



# Microbial ocean-atmosphere transfer: The influence of sewage discharge into coastal waters on bioaerosols from an urban beach in the subtropical Atlantic

Jamille da Silva Rabelo<sup>a</sup>, Fátima Cristiane Teles de Carvalho<sup>a</sup>, Rosa Helena Rebouças<sup>b</sup>, Oscarina Viana de Sousa<sup>a,\*</sup>

<sup>a</sup> Institute of Marine Sciences (LABOMAR), Federal University of Ceará (UFC), Ceará, Brazil

<sup>b</sup> Department of Fisheries Engineering, University of Delta do Parnaíba (UFDPAr), Piauí, Brazil

## ARTICLE INFO

### Keywords:

Bacterioneuston  
Marine surface  
Coastal region  
Aerosolization  
Domestic wastewater  
Air quality

## ABSTRACT

All over the world, the oceans are the final destination of sewage transported by river estuaries, rainwater and other coastal discharges. The risks to human health related to direct contact with water and consumption of contaminated fish are well known, but little is known about the potential for atmospheric exposure to pollutants and pathogens from contaminated seawater. The release of microbial particles from the sea into the atmosphere occurs mainly by the eruption of rising bubbles through the sea surface microlayer (SML) or by sea spray. We investigated the heterotrophic bacteria density and relative abundance in SML and bioaerosols originated on the seafront of Fortaleza (Atlantic coastal zone, northeastern Brazil) influenced by wastewater disposal. There was a difference in the density of total heterotrophic bacteria (THB) according to the matrix analyzed during two seasons: the bacterial count was highest in the SML during the rainy season while the highest number of bacteria in bioaerosols samples was recorded during the dry season. Twenty-nine bacterial taxonomic groups were identified with variable abundance for both environments. These were the same in both matrices, with environmental variables influencing their abundance and composition. The contribution of the marine and continental environments in shaping the microbiota of the SML and coastal bioaerosols was clear, with the constant and representative presence of Enterobacteria standing out. The aerosolization of bacteria resulting from the discharge of untreated sewage is an important issue related to coastal environmental health and ecological safety.

## 1. Introduction

Our knowledge about the role of oceanic microorganisms in atmospheric microbiome and the anthropogenic interference with these natural processes is still basic.

The sea-air interaction manages the planet's processes, regulating meteorological and oceanographic conditions. The microorganisms present in this transition environment act under several factors and conditions. The sea surface microlayer (SML) corresponds from 1 to 1000  $\mu\text{m}$  of water separating water/atmosphere interface, it is frequently enriched with organic matter, showing distinct physico-chemical features and more microbial abundance and diversity compared with the underlying waters (Aller et al., 2005; Cunliffe et al., 2013; Karavoltzos et al., 2015; Wurl et al., 2017). This abundance is due

to the rise of bubbles from the lower layers of the ocean, which aggregate dissolved and particulate organic matter and microorganisms present in the water column. When these bubbles reach the surface, they burst, releasing their contents into the surface and the atmosphere (Kuznetsova; Lee, 2002; Aller et al., 2005; Alves, 2014). The microorganisms aerosolized from the SML into the atmosphere form marine bioaerosols (Cho and Hwang, 2011). These biological particles are transported over long distances and represent an important mechanism for dispersion and distribution of marine microorganisms that can contribute to the cosmopolitan maintenance of some bacteria (Cunliffe et al., 2013). Bioaerosols are also indicators of pollution and spreading a wide range of diseases (Polymenakou, 2012; Qi et al., 2014; Xia et al., 2015).

In this case, air is an important carrier of bacterial pathogens and it is

\* Corresponding author.

E-mail address: [oscarinavs@ufc.br](mailto:oscarinavs@ufc.br) (O.V. Sousa).

<https://doi.org/10.1016/j.marenvres.2024.106765>

Received 30 May 2024; Received in revised form 21 September 2024; Accepted 22 September 2024

Available online 27 September 2024

0141-1136/© 2024 Elsevier Ltd. All rights reserved, including those for text and data mining, AI training, and similar technologies.

essential to understand and identify their origin, survival capacity, dispersion rate and relationship with the environment (Smets et al., 2016).

Coastal dynamics include factors absent in oceanic regions that contribute to the distribution of microorganisms in the SML and atmosphere, such as wave breaking and a significant continental contribution. There is higher primary productivity in coastal regions due to a greater input of available nutrients (Zewde et al., 2018). When a wave breaks, it generates sea spray and bursting bubbles, providing particles, including bacteria, in the air and water surface (Park et al., 2014). In addition, tidal cycles affect the dynamics of coastal and estuarine regions. During low tide, there is a flow of nutrients and microorganisms from the mainland to the sea, while at high tide particles from the sea to the mainland prevail, as well as greater mixing of waters (Kolm and Andretta, 2003; Anwar et al., 2014).

Amplifying the natural aspects of the environment, human activities play an important role in coastal regions, especially in densely populated coastal cities. For example, storm drains are point sources for discharging illegal domestic sewage into the sea, modifying and polluting the marine environment and putting the health of the population at risk (Fröhlich-Nowoisky et al., 2016). The aim of this study was to establish the relationship between abundance and diversity of culturable bacteria present in the microlayer of the sea surface and marine bioaerosols on a city at waterfront with a punctual source of sewage contamination and the influence of oceanographic factors.

## 2. Methods

### 2.1. Study area and sampling sites

The SML and bioaerosols samplings occurred in two points of a populated beach area in Fortaleza coast, Ceará, Brazil. The region is characterized by subtropical climate, which is led by the Intertropical Convergence Zone (ITCZ) dividing the year into two seasons, dry (August–January) and rainy (February–July) ones (Tsoar et al., 2009).

The mouth of a stream (Riacho Maceió) was chosen as a reference point for point-source urban pollution on the Fortaleza waterfront. Previous studies and monitoring of water quality on the city's beaches show contamination resulting from anthropogenic activity and clandestine sewage connections (Vieira et al., 2012). The sampling points were located upstream (P1) and downstream (P2) of the creek, considering the direction of the coastal current (Fig. 1).

Sampling was carried out during the dry and rainy seasons (DS and RS) in 2017 on spring tides for a better contrast between low and high

tides (LT and HT). The Ceará coast has semi-diurnal tides, which means that there are two LT and two HT tides on the same day (Frota et al., 2016), with an average sea surface temperature (SST) above 27 °C, characteristic of the tropical Atlantic (Bomventi et al., 2006).

### 2.2. Methods of sampling and inoculating bacteria

The sampling of SML bacteria was made to a minimum distance of 5 m from the Maceió stream discharge and in an undisturbed area of sea, before break point of waves on the coast. SML microbiota was collected with a 47-mm hydrophilic polycarbonate membrane filter according to Kurata et al. (2016) adapted from Franklin et al. (2005). The sampling was conducted in triplicate, where the membrane filters were placed on the SML using sterile tweezers and, after surface contact, removed immediately to avoid sinking and coming into contact with the lower layers. After, the membrane filters were stored aseptically in sterile Petri dishes for transport to the lab. For microbiological enumeration and isolation, the filter samples were inoculated by Pour Plate technique in Plate Count Agar (PCA) medium prepared with seawater, and the plates incubated at 48h at 35 °C.

For marine bioaerosols sampling, the aerolized biological particles were collected by the passive sedimentation technique, using open petri dishes containing selective culture medium for bacteria (Plate Count Agar - PCA, Difco®, diluted in seawater with salinity adjusted to 20 ppm) exposed to the air, at a height of 2 m, for 30 min. The plates were then closed, conditioned and transported to the laboratory (Manibusan; Mainelis, 2022; Rastmanesh et al., 2024). After sampling, the plates were incubated at 35 °C for 48 h. Sampling was carried out in triplicate and synchronously with SML sampling.

After incubating the plates, the bacterial colonies that had grown were counted and the results were expressed as number of colony-forming units (CFU) per square meter (m<sup>2</sup>) for SML. For bioaerosols, the counts have been converted into CFU per cubic meter (m<sup>3</sup>) using the formula  $a\text{ CFU/m}^3 = [\text{CFU/p (m}^2)] \times \text{SAR (1/23)}$ , where CFU = average number of colony-forming units, p = Petri plate area, SAR = surface/air ratio (Friberg et al., 1999; Pasquarella et al., 2007).

### 2.3. Sample processing: bacteria characterization

Bacterial colonies were isolated on Tryptic Soy Agar (TSA) diluted with seawater (salinity adjusted to 10 ppm), incubated for 24 h in 35 °C and characterized by morphological (Gram staining) and biochemical tests following mainly Bergey's Manual of Systematic Bacteriology (Garrity et al., 2005).

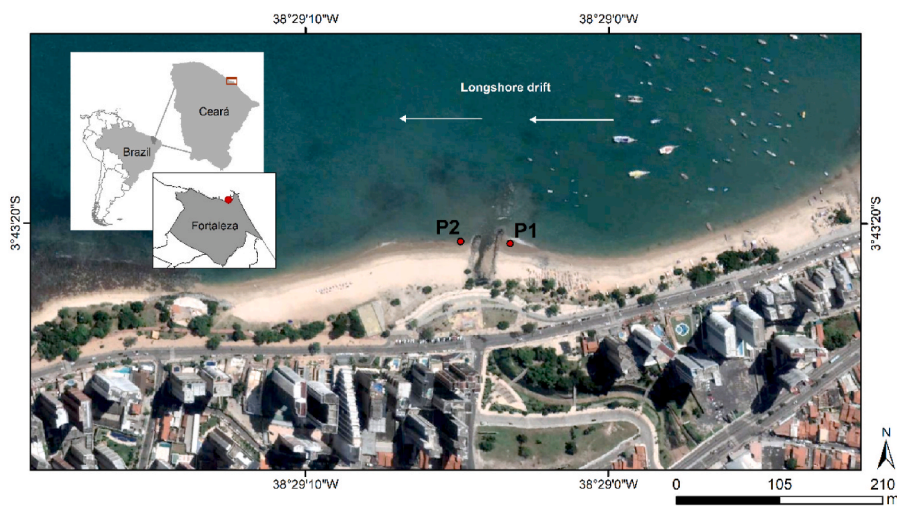


Fig. 1. Sampling location in Fortaleza, northeast Brazil. P1 localized upstream and P2 localized downstream of the discharge of the Maceió stream.

### 2.4. Environmental variables

Physical and chemical parameters, such as salinity, pH, water and air temperature were measured *in situ* during sampling, using a salinity refractometer (ATAGO S/MILL), pH meter (MARCONI - PA 200P) and thermometer (INCOTERM), respectively. Data from wind and relative humidity came from the institute from the National Institute of Meteorology (INMET) website (<http://inmet.gov.br/>).

### 2.5. Statistical analysis

Pearson's correlation analysis was performed to analyze the correlation between bacterial concentrations and physical and chemical parameters for both SML and bioaerosols, where a positive correlation means a direct correlation whereas a negative correlation means an inverse correlation. The significance calculated by one-way ANOVA and *P* values < 0.05 were considered as significant.

## 3. Results

### 3.1. Parameters mean values

Table 1 shows information of collected parameters at the time of sampling. The interval between collections varied by 6 h for each day, according to the tides. The mean tidal range for LT was 0.1 m and for HT was 2.9 m. Mean wind speed for DS (September and November) was 3.8 m/s and for RS (March and April) was 1.5 m/s. In addition, the wind direction was predominant to northwest for both seasons, showing the domination of trade winds in Fortaleza coast.

Mean values for relative humidity (RH) during DS was 62% while RS was 81%. Water temperature (WT) did not show much variation between seasons, points or tides. Air temperature (AT) had 2 °C of difference for mean values between tides and seasons, where LT and DS showed higher temperatures.

Salinity varied from 36 to 39, except for an outlier in P2 during april, which can be explained by the rain and heavy discharge of Maceió stream prior the moment of the sampling. pH showed lower values for RS.

### 3.2. Cultivable bacteria abundance in sea surface microlayer and bioaerosols

The mean values for CFU (colony forming unit) in SML samples varied between samples conditions. The concentration of bacteria for DS had the lowest mean values, reaching 1913 CFU/m<sup>2</sup> in P1 during LT and 3012 CFU/m<sup>2</sup> during HT for the same point. For P2, during the same season, the mean value was 2568 CFU/m<sup>2</sup> during LT and 3722 CFU/m<sup>2</sup> during HT. On the other hand, the mean values during RS showed higher bacteria concentration, where P1 reached 90797 CFU/m<sup>2</sup> and 50116 CFU/m<sup>2</sup> for LT and HT, respectively. Whereas P2 reached 100652 CFU/

m<sup>2</sup> and 38085 CFU/m<sup>2</sup> for LT and HT, respectively (Fig. 2).

In the coastal bioaerosol samples, the average bacterial CFU values were highest during HT for both points and stations, reaching 3311 CFU/m<sup>3</sup> and 5241 CFU/m<sup>3</sup> for P1 and P2 during DS, respectively. While during LT in DS, the average values were 2112 CFU/m<sup>3</sup> and 2522 CFU/m<sup>3</sup> for P1 and P2, respectively (Fig. 2). The samples were collected at tides with similar heights (low and high), according to the tide table. For LT, the mean values reached 77 CFU/m<sup>3</sup> and 107 CFU/m<sup>3</sup> for P1 and P2, respectively. Whereas during HT the mean values were 6636 CFU/m<sup>3</sup> and 3285 CFU/m<sup>3</sup> for P1 and P2, respectively.

### 3.3. Pearson correlation analysis

The correlation between bacterial concentrations and the physical and chemical parameters for both sample types was performed by Pearson correlation analysis (Table 2). The correlation of the sampling conditions was analyzed separately (points, tides and seasons) and together (points + tides and points + seasons).

### 3.4. Identification and relative abundance

In total, 164 bacterial strains belonging to 29 taxonomic groups were isolated and identified with varying frequency for SML and bioaerosols. In the SML samples, the relative abundance varied according to the seasons, points and tidal conditions. Between the rainy and dry periods of the year, DS showed lower relative abundance when compared to RS, with the species *Corynebacterium kutscheri* being dominant in DS, while the genus *Bacillus* was dominant in RS. The relative abundance of bacteria was similar between P1 and P2. In terms of tidal phases, the samples collected in LT showed twice the richness of bacterial groups compared to those in HT (Fig. 3a). The genus *Vibrio* was abundant for both seasons and predominant in all other conditions on SML samples. Species of *Corynebacterium*, *Bacillus* and *Pseudomonas* had a notable presence in all conditions.

For bioaerosols, there was a shift in dominant groups, where *Serratia liquefaciens* appeared as the most abundant, followed by the genus *Bacillus* and *Corynebacterium*. When compared, conditions shared similar relative abundance, except for tides, where LT showed higher relative abundance than HT (Fig. 3b). The frequency of the identified groups was very similar to the groups identified in the marine surface layer at the analyzed site. (Fig. 3b). More than 70% of the bacterial taxonomic units were detected in both matrices. Of these, more than half are bacteria related to the human microbiome, the intestinal tract (Enterobacteriaceae family) or the skin and mucous membranes (*Micrococcus*, *Staphylococcus*, *Corynebacterium*). Some others are ubiquitous in the marine environment, water and soil.

**Table 1**  
Environmental and meteorological parameters, space-temporal data of sampling points in the coast from Fortaleza city (northeast of Brazil).

	Dry season								Rainy season							
	September				November				March				April			
	1pm		7am		10am		4pm		11am		5pm		10am		4pm	
Tidal Range (m)	0.2	2.9	0.0	3.2	0.2	2.9	0.3	2.8	0.2	2.9	0.3	2.8	0.2	2.9	0.3	2.8
Wind Speed (m/s)	4.6	2.3	3.6	4.8	1.2	1.6	1.3	2.0	1.2	1.6	1.3	2.0	1.2	1.6	1.3	2.0
Wind direction (°)	111	120	130	98	167	97	175	143	167	97	175	143	167	97	175	143
Relative Humidity (%)	51	77	68	53	86	71	89	76	86	71	89	76	86	71	89	76
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
Water Temperature(°C)	29	28	27	27	29	29	29	29	29	29	29	29	29	29	29	29
Air Temperature (°C)	30	30	25	25	30	30	27	27	27	27	26	26	28	28	26	27
Salinity	40	39	40	40	39	36	38	40	35	33	35	38	36	13*	36	39
Water pH	8.14	8.19	8.12	8.14	8.08	8.09	8.06	8.07	8.03	8.05	8.03	8.08	8.11	7.78	7.9	8.01

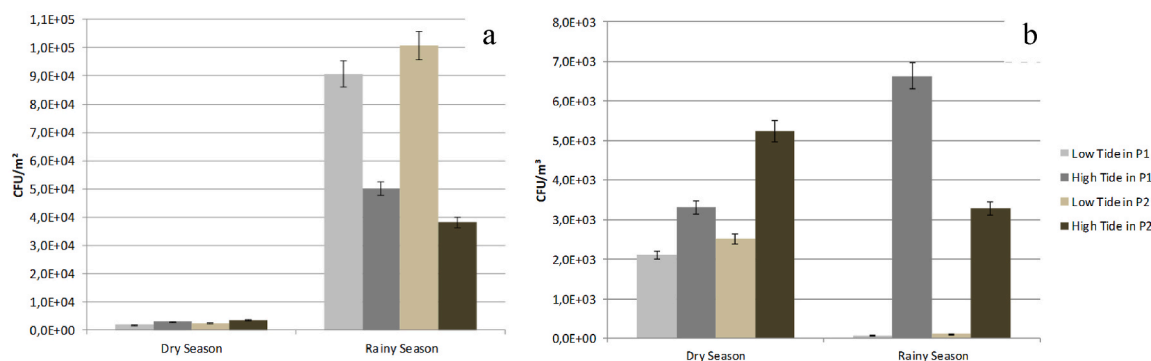


Fig. 2. Seasonal changes in cultivable bacterial abundance in SML (a) and coastal bioaerosol (b) samples on the waterfront of Fortaleza (Brazil). The results were expressed as CFU/m<sup>2</sup> for SML and CFU/m<sup>3</sup> for bioaerosols.

Table 2

Pearson's correlation coefficient matrix between the concentration of bacteria in SML and bioaerosol samples and physicochemical factors during sampling. \*p < 0.01, \*\*p < 0.05.

	SML				Bioaerosols			
	WT	pH	S	TR	AT	WS	RH	
P1	0.32	-0.44	-0.73**	-0.24	-0.28	0.02	-0.27	+1 0 -1
P2	0.45	-0.69	-0.82**	-0.13	-0.1	0.59	-0.67	
HT	0.45	-0.91**	-0.5	-0.63	0.36	0.27	-0.58	
LT	0.35	-0.53	-0.87**	0.66**	0.76**	0.62	-0.49	
P1 + HT	0.43	-0.96**	-0.55	-0.64	0.33	-0.19	-0.23	
P2 + HT	0.52	-0.86**	-0.54	-0.66	0.44	0.75*	-0.95**	
P1 + LT	0	-0.36	-0.92*	0.73*	0.65	0.48	-0.33	
P2 + LT	0.51	-0.73*	-0.99**	0.49	0.89*	0.79*	-0.68	
DS	0.24	-0.72	-0.12	0.53	-0.1	0.46	-0.33	
RS	0	-0.04	-0.33	-0.73*	-0.72**	0.33	-0.81**	
P1 + DS	0.13	-0.87	-0.73*	0.58	-0.11	0.28	-0.08	
P2 + DS	0.67	-0.89*	-0.5	0.54	-0.11	0.61	-0.49	
P1 + RS	0	0.14	-0.69	-0.67*	-0.68	0.37	-0.88*	
P2 + RS	0	-0.48	-0.73*	-0.85*	-0.85*	0.35	-0.87*	

Notes: P1 - Point 1; P2 - Point 2; HT - High Tide; LT - Low Tide; DS - Dry Season; RS - Rainy Season; WT - Water temperature; S - Salinity; TR - Tidal Range; AT - Air Temperature; WS - Wind Speed; RH - Relative Humidity.

## 4. Discussion

### 4.1. Bacterial abundance in SML and bioaerosols samples and environmental conditions

When comparing the means of bacterial counts in SML according to seasonal period (Fig. 2a), the highest values appeared in the period corresponding to the rainy season for the two sampling points, emphasizing the role of rain in the contribution of bacterial abundance for SML. P2 presented the highest values, showing the contribution of Maceió stream, which may act as an input of organic materials from freshwater and anthropogenic sources. The properties of the sea surface microlayer may vary according to the weather and seasons (Agogue et al., 2005; Stolle et al., 2010). During dry season, the highest mean values for bacterial counts occurred during HT for both collection points (Fig. 2a), which can be explained by larger tidal amplitudes, revolving more substances and microorganisms to the surface, through bursting bubbles and turbulence from waves (Alves, 2014; Engel et al., 2017).

Pearson's correlation explained the CFU concentrations for SML with pH and salinity, being in agreement with the other studies for these environments. Salinity had a strong negative Pearson correlation, meaning that a low salinity, especially during RS, benefits a higher bacterial concentration. As salinity increases the osmotic potential of

water, non-marine bacteria are severely affected. In addition to the osmotic effect, salinity interacts with other parameters such as nutrient deprivation and solar radiation (producing viable but nonculturable bacteria) (Carneiro et al., 2018). The abundance of bacteria during LT was higher during the rainy season, due to less mixing in water layers and aerosol deposition. The pH also showed a strong negative correlation with the abundance of microorganisms in SML, meaning bacterial growth benefited in a near neutral pH, especially during DS. Krause et al. (2012) investigated small changes in pH on marine bacteria, the authors didn't find interference in bacterial abundance in lower pH either, although bacterial communities shifted according to pH changes. P2 had the highest concentrations of bacteria in the water, which is probably influenced by the current of the Maceió stream and by coastal drift.

### 4.2. The effect of rain and tides on cultivable bacterial abundance

The SML has contributions from both sea (bursting bubbles, waves, e. g.) and continent (deposition) to form its microbiota. Winds and rain assist in the continental transportation to this environment (Cho and Hwang, 2011; Xia et al., 2015). There was a notable change in bacterial concentration between DS and RS, showing the strong depositional effect of rain and increased water flow in the Maceió stream, where it increased bacterial concentration in the SML. This is noticeable when comparing the concentration during RS (Fig. 1), where the highest concentrations occurred in LT and P2, whereas for HT, P1 had more bacterial concentration. The constant discharge of the Maceió stream influenced the increase in P2 while in the HT there was a greater mixing favoring the increase in P1.

Rainfall leads to the deposition of aerosolized particles (Qi et al., 2014; Zhen et al., 2017), resulting in a decrease in the concentration of bacteria in the air, while high tide contributes inversely, resuspending particles from the sea into the atmosphere (Fig. 2). In addition, the low intensity of winds during this period would also prevent the interaction between marine and continental bacteria. Zhen et al. (2017) studied the effect of meteorological conditions on the abundance and diversity of bacteria present in the air, also concluding that there is great variation between seasons. The interaction between wind, water and land surfaces controls the formation of microbial aerosols, allowing them to be transported regionally and globally (Dueker et al., 2018). There was an abrupt drop in bacterial counts during low tides in RS, demonstrating the role of rain in depositing bioaerosols.

Studies indicate that bioaerosols present different distributions according to the climatic conditions of the region, the low relative humidity being one of the most important for their diffusion. High levels of water vapor in the air, however, would force bioaerosols to adsorb onto water molecules, making their spread more difficult (Qi et al., 2014; Zhen et al., 2017). Winds act in the dispersion of bacteria of the sea surface microlayer and bioaerosols, helping in the exchange of the continent and ocean, being able to carry them by thousands of

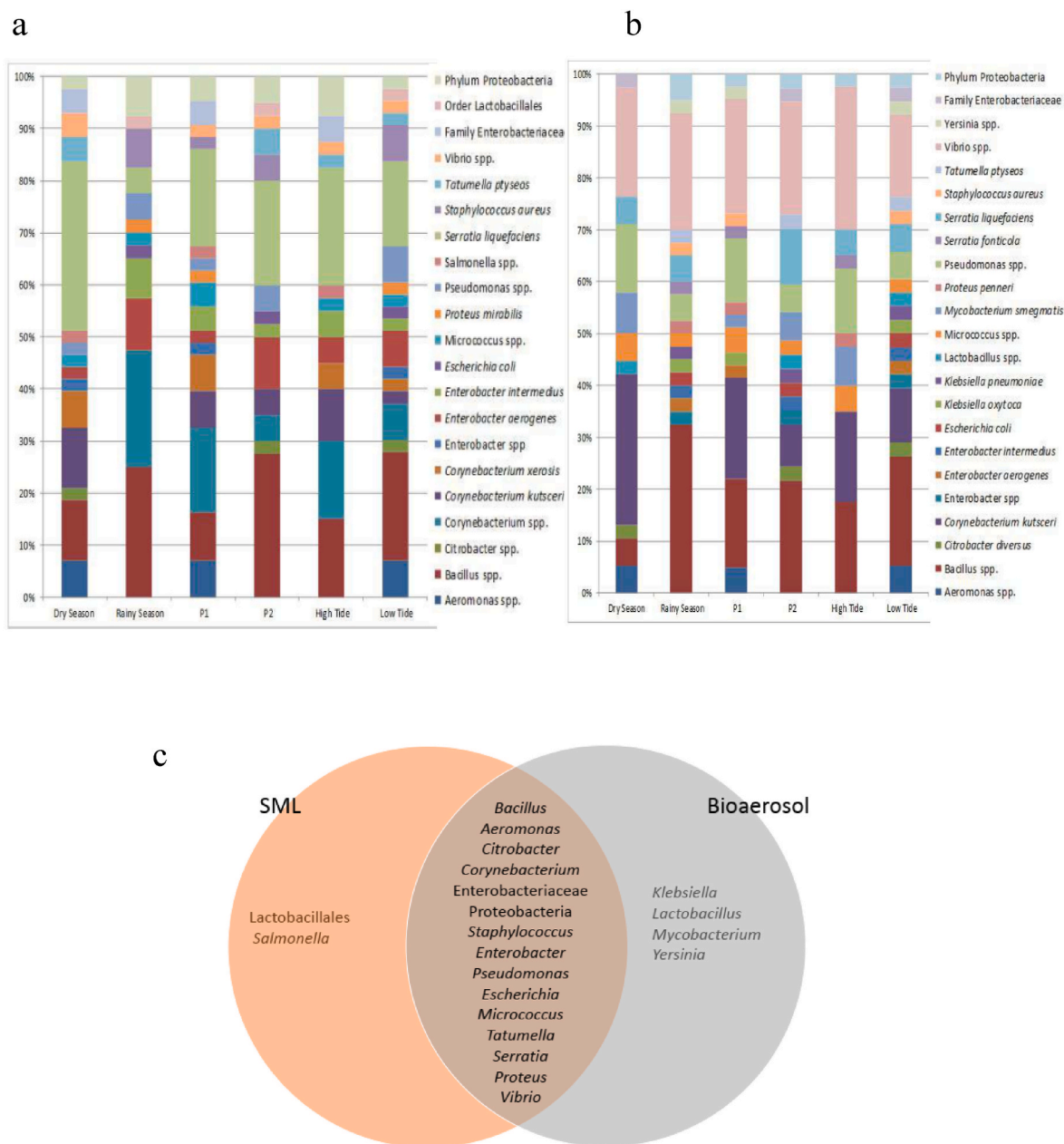


Fig. 3. Relative abundance of bacterial taxonomic units isolated from samples of SML (a) and bioaerosols (b) for each season, point and tidal condition; (c) and bacterial taxonomic units shared by the two samples on the seafrent of Fortaleza city (Brazil).

kilometers, as well as to keep them locally (Cho; Hwang, 2011; Zhen et al., 2017).

Pearson’s correlation explained the CFU concentrations for bioaerosols with AT, RH and WS being the most significant parameters, also in agreement with the other studies for these environments. AT had a strong positive correlation for LT ( $p < 0.05$ ) meaning that a warmer temperature favored a higher bacterial concentration for this tide, whereas AT and RH showed a strong negative correlation for RS ( $p < 0.05$ ), meaning that the lowest air temperature and drier air benefit a higher concentration of bacteria in the bioaerosols. This is an expected pattern because the rainfall cleans out aerosol particles, on which bacteria mainly adhere to and the relative humidity influences negatively the dispersion of bacteria and fungi in the air (Qi et al., 2014). WS had a strong positive correlation in P2 for both LT and HT, which may have been enriched by the influence of the Maceió stream, carried downstream by the wind. This demonstrates the importance of wind transporting particles from different environments, particularly the trade

winds in coastal cities.

#### 4.3. Bacterial diversity and environmental conditions

The genus *Bacillus* had a constant presence in all sampling conditions for this work Previous studies on the bacterial components of SML and aerosols have consistently mentioned the presence of *Bacillus* species (Agoqué et al., 2005; Cho and Hwang, 2011; Xia et al., 2015). The ability to form desiccant-tolerant spores may explain the frequency of bacilli in aerosols. *Bacillus* species were found in aerosols and foams as a result of aeration remediation in a polluted urban watercourse of the Hudson River Estuary (Jacob et al., 2024); they were found at 20 km altitude as dominant bacteria (99% frequency) (Griffin, 2004) and in aerosols over Northwestern Pacific Ocean (Zhang et al., 2023).

The genus *Vibrio* is widely known as resident in ocean waters, coasts, marine sediments and associated with marine animals, as pathogens. They are Gram-negative bacteria that have great ability to produce

biofilm and remain in the environment for years, depending on variation of temperature and salinity (Munn, 2011; Menezes et al., 2018). Microorganisms with survival adaptations in biofilms have significant advantages in thriving within SML, due to the presence of compounds in this environment that promote the formation of gels and films (Cunliffe and Murrell, 2009). Franklin et al. (2005) studied the composition of the SML in the North Sea and found the dominance of *Vibrio* and *Pseudoalteromonas* in the environment, differing from the diversity of the 0.4 m underlying waters. Rahlff et al. (2019) found similar data indicating that marine foams represent the compressed microlayer of sea surface with distinct bacterial communities. Despite the difference between the techniques used (they used 16S rRNA library and sequencing approach and we used culture-dependent analysis) and climatic factors, we found similarity between the dominant bacterial groups detected.

Species from genera *Serratia* and *Corynebacterium* were often isolated from our samples. These bacteria are ubiquitous in environment, related to anthropic activities (Oyetibo et al., 2010; Alvarez et al., 2017) and often listed as bioaerosols components from different sources (Han et al., 2020; Górný, 2020). Human activity on the beach and the discharge of effluent from the Macelió stream may be responsible for the high abundance of these genera. The isolation of the intestinal bacterium *Escherichia coli* reinforces this understanding. Sewage contamination has already been detected (Vieira et al., 2012) in the section of beach affected by the estuary.

#### 4.4. Interactions between SML and bioaerosols microbiota

Studies on the seasonal variation of the microbiota of SML have found correlations with climatic and meteorological factors (Frka et al., 2009; Dreshchinskii and Engel, 2017). The seasonality and the factors that vary from it prove to be crucial in the distribution of bacteria in SML. Considering the little variation of other physicochemical parameters between the seasons, the greater diversity during the rainy season reinforces the contribution of rain as an agent in the deposition of bacteria in the sea, as well as the low intensity of the winds can disadvantage the distribution of particles, causing a greater concentration of microorganisms in that environment.

Cultivation based methods are only capable of detecting certain viable microorganisms but despite these limitations, cultivation is particularly useful for targeting individual species or specific groups. We identified bacteria of SML in coastal environment belonging to the phyla Proteobacteria, Actinobacteria and Firmicutes and the genera *Vibrio*, *Corynebacterium* and *Bacillus* being prevalent. The genus *Bacillus* was widely present in the rainy season in all points and tides collected. The genus *Vibrio* also appeared in all points and amplitudes of tide collected, with the exception of the low tide at P1. Low tides showed higher diversity for both P1 and P2. The rain effect on the deposition of continental bacteria in the sea may explain the great difference for the presence of this genus between the dry and the rainy seasons.

Studies on the abundance and composition of bioaerosols have shown that they are strongly influenced by the seasonality and meteorological factors (Almaguer et al., 2014; Qi et al., 2014; Zhong et al., 2016; Innocente et al., 2017; Zhen et al., 2017). Wind, temperature and relative humidity appear as the main parameters that vary with seasonality and lead the diversity of component species from bioaerosols. The size of these cells generally is in the range of 1–5 µm classified as medium and fine inhalable particles (MP2.5-10 and MP2.5) and predominant size of airborne pathogens (Fennelly, 2020).

In our results, bacteria concentrations showed statistically significant positive correlation with wind speed in the coastal area suggesting a double role for the wind producing and transporting biological particles from SML at the urban waterfront. The intensity of the wind influences the diversity of the bioaerosol: weaker winds bring bacteria of continental origin, while stronger winds bring marine bacteria. It is therefore possible that Gram-positive bacteria of terrestrial origin are present during rainy seasons with low-intensity winds. Bacteria detected in

marine bioaerosols belong mainly to the Proteobacteria, Firmicutes and Bacteroidetes phyla (Innocente et al., 2017; Després et al., 2012).

Marine microbiota and bioaerosol monitoring studies in coastal cities affected by river flows with high levels of sewage pollution found similar patterns: significant relationships between the concentration of bacteria in water and air and environmental factors (humidity and temperature, wind speed and direction) (Michalska et al., 2021; Pendergraft et al., 2023).

Enteric bacteria were among the neustonic isolates and were dominant in the aerolized culturable microbiota on the coast, mainly at the point downstream of the pollutant discharge. It is possible to verify the similarity of almost 70% between the most frequently bacterial genera identified in the two environmental matrices. This highlights SML as a contributor to the aerial microbiota. This study did not determine the pathogenicity of bacterial isolates; however, many cultivable bacteria present in the air and water belong to genera with species recognized as human pathogens. This is indicative that they are resistant to aerolization and are among the respirable particles in the atmosphere. Other studies have already demonstrated a microbial connection between water and air in the coastal urban environment (Dueker et al., 2017, 2018). Similarly, the pressure of human activities on the composition of the microbiota has also been demonstrated in areas outside the coastal marine environment (Moura et al., 2023).

During dry season, *S. liquefaciens* was present in all points and tides collected, being more abundant at low tide at P1 and high tide at P2. The ability to produce pigment can explain the competence and abundance of this bacterial species in the atmosphere during the dry season, as a protection against UV rays effects (Schwieterman et al., 2015). Gram-positive bacteria of the genera *Bacillus*, *Corynebacterium* and *Staphylococcus* were in most samples. They are very resistant to high concentrations of salts, heat and can stay in the environment for months (Huertas et al., 2018), clearly showing the advantage of Gram-positive bacteria in surviving under environmental stress conditions.

Our results confirm the significant influence of the sea on the formation of bioaerosols and bacterial abundance in the coastal zone and, more importantly, the impact of untreated sewage discharges on the aerolized microbiota.

## 5. Conclusions

In summary, the meteorological, oceanographic and continental variables influenced the abundance and diversity of heterotrophic bacteria present in the SML and marine bioaerosols formed in a coastal city in northeastern Brazil. Physical (tides and longshore drift current) and climatic (seasonal period) parameters and anthropic pressure influence the bacterial abundance and diversity in both environments.

The contributors to the abundance of bacteria in marine bioaerosols are the high tide and wind speed, which result in the availability of the microbiota in the air. The bacterial taxonomic groups identified make clear the marine and continental contributions in the formation of the microbiota of the SML and marine bioaerosols, emphasizing the constant and representative presence of the genera *Bacillus* and *Corynebacterium* in these two environments.

Human enteric bacteria are constituents of these marine microcosms influenced by the rainfall regime, with the expansion of continental discharges and the depositional action of biological particles in the atmosphere. The aerosolization of bacteria resulting from the discharge of untreated sewage is an important issue related to the pollution of coastal waters and a global environmental problem.

## CRedit authorship contribution statement

**Jamille da Silva Rabelo:** Visualization, Investigation. **Fátima Cristiane Teles de Carvalho:** Project administration, Formal analysis. **Rosa Helena Rebouças:** Writing – review & editing, Validation. **Oscarina Viana de Sousa:** Writing – original draft, Supervision,

Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgments

This research was supported by the Brazilian National Council for Scientific and Technological Development (CNPq) through The Institutional Scholarship Program (PIBIC). Sousa, O.V is grateful to the CNPq by the Fellowship of Research Productivity (PQ2: 316342/2021-1)

## References

- Agogue, H., Casamayor, E.O., Bourrain, M., Obernosterer, I., Joux, F., Herndl, G.J., Lebaron, P., 2005. A survey on bacteria inhabiting the sea surface microlayer of coastal ecosystems. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol.* 54, 269–280. <https://doi.org/10.1016/j.femsec.2005.04.002>.
- Aller, J.Y., Kuznetsova, M.R., Jahns, C.J., Kemp, P.F., 2005. The sea surface microlayer as a source of viral and bacterial enrichment in marine aerosols. *J. Aerosol Sci.* 36, 801–812. <https://doi.org/10.1016/j.jaerosci.2004.10.012>.
- Almaguer, M., Aira, M.J., Rodríguez-Rajo, F.J., Rojas, T.I., 2014. Temporal dynamics of airborne fungi in Havana (Cuba) during dry and rainy seasons: influence of meteorological parameters. *Int. J. Biometeorol.* 58, 1459–1470. <https://doi.org/10.1007/s00484-013-0748-6>.
- Alvarez, A., Saez, J.M., Costa, J.S.D., Colin, V.L., Fuentes, M.S., Cuozzo, S.A., Benimeli, C. S., Polti, M.A., Amoroso, M.J., 2017. Actinobacteria: current research and perspectives for bioremediation of pesticides and heavy metals. *Chemosphere* 166, 41–62. <https://doi.org/10.1016/j.chemosphere.2016.09.070>.
- Alves, C.A., 2014. Chemistry between the sea surface microlayer and marine aerosols. *Quim. Nova* 37, 1382–1400. <https://doi.org/10.5935/0100-4042.20140223>.
- Anwar, N., Robinson, C., Barry, D.A., 2014. Influence of tides and waves on the fate of nutrients in a nearshore aquifer: numerical simulations. *Adv. Water Resour.* 73, 203–213. <https://doi.org/10.1016/j.advwatres.2014.08.015>.
- Bomventi, T.N., Wainer, I.K.C., Taschetto, A.S., 2006. Relação entre a radiação de onda longa, precipitação e temperatura da superfície do mar no oceano Atlântico tropical. *Braz. J. Genet.* 24, 513–524. <https://doi.org/10.1590/S0102-261X2006000400005>.
- Carneiro, M.T., Cortes, M.B.V., Wasserman, J.C., 2018. Critical evaluation of the factors affecting *Escherichia coli* environmental decay for outfall plume models. *Revista Ambiente & Água* 13, e2106. <https://doi.org/10.4136/ambi-agua.2106>.
- Cho, B.C., Hwang, C.Y., 2011. Prokaryotic abundance and 16S rRNA gene sequences detected in marine aerosols on the East Sea (Korea). *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol.* 76, 327–341. <https://doi.org/10.1111/j.1574-6941.2011.01053.x>.
- Cunliffe, M., Engel, A., Frka, S., Gašparovic, B., Guitart, C., Murrell, C., Salter, M., Stolle, C., Upstill-Goddard, R., Wurl, O., 2013. Sea surface microlayers: a unified physicochemical and biological perspective of the air-ocean interface. *Prog. Oceanogr.* 109, 104–116. <https://doi.org/10.1016/j.pocean.2012.08.004>.
- Cunliffe, M., Murrell, J.C., 2009. The sea-surface microlayer is a gelatinous biofilm. *ISME J.* 3, 1001–1003. <https://doi.org/10.1038/ismej.2009.69>.
- Després, V.R., Huffman, J.A., Burrows, S. M. Corinna Hoese, Safatov, A.S., Buryak, G., Fröhlich-Nowoisky, J., Elbert, W., Andreae, M.O., Pöschl, U., Jaenicke, R., 2012. Primary biological aerosol particles in the atmosphere: a review. *Tellus B* 64, 15598. <https://doi.org/10.3402/tellusb.v64i0.15598>.
- Dreshchinskii, A., Engel, A., 2017. Seasonal variations of the sea surface microlayer at the boknis eck times series station (baltic sea). *J. Plankton Res.* 39, 943–961. <https://doi.org/10.1093/plankt/fbx055>.
- Dueker, M.E., French, S., O'Mullan, G.D., 2018. Comparison of bacterial diversity in air and water of a Major Urban Center. *Front. Microbiol.* 9, 2868. <https://doi.org/10.3389/fmicb.2018.02868>.
- Dueker, M.E., O'Mullan, G.D., Martínez, J.M., Juhl, A.R., Weathers, K.C., 2017. Onshore wind speed modulates microbial aerosols along an urban waterfront. *Atmosphere* 8, 215. <https://doi.org/10.3390/atmos8110215>.
- Engel, A., Bange, H.W., Cunliffe, M., Burrows, S.M., Freidrichs, G., Galgani, L., Herrmann, H., Hertkorn, N., Johnson, M., Liss, P.S., Quinn, P.K., Schartau, M., Soloviev, A., Stolle, S., Upstill-Goddard, R.C., van Pinxteren, M., Zänker, B., 2017. The Ocean's vital skin: toward an integrated understanding of the sea surface microlayer. *Front. Mar. Sci.* 4, 165. <https://doi.org/10.3389/fmars.2017.00165>.
- Fennelly, K.P., 2020. Particle sizes of infectious aerosols: implications for infection control. *Lancet Respir. Med.* 8, 914–924. [https://doi.org/10.1016/S2213-2600\(20\)30323-4](https://doi.org/10.1016/S2213-2600(20)30323-4).
- Franklin, M.P., McDonald, I.R., Bourne, D.G., Owens, N.J., Upstill-Goddard, R.C., Murrell, J.C., 2005. Bacterial diversity in the bacterioneuston (sea surface microlayer): the bacterioneuston through the looking glass. *Environ. Microbiol.* 7, 723–736. <https://doi.org/10.1111/j.1462-2920.2004.00736.x>.
- Friberg, B., Friberg, S., Burman, L.G., 1999. Correlation between surface and air counts of particles carrying aerobic bacteria in operating rooms with turbulent ventilation: an experimental study. *J. Hosp. Infect.* 42, 61–68. <https://doi.org/10.1053/jhin.1998.0542>.
- Frka, S., Kozarac, Z., Cosovic, B., 2009. Characterization and seasonal variation of surface active substances in the natural sea surface micro-layers of the coastal Middle Adriatic stations. *Estuar. Coast Shelf Sci.* 85, 555–564. <https://doi.org/10.1016/j.ecss.2009.09.023>.
- Fröhlich-Nowoisky, J., Kampf, C.J., Weber, B., Huffman, J.A., Pöhlker, C., Andreae, M. O., Lang-Yona, N., Burrows, S.M., Gunthe, S.S., Elbert, W., Su, H., Hoor, P., Thines, E., Hoffmann, T., Després, V.R., Pöschl, U., 2016. Bioaerosols in the Earth system: climate, health, and ecosystem interactions. *Atmos. Res.* 182, 346–376. <https://doi.org/10.1016/j.atmosres.2016.07.018>.
- Frota, F.F., Truccolo, E.C., Schettini, C.A.F., 2016. Tidal and sub-tidal sea level variability at the northern shelf of the Brazilian Northeast Region. *Annals of the Brazilian Academy of Sciences* 88, 1371–1386. <https://doi.org/10.1590/0001-3765201620150162>.
- Garrity, G., Brenner, D., Kreig, N., Staley, J., 2005. Bergey's manual of systematic Bacteriology vol. 2 Part C. The Alpha-, Beta-, Delta-, and Epsilonproteobacteria. <https://doi.org/10.1007/0-387-29298-5>.
- Górny, R.L., 2020. Microbial aerosols: sources, properties, health effects, exposure assessment—a review. *Kona Powder Part J*, 2020005. <https://doi.org/10.14356/kona.2020005>.
- Griffin, D.W., 2004. Terrestrial microorganisms at an altitude of 20,000 m in Earth's atmosphere. *Aerobiologia* 20, 135–140. <https://doi.org/10.1023/B:AERO.0000032948.84077.12>.
- Han, Y., Yang, T., Xu, G., Li, L., Liu, J., 2020. Characteristics and interactions of bioaerosol microorganisms from wastewater treatment plants. *J. Hazard Mater.* 391, 122256. <https://doi.org/10.1016/j.jhazmat.2020.122256>.
- Huertas, M.E., Acevedo-Barrios, R.L., Rodríguez, M., Gaviria, J., Arana, R., Arciniegas, C., 2018. Identification and Quantification of Bioaerosols in a Tropical Coastal Region: Cartagena de Indias, Colombia. *Aerosol Science and Engineering* 2, 206–215. <https://doi.org/10.1007/s41810-018-0037-1>.
- Innocente, E., Squizzato, S., Visin, F., Facca, C., Rampazzo, G., Bertolini, V., Gandolfi, I., Franzetti, A., Ambrosini, R., Bestetti, G., 2017. Influence of seasonality, air mass origin and particulate matter chemical composition on airborne bacterial community structure in the Po Valley, Italy. *Sci. Total Environ.* 593–594, 677–687. <https://doi.org/10.1016/j.scitotenv.2017.03.199>.
- Jacob, J., Veras, I., Porter-Morgan, H.A., Tan, J., Aguilar, H.E., Elkins, W.T., Martínez Castro, V.P., Fulton, V., Younsi, W.K., 2024. Possibly pathogenic bacteria in aerosols and foams as a result of aeration remediation in a polluted urban waterway. *Folia Microbiol.* 69 (1), 235–246. <https://doi.org/10.1007/s12223-023-01096-2>.
- Karavoltos, S., Kalambokis, E., Sakellari, A., Plavšić, M., Dotsika, M., Karalis, P., Leontiadis, L., Dassenakis, M., Scoullous, M., 2015. Organic matter characterization and copper complexing capacity in the sea surface microlayer of coastal areas of the Eastern Mediterranean. *Mar. Chem.* 173, 234–243. <https://doi.org/10.1016/j.marchem.2014.12.004>.
- Kolm, H.E., Andretta, L., 2003. Bacterioplankton in different tides of the Perequê tidal creek, Pontal do Sul, Paraná, Brazil. *Braz. J. Microbiol.* 34, 97–103. <https://doi.org/10.1590/S1517-83822003000200002>.
- Krause, E., Wichels, A., Giménez, L., Lunau, M., Schilhabel, M.B., Gerds, G., 2012. Small changes in pH have direct effects on marine bacterial community composition: a microcosm approach. *PLoS One* 7, 10. <https://doi.org/10.1371/journal.pone.0047035>.
- Kurata, N., Vella, K., Hamilton, B., Shivji, M., Soloviev, A., Matt, S., Tartar, A., Perrie, W., 2016. Surfactant-associated bacteria in the near-surface layer of the ocean. *Sci. Rep.* 6, 1–8. <https://doi.org/10.1038/srep19123>.
- Kuznetsova, M., Lee, C., 2002. Dissolved free and combined amino acids in nearshore seawater, sea surface microlayers and foams: influence of extracellular hydrolysis. *Aquat. Sci.* 64, 252–268. <https://doi.org/10.1007/s00027-002-8070-0>.
- Manibusan, S., Mainelis, G., 2022. Passive bioaerosol samplers: a complementary tool for bioaerosol research. A review. *J. Aerosol Sci.* 163, 105992. <https://doi.org/10.1016/j.jaerosci.2022.105992>.
- Menezes, F.G.R., Barbosa, W.E., Vasconcelos, L.S., Rocha, R.S., Maggioni, R., Sousa, O.V., Hofer, E., Vieira, R.H.S.F., 2018. Genotypic assessment of a dichotomous key to identify *Vibrio coralliilyticus*, a coral pathogen. *Dis. Aquat. Org.* 128, 87–92. <https://doi.org/10.3354/dao03209>.
- Michalska, M., Zorena, K., Marks, R., Wąz, P., 2021. The emergency discharge of sewage to the Bay of Gdańsk as a source of bacterial enrichment in coastal air. *Sci. Rep.* 11 (1), 1–13. <https://doi.org/10.1038/s41598-021-00390-8>.
- Moura, G.C.C., Ayres, Y.M., Brito, A.L.D.C., Júnior, E.F.D.S., Rocha, R.D.S., De Sousa, P. M.V., Ferreira, A.G., Sousa, O.V., Veleda, D., 2023. Characterization of the cultivable microbiota components of marine bioaerosols in the North tropical Atlantic. *Atmosphere* 14 (10), 1470. <https://doi.org/10.3390/atmos14101470>.
- Munn, C.B., 2011. *Marine Microbiology: Ecology & Applications*, second ed. Garland Science, New York.
- Oyetibo, G.O., Ilori, M.O., Adebuseye, S.A., Obayori, O.S., Amund, O.O., 2010. Bacteria with dual resistance to elevated concentrations of heavy metals and antibiotics in Nigerian contaminated systems. *Environ. Monit. Assess.* 168, 305–314. <https://doi.org/10.1007/s10661-009-1114-3>.
- Park, J.Y., Lim, S., Park, K., 2014. Mixing state of submicrometer sea spray particles enriched by insoluble species in bubble-bursting experiments. *J. Atmos. Ocean. Technol.* 31, 93–104. <https://doi.org/10.1175/JTECH-D-13-00086.1>.

- Pasquarella, C., Sansebastiano, G.E., Ferretti, S., Saccani, E., Fanti, M., Moscatu, U., Giannetti, G., Fornia, S., Cortellini, P., Vitali, P., Signorelli, C., 2007. A mobile laminar airflow unit to reduce air bacterial contamination at surgical area in a conventionally ventilated operating theatre. *J. Hosp. Infect.* 66, 313–319. <https://doi.org/10.1016/j.jhin.2007.05.022>.
- Pendergraft, M.A., Belda-Ferre, P., Petras, D., Morris, C.K., Mitts, B.A., Aron, A.T., Bryant, M., Schwartz, T., Ackermann, G., Humphrey, G., Kaandorp, E., Dorrestein, P. C., Knight, R., Prather, K.A., 2023. Bacterial and chemical evidence of coastal water pollution from the Tijuana river in sea spray aerosol. *Environ. Sci. Technol.* 57 (10), 4071–4081. <https://doi.org/10.1021/acs.est.2c02312>.
- Polymenakou, P.N., 2012. Atmosphere: a source of pathogenic or beneficial microbes? *Atmosphere* 3, 87–102. <https://doi.org/10.3390/atmos3010087>.
- Qi, J., Shao, Q., Xu, W., Gao, D., Jin, C., 2014. Seasonal distribution of bioaerosols in the coastal region of Qingdao. *J. Ocean Univ. China* 13, 57–65. <https://doi.org/10.1007/s11802-014-1951-8>.
- Rahlf, J., Herlemann, D., Giebel, H.A., Mustafa, N.I.H., Wurl, O., Stolle, C., 2019. Marine foams represent compressed sea-surface microlayer with distinctive bacterial communities. *bioRxiv* 820696. <https://doi.org/10.1101/820696>.
- Rastmanesh, A., Boruah, J.S., Lee, M.S., Park, S., 2024. On-site bioaerosol sampling and airborne microorganism detection technologies. *Biosensors* 14 (3), 122. <https://doi.org/10.3390/bios14030122>.
- Schwieterman, E.W., Cockell, C.S., Meadows, V.S., 2015. Nonphotosynthetic pigments as potential biosignatures. *Astrobiology* 15, 341–361. <https://doi.org/10.1089/ast.2014.1178>.
- Smets, W., Morettia, S., Denys, S., Lebeer, S., 2016. Airborne bacteria in the atmosphere: presence, purpose and potential. *Atmos. Environ.* 139, 214–221. <https://doi.org/10.1016/j.atmosenv.2016.05.038>.
- Stolle, C., Nagel, K., Labrenz, M., Jürgens, K., 2010. Succession of the sea-surface microlayer in the coastal Baltic Sea under natural and experimentally induced low-wind conditions. *Biogeosciences* 7, 2975–2988. <https://doi.org/10.5194/bg-7-2975-2010>.
- Tsoar, H., Levin, N., Porat, N., Maia, L.P., Herrmann, H.J., Tatumi, S.H., Claudino-Sales, V., 2009. The effect of climate change on the mobility and stability of coastal sand dunes in Ceará State (NE Brazil). *Quat. Res.* 71, 217–226. <https://doi.org/10.1016/j.yqres.2008.12.001>.
- 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., 2012. Storm drains as a source of fecal-derived pollution to Fortaleza city's coastal zone. *Arq. Ciências do Mar* 44, 5–12.
- Wurl, O., Ekau, W., Landing, W.M., Zappa, C.J., 2017. Sea surface microlayer in a changing ocean—A perspective. *Elementa: Science of the Anthropocene* 5, 31. <https://doi.org/10.1525/elementa.228>.
- Xia, X., Wang, J., Ji, J., Zhang, J., Chen, L., Zhang, R., 2015. Bacterial communities in marine aerosols revealed by 454pyrosequencing of the 16S rRNA gene. *J. Atmos. Sci.* 72, 2997–3008. <https://doi.org/10.1175/JAS-D-15-0008.1>.
- Zewde, A.A., Zhang, L., Ghebresilasse, H., Mantay, I.M., 2018. A comparative study on pelagic primary productivity in the coastal areas of Eritrean red sea. *J. Mar. Sci. Res. Dev.* 8, 247. <https://doi.org/10.4172/2155-9910.1000247>.
- Zhang, B., Zhen, Y., Mi, T., Qi, J., Yuan, G., 2023. Characterization of bacterial communities in aerosols over northern Chinese marginal seas and the northwestern Pacific Ocean in autumn. *J. Ocean Univ. China* 22 (1), 136–150.
- Zhen, Q., Deng, Y., Wang, Y., Wang, X., Zhang, H., Sun, X., Ouyang, Z., 2017. Meteorological factors had more impact on airborne bacterial communities than air pollutants. *Sci. Total Environ.* 601–602, 703–712. <https://doi.org/10.1016/j.scitotenv.2017.05.049>.
- Zhong, X., Qi, J., Li, H., Dong, L., Gao, D., 2016. Seasonal distribution of microbial activity in bioaerosols in the outdoor environment of the Qingdao coastal region. *Atmos. Environ.* 140, 506–513. <https://doi.org/10.1016/j.atmosenv.2016.06.034>.