + + +	***Enterobacter aerogenes* - Variedade II** ***Enterobacter cloacae*** ***Citrobacter freundii*- Variedade I** ***Klebsiella pneumoniae*** ***Proteus vulgaris***

P2	– + –	– + +	+ + –	+ + +	***Enterobacter aerogenes* - Variedade I** ***Proteus vulgaris*** ***Salmonella* sp.**
P3	– + – –	– – – +	– + – –	– + + +	***Enterobacter aerogenes* - Variedade I** ***Enterobacter aerogenes* - Variedade II** ***Enterobacter aerogenes*** ***Citrobacter freundii*- Variedade I**

The classification of the beaches is based on what is established by CONAMA Resolution No. 274/00, which defines water quality standards for bathing. The framing criterion is based on the concentrations of thermotolerant coliforms. 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 criterion for own waters is not met or present more than 2500 thermotolerant coliforms in the last sampling).

However, the presence of total and faecal coliforms and *Escherichia coli* In the marine environment it may be due to microbial contamination of fecal origin, so it is indicative of inadequate sanitary conditions. The presence of animals, dumping of sanitary sewage, change of season, and many bathers contribute to the survival and dispersal of pathogenic microorganisms in saline waters (DESTRO et al., 2020; KOCHINSKI; BARBOSA; ROMANELLO, 2020).

The physicochemical analyses showed that only the variables of total nitrogen $(N2)$ and nitrite $(NO2^{-})$, nitrate $(NO3^{-})$ and ammonium $(NH4+)$ were within the parameters specified by the legislation in all points (P1, P2 and P3) analyzed.

When analyzing the microbiological parameters, it was observed that in the quantification of total and fecal coliforms at all points (P1, P2 and P3) there were indices above quality control. In the confirmatory test for *Escherichia coli* 100% of the samples presented values above the tolerable limits of contamination.

In this context, the areas analyzed on 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, recommending the interdiction of areas for leisure.

4.2 PHYSICO-CHEMICAL PARAMETERS

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

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

Parameters (Unit)	Point P1	Point P2	Point 3
ph	7,46	7,81	7,92
Electrical Conductivity (µS/cm)	66.060,00	65.010,00	64.730,00
Corus apparent (mg/L Pt-Co)	1,00	1,50	0,00
Turbidity (mg/L SiO2)	0,00	0,00	0,00
Temperature (^{oC})	27,50	28,00	27,50
Chlorides (mg/L Cl^-)	8.450,00	8.445,00	8.505,00
Total hardness (mg/L CaCO3)	2.800,00	3.000,00	3.100,00
Sodium (mg/L Na+)	10.787,00	11.397,40	10.594,40
Potassium (mg/L K+)	374,62	539,96	446,52
Total solids (mg/L)	34,07	26,72	29,44
Dissolved oxygen (mg/L O2)	4,80	5,00	3,60
DQO (mg/L O2)	89,88	294,37	282,70
DBO (mg/L O2)	12,35	95,02	32,61

Source: Authors (2023).

Second Von Sperling (2007) The electrical conductivity of water is an important indicator of possible polluting sources, however, it does not discriminate which ions are present in the water. The electrical conductivity above 0.100 mS/cm, according to CETESB, indicates corrosive characteristics of the water and environmental impacts by anthropic actions. In this study, according to Table 5, it can be observed that the P3 point presented high pH (7.92) and the P1 point electrical conductivity with 64.73 mS, which can be justified by the dilution of fluvial water to domestic sewage released into the sea. According to the Resolution of the National Environment Council (CONAMA) No. 357/2005, the pH of saline waters should be in the range of 6.5 to 8.5, and natural variations greater than 0.2 units should not occur. In this work, all points were below the specifications for class 1,2 and 3 saline waters.

According to the data obtained through the analyses, it is noted from Table 5 that the values of dissolved solids are high due to the high contents 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 fluvial waters, making the study area unsuitable for use in primary and secondary contact, according to what is recommended by resolution 357/2005 of CONAMA.

The water temperature determined was 27.5ºC for points P1 and P3 and 28ºC for point P2. Temperature is one of the most important abiotic factors for the survival and growth of marine organisms, since the higher it is, the less dissolved oxygen the water will have. The legislation does not contain maximum or minimum values stipulated for this variable. However, the results obtained for this variable are in accordance with the climatic condition of the region, whose climate is considered transitional between tropical and subtropical climates and the annual seasons are not well defined.

The values found for dissolved oxygen (DO) for points P1, P2 and P3 were 4.8, 5.0 and 4.6 mg/L respectively (Table 5), being within the values recommended for resolution No. 357/2005 of CONAMA for saline waters, which should not be less than 6.0 mg/L. The results of chemical oxygen demand (COD)

and biochemical oxygen demand (BOD) depict a large amount of organic matter at points P2 and P3, thus confirming the presence of domestic sewage dumps in the study area due to river interference and the proximity of bars and restaurants, in addition, the CPRH (State Agency for the Environment) identified in March 2022 the poor sanitation in these points, whose untreated sewage is released into the sea clandestinely.

5 FINAL CONSIDERATIONS

From the results presented, it was possible to conclude that the waters of the beaches of Olinda present contamination by pathogenic agents. The determining factor is directly linked to open sewage, being one of the main factors of pollution, together with the anthropic actions and the presence of animals. Therefore, it is believed that a control study is necessary, where monitoring and sanitation are frequent, in order to have a certification of the quality of the environment, making these areas appropriate for leisure.

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