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Microbiology Water Quality of Three Coastal Beaches at Madeira Island, Atlantic Ocean

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Abstract

The microbial water quality of three different coastal beaches located in the south coast of Madeira Island, were monitored from May to December 2005 (Formosa and São Roque), and from October 2006 to June 2007 (Gavinas). Total and fecal *coliforms* and *enterococci* counts in two of the beach sites, showed a seasonal dependence, with highest values in autumn and winter suggesting a positive relationship with rainfall and a negative relationship with temperature. Of the three sampled beaches, Formosa beach showed the highest water quality and São Roque beach the poorest water quality. Impacted freshwater discharges and deficient water circulation in this recreational zone could be the causes for the lower quality of this beach. However beach rating was shown to be dependent on the indicator microbe chosen. The results obtained emphasized the need of differential sampling strategies according to beach specific characteristics, when assessing the water quality, as well as further sampling taking into consideration other variables, such as time of the day, tide, and microbial analysis of the freshwater inputs.

Keywords: *Enterococci*; Fecal *coliforms*; Madeira Island; Microbial indicators; Water quality

Introduction

Coastal marine environments are highly vulnerable to anthropogenic pollution from municipal sewage, industrial effluents as well as agriculture run-off and river discharges [1-3]. Fecal contamination not only impairs water quality but also potentiates human health risks [4,5]. In urbanized areas, possibly sources of fecal pollution can include deficient sewage treatment and leaks due to wastewater treatment plant outflows [3,6,7]. Many coastal beaches are located near urban areas, others near river discharges, with input from agricultural and industrial wastes, so that potential risks of contamination may exist, whenever sewage treatment is not effective [1,8]. Therefore regular monitoring of the quality of coastal waters has been suggested [9,10], employing different sampling strategies, according to the specific characteristics of the recreational area in terms of physical-chemical parameters and number of bathers [11,12]. Two European directives recommended the microbial parameters to be taken into consideration, when assessing the quality of bathing waters (76/160/EEC and 2006/7/EEC). These directives are being followed by member states in order to determine the quality of their bathing waters, and investigate potential health risks associated with fecal polluted waters. Madeira Island, located in the Macaronesian Biogeographic Region, is a popular touristic destination for many European and non-European countries, with a coastline composed of several beaches and sea resorts, highly appreciated for its round the year water warm temperatures from 18 up to 23°C. Bathers, due to the warm weather conditions, attend beaches almost all year, including in autumn and winter, when water quality monitoring is not done by official mandate institutions. The aim of the present study was to evaluate the microbiological quality of coastal waters in three different beaches in the south coast of Madeira, located near populated areas, in different times of the year, based on the total and fecal *coliform* counts, as well as on the fecal *enterococci*. The influence of environmental parameters on the indicator values and their significance for the evaluation of the quality of waters is discussed.

Methods

Sampling collection and sampling site characterization

Sampling sites were located in three different points at the south coast of Madeira Island, located in Central North Atlantic Ocean (33°7ʹ30ʺ-32°22ʹ20ʺN and 16°16ʹ30ʺ-17°16ʹ38ʺW) (Figure 1). Surface water samples, at 1 meter depth, were collected at all sites during the morning, using sterile 500 ml bottles. Sampling in Formosa (Funchal) and São Roque (Machico) beaches was done from May to December 2005, twice a month, except for May when 3 samples were taken. Sampling in Gavinas (Lido) beach was conducted from October 2006 to June 2007. Seventeen water samples were collected from both beaches Formosa and São Roque. From Gavinas beach, 9 water samples were collected from October 2006 to June 2007. Formosa is an open sea beach with about 1 km in extension, São Roque beach is limited by two piers and with a total extension of 330 meters, while Gavinas beach, is a smaller bay with 80 meters in total length. All beaches are predominantly pebble and rocky with just a few sandy patches. Air and water temperature as well as rainfall data were either recorded on site or obtained from the Regional Meteorological Institute. Water conductivity was measured with a probe meter. Turbidity was measured as NTUs. At the laboratory the water pH was measured with a pH meter.

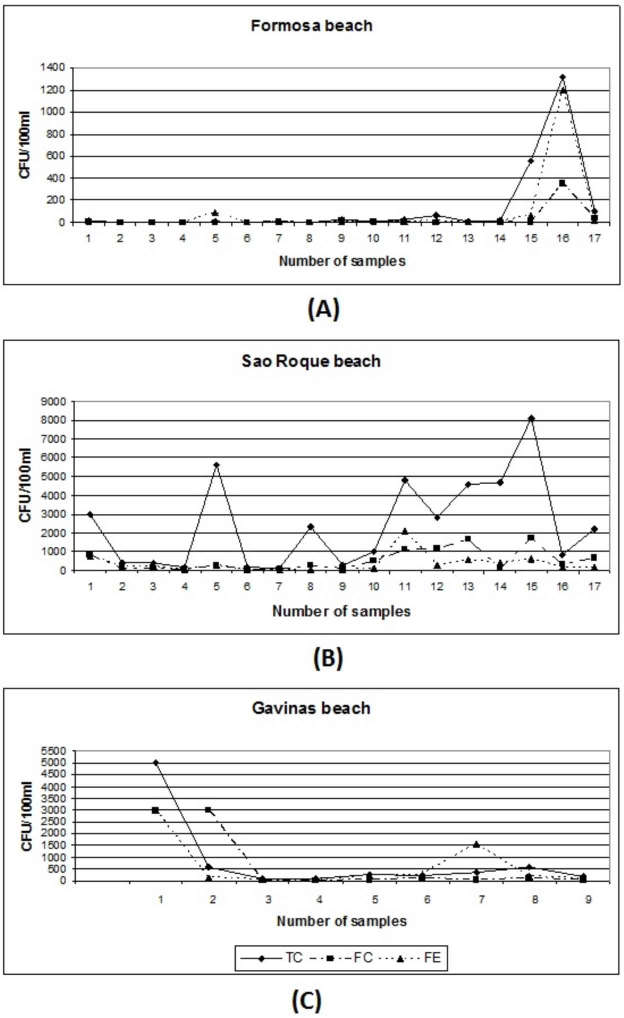

Figure 1: Map of Madeira Island with indication of the three sampled marine beaches, Formosa (A) Gavinas (B) and São Roque, Machico (C).

Determination of microbial parameters

Total *coliform* (TC), fecal *coliform* (FC) and fecal *enterococci* (FE) counts were enumerated using the membrane filtration method (MF). Water samples (10-100ml) were filtered through 0.45 µm membrane filters (Pall Corporation) and filters transferred to membrane lauryl sulphate agar (OXOID), mFC agar (DIFCO) and either KF *Streptococcus* agar or Slanetz Bartley agar (OXOID). Plates were incubated at 35-37°C for TC and FE, while for FC plates were incubated at 44°C for 24-48hr. After that period, filters with red colonies in KF *Streptococcus* agar or Slanetz Bartley agar, were transferred to bile aesculin azide agar (OXOID) for confirmation. Dark brown colonies were considered positive. Positive colonies in membrane lauryl sulphate agar (yellow) were submitted to the oxidase test (OXOID), inoculated in Kliger iron agar (OXOID) and identified with the strips API 20E (Biomérieux). Dark blue colonies in mFC agar were counted as fecal *coliforms*.

Statistical analysis

Water samples of Gavinas beach were taken monthly in triplicate and numbers of indicator bacteria were averaged to obtain a single value, whereas in Formosa and São Roque beaches single water samples were taken twice a month. The correlation between the indicator parameters and the environmental variables were examined using the Spearman's correlation coefficient. For the estimation of mean abundances and the 95% confidence intervals of total and fecal *coliforms* and fecal *enterococci*, data were log transformed [13]. All statistical analysis was performed with SPSS 11.0.

Results

The two coastal beaches, Formosa and São Roque were characterized by a constant salinity of 35% and pH=8.2. Water temperature varied from 18.0 to 22.5°C in Formosa, and 18.5 to 23.5°C in São Roque. Waters of both beaches did not show presence of mineral oils and suspended solids, no abnormal odors and were transparent in appearance. In Gavinas beach the water temperature varied from 17.5 to 21.5°C, pH was 8.0, and salinity was also 35%. During the sampling period, in both beaches Formosa and São Roque, from May 2005 to December 2006, only in December substantial rainfall occurred (63 mm/day) and some rainfall occurred in September (4.1 mm/day), while during the remaining of sampling periods no rainfall or negligible rainfall less than 0.5 mm/day occurred. From October 2006 to June 2007, (sampling in Gavinas beach) rainfall was less than 0.5 mm/day except in March and April when 7.7 mm and 3.3 mm/day were registered. Monthly variations in numbers of the indicator parameters were found in all beaches, with higher values observed in São Roque beach (Figure 2). The highest values for TC, FC and FE for Formosa were found in December (1315, 352 and 1199 cfu/100ml respectively). In the other months of the year values were below the EU guide value of 500 cfu/100ml for TC and 100 cfu/100ml for FC and FE. In this beach only 2 samples exceeded the recommended limits of 500 cfu/100ml for TC, so that a percentage of compliance of 88% was obtained. Regarding the FC and FE counts only one sample was above the guide limit of 100 cfu/100ml for these parameters. São Roque showed higher values of the indicator parameters, well above the guide values, in several occasions (Figure 2). Although these values did not surpass the maximum allowed value of 10,000 cfu/ml for TC, and 2000 cfu/ml for FC, they were above the guide limits for TC and FC counts, 11 times and 13 times respectively. Identically the values of fecal *enterococci* in this site were above the limit of 100 cfu/100ml in 11 of the 17 water samples. In Gavinas beach, for the parameter TC, 6 out of 9 samples had values above the guide limit. Furthermore the TC and FC values in October and November 2006, were too numerous to be accurately counted. The fecal *coliform* counts (FC) were above the guide limit in 3 of the water samples and the fecal *enterococci* values (FE) were 5 times above the guide limit (Figure 2). The mean values of the three indicator parameters and their 95% confidence intervals are shown on Table 1. Based on the values of the indicator parameters, beach Formosa were rated as excellent for all three parameters, São Roque was rated as poor for FC and FE and moderate considering the TC counts, and Gavinas beach was cautiously classified as moderate for TC and FC, but poor in relation to FE counts. Positive and significant correlations were found between the indicator parameters in the three beaches (Table 2). The response of indicator bacteria to the environmental parameters (temperature, turbidity and rainfall) was variable, with higher values in winter at Formosa beach, higher values in autumn and spring at

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Gavinas beach, whereas in São Roque beach fluctuations occurred all through the sampling period, with higher values of the indicator parameters obtained in winter (Figure 2). For Formosa and São Roque beaches, positive and significant correlations were found between total *coliform* and fecal *enterococci* densities, with rainfall and turbidity (Table 2). The influence of temperature on indicator microbes, although showing a negative correlation, was not significant in all occasions as seen in Table 2. Accordingly, total *coliforms* decreased with increasing temperatures in both beaches, whereas fecal *coliforms* and *enterococci* decreased with increasing temperatures only in São Roque beach. In Gavinas beach no significant correlations were found between either rainfall or temperature, with the indicator microbes. Rainfall and turbidity were the variables, with greater effect on densities of indicator bacteria in Formosa and São Roque beaches. Nevertheless the significance of correlation values in São Roque beach and the high counts of indicator microbes found in both wet and dry seasons, suggests that other factors can influence the densities of indicator bacteria. Likewise in Gavinas beach, water quality did not seem to be influenced solely by rainfall, as high microbe counts were found in the dry and wet periods. The identification of the bacteria to species level in Formosa beach demonstrated a predominance of *Escherichia coli* (45%), followed by *Enterococcus faecium* (28.9%), *Klebsiella pneumoniae* (14.1%), *Klebsiella oxytoca* (5.2%), *Enterobacter cloacae* (5.3%) and *Enterococcus faecalis* (1.26%). In São Roque beach *Klebsiella spp*. occurred in higher percentage (28.6%), followed by *Enterobacter cloacae* (28.5%), *E. coli* (11.2%), *Aeromonas spp* (15.9%), *Klebsiella pneumoniae* (9.9%), *E. faecalis* (2.5%), *E. faecium* (0.93%) and *E. gallinarum* (0.86%).

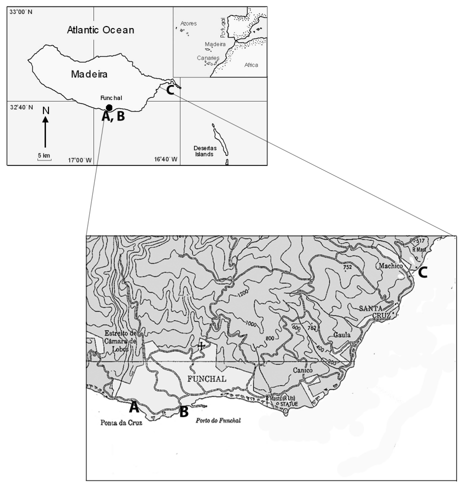

Figure 2: Distribution of total coliforms (TC), fecal coliforms (FC) and fecal enterocci (FE) in three different marine beaches at the south coast of Madeira Island, along the year.

Parameter	Beach	Mean (95% CI)	Min-Max.	Rating
Total	Formosa	128.41 (47.91-344.14)	(0-1315)	Excellent
coliform	São Roque	2439.65 (1167.3-5098.87)	(104-8100)	Moderate
counts (TC)	Gavinas	294.75 (161.95-536.40)*	(87-580)	Moderate
Fecal	Formosa	27.12 (12.85-57.22)	(0-352)	Excellent
coliforms	São Roque	536.53 (286.91-1003.3)	(39-1710)	Poor
(FC)	Gavinas	32.14 (16.48-62.67)*	(10-75)	Excellent
Fecal	Formosa	84.88 (33.29-216.44)	(0-1199)	Excellent
Enterococci	São Roque	358.06 (188.75-679.24)	(14-2080)	Poor
(FE)	Gavinas	315.00 (76.46-1297.8)*	(7-1587)	Poor

Table 1: Abundance of total coliforms (TC), fecal coliforms (FC) and fecal enterococci (FE) in three different beaches in the south coast of Madeira Island, and beach rating according to bacteriological indicators.

Discussion

An increased risk for the transmission of waterborne diseases may exist in waters with high levels of indicator bacteria, which are associated with fecal contamination [4,10,14,15]. Our results showed that the levels of indicator bacteria in the recreational waters sampled were highly variable between beaches and with the time of the year (Figure 2 and Tables 1 and 2). The high levels of indicator bacteria found in December 2005 in Formosa beach could be due to the intense rainfall that occurred at that time, and the consequent input of run-off from land. This however was not the only factor affecting the water quality of São Roque beach, where the levels of indicator bacteria were above regulatory limits in several occasions, with and without rainfall (Figure 2). The topography of this beach might cause poor water exchange with a consequent decrease in water quality. Nevertheless a significant effect of rainfall on the indicator bacteria levels was found in this beach (Table 2). During rainfall events not only water turbidity might increase due to input of particulate matter from freshwater sources, but also the intense rainfall might lead to an input of untreated sewage into the sea and a consequent rise in indicator bacteria [6,8,16]. Turbidity has shown to influence the *coliform* levels due to re-suspension of sediment-bound bacteria [17]. Rainfall and storm events are implicated also in re-suspension of bacteria and might explain the high densities observed in December 2006 in

Formosa and in April 2007 in Gavinas beach [17-19]. Additionally in temperate-tropical regions, such as Madeira Island, the persistence and re-growth of indicator microbes can contribute to an increase in their numbers [10]. Nevertheless temperature was in some occasions significantly negatively correlated with indicator bacterial levels. In fact increasing temperatures are associated with higher solar radiation exposure, which was proved to affect the survival of indicator bacteria [6,8,10,20,21]. Although no measurements of the levels of solar radiation were done in the present study, our data implied that densities of indicator bacteria for Formosa and Gavinas were generally lower in spring and summer. This, combined with the negative correlation between temperature and indicator bacteria counts, suggested indeed a sensitivity of bacteria to solar radiation, with total *coliforms* and fecal *coliforms* been mostly affected, whereas fecal *enterococci* were less sensitive to solar radiation (Table 2). A number of studies demonstrated that *Enterococci* were the group with higher resistance to environmental stress [21,22-25]. They may therefore be more suitable as indicators of fecal contamination due to their higher survival in water and their inability of multiplying in polluted waters [26]. The bacterial species found in Formosa and São Roque beaches, suggested fecal and non-fecal pollution, as the identified bacteria are common in the intestines of humans and other animals as well as in the environment. In São Roque beach the relative high percentage of *Aeromonas spp* (15.9%) could denote an input of freshwater and sewage into this beach [9,27]. In order to determine the real source of these microorganisms, whether they were from human fecal origin, environment or feces of other animals, specific techniques such as ribotyping have been recommended [9] or the choice of alternative indicators such as coliphages [28]. Even so no indicator so far chosen, proved to be perfect for microbial water quality characterization, so that when assessing the quality of waters one has to balance costs, speed and effectiveness of results [3,29]. It is also important to increase the sampling effort, with collection of waters samples in different times of the day, to have a clear picture of the water quality of a given water course, as bacterial counts may change from morning to afternoon due to tidal circulation [12,30].

Parameter	Rainfall	Temperature	Turbidity	TC	FC	FE
Rain	1.000					
	-0.292; p=0.128 (A)	1.000				
	-0.156; p=0.688 (B)					
Temp	-0.425; p=0.045 (C)					
	0.617;p=0.004 (A)	-0.498; p=0.021 (A)	1.000			
	-	-				
Turbidity	0.616; p=0.004 (C)	-0.567; p=0.009 (C)				
	0.609; p=0.005 (A)	-0.516; p=0.017 (A)	0.664; p=0.002 (A)	1.000		
	0.110; p=0.779 (B)	0.479; p=0.192 (B)	-			
TC	0.447; p=0.036 (C)	-0.410; p=0.051 (C)	0.422; p=0.046 (C)			
	0.282; p=0.136 (A)	-0.182; p=0.242 (A)	0.357; p=0.080 (A)	0.768; p=0.000 (A)		
	0.350; p=0.356 (B)	0.386; p=0.305 (B)	-	0.790; p=0.010 (B)		
FC	0.661, p=0.002 (C)	-0.588; p=0.007 (C)	0.627; p=0.004 (C)	0.799; p=0.000 (C)	1.000	
	0.489; p=0.023 (A)	-0.212; p=0.207 (A)	0.450; p=0.035 (A)	0.733; p=0.001 (A)	0.618; p=0.008 (A)	1.000
	0.621; p=0.074 (B)	0.034; p=0.932 (B)	-	0.733; p=0.025 (B)	0.723; p=0.028 (B)	
FE	0.512; p=0.018 (C)	-0.549; p=0.011 (C)	0.396; p=0.058 (C)	0.856; p=0.000 (C)	0.768; p=0.000 (C)	

Table 2: Correlations (rs) between physico-chemical parameters and indicator bacteria at the three beach sites. Beaches are indicated as (A) Formosa, (B) Gavinas and (C) São Roque:

Conclusions

The results obtained suggested that the quality of the recreational waters, examined in this study, can be strongly influenced by rainfall and consequent increase in water turbidity, during autumn and winter months. Given the variability in the water quality in the three beaches monitored, specifically in Gavinas and São Roque beaches, with poor water quality in several occasions, further studies are recommended with an increased periodicity of sampling, and the inclusion of additional variables, such as time of the day and tidal regime, as well as an assessment of the quality and quantity of freshwater input into those beaches.

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