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Comparing different methods for fast screening of microbiological quality of beach sand aimed at rapid-response remediation

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ABSTRACT

There is scientific evidence that beach sands are a significant contributor to the pathogen load to which visitors are exposed. To develop beach quality guidelines all beach zones must be included in microbiological evaluations, but monitoring methods for beach sand quality are relatively longstanding, expensive, laborious and require moderate laboratory infrastructure. This paper aimed to evaluate the microorganism activity in different beach zones applying and comparing a classical method of membrane filtration (MF) with two colorimetric screening methods based on fluorescein (FDA) and tetrazolium (TTC) salt biotransformation to evaluate a new rapid and low-cost method for beach sand microbiological contamination assessments. The colorimetric results can help beach managers to evaluate rapidly and at low cost the microbiological quality of different beach zones in order to decide whether remedial actions need to be adopted to prevent exposure of the public to microbes due to beach sand and/or water contamination.

1. Introduction

Due to intensive land use in the coastal zone and to the economic importance of beaches, concern regarding environmental and public health has increased considerably in recent years. To address this issue, actions have emerged internationally to improve the sustainable recreational use of beaches, such as the Blue Flag program, aimed at connecting the public with their surroundings and encouraging them to learn more about their environment (ABAE, 2011). With respect to beach sand quality, it is well established that microorganisms can be naturally present (indigenous biota) or originate from anthropogenic contamination (sanitary discharges, runoff). There are many different types of microorganisms that humans can be exposed to through contact with water or sand, such as bacteria, viruses, fungi, protozoans and trematodes (WHO, 2003; Whitman et al., 2014; Heaney et al., 2012; Solo-Gabriele et al., 2016).

Unfortunately, in the microbiological monitoring of beaches worldwide only water quality is considered, despite several authors and organizations highlighting the urgent need for the assessment of beach sand quality (Mendes et al., 1993; WHO, 2003; Sabino et al., 2014; Whitman et al., 2014; Solo-Gabriele et al., 2016). For instance, there are no public policies associating epidemiological data with the health problems caused by primary skin contact with contaminated sands. However, correlations between beach sand exposure and infectious disease have been identified (Phillips et al., 2011; Heaney et al., 2012; Solo-Gabriele et al., 2016), and it should be emphasized that microbiologically contaminated water can contaminate the sand where children often spend most of their leisure time on the beach (Wade et al., 2010; Solo-Gabriele et al., 2016). An additional issue is that only a few studies have investigated a relationship between the water quality and the quality of the wet sand (intertidal zone) or dry sand (supratidal zone), and some of these have shown contradictory results (Chabasse et al.,

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1986; Aulicino et al., 1985; Roses-Codinachs et al., 1988; Sato et al., 2005).

It should be noted that sand quality is not dependent solely on water quality. The use of the sandy beach zones by a large number of visitors can lead to the proliferation of microorganisms nourished by food waste, which can be aggravated by the lack of good personal hygiene habits and sometimes by the presence of domestic animals (Mancini et al., 2005; Roca et al., 2009). Also, it is well known that floating solid waste can be brought to the beach by the seawater, affecting the visitors' perception of the beach quality (Santos et al., 2009; Díaz-Mendoza et al., 2014). In this regard, WHO have provided scientific evidence that beach sands can contribute significantly to the exposure of visitors to microbes through direct contact between beach sand and human skin, which can lead to health problems (Sabino et al., 2014; Lamparelli et al., 2015).

From the management point of view, very few initiatives have been adopted internationally in relation to beach sand quality. In Brazil, Rio de Janeiro is the only city that has embraced a classification for recreational sands with primary contact based on the results of the densities of total coliforms and *Escherichia coli* (SMAS, 2010). Furthermore, the methods currently available for monitoring the microbiological quality of beach sand are relatively time-consuming (e.g., microorganism cultivation in Petri dishes) and expensive (e.g., quantitative polymerase chain reaction (qPCR)). In this regard, microorganism communities in beach sand are heterogeneous and include bacteria, viruses, protozoa, helminthes (worms) and fungi, which require, in some cases, specific procedures and at least moderate laboratory infrastructure for their identification and/or quantification (WHO, 2003; Solo-Gabriele et al., 2016).

It thus seems reasonable to propose a tiered approach to beach management, starting with a screening method for microbiological contamination prior to applying a method that considers a direct connection with human health. Thus, to ensure effective beach management, municipal managers should consider applying a sand cleaning process at the end of the day on highly frequented beaches. This could involve simply sweeping the sand and/or turning it over. This is pertinent with regard to the situation in Santa Catarina State (Southern Brazil, Lat. 25°58′ S to 29°19′S), which has 36 coastal touristic municipalities, encompassing around 600 km of coastline. There are hundreds of beaches with different morphodynamics, and the intensity of visitors varies considerably (Polette and Vianna, 2006). Every year, the beach towns together receive >7 million visitors during the summer season, which is economically important for the coastal region (Polette and Vianna, 2006). In 2015–2016, two beaches located in Santa Catarina State had the Blue Flag certification granted by the Foundation for Environmental Education (http://www.blueflag.global/), and a third is in the pilot stage of the certification process. In addition, the State Environmental Protection Agency (FATMA-SC) carry out weekly microbial monitoring during the summer season to assess the water quality of beaches in 27 municipalities, where 211 sampling points are evaluated. However, a rapid and simple test could also be conducted to determine if sand zones are microbiologically contaminated, requiring a more powerful sand remediation process. In this context, the goal of this study was to evaluate the microorganism activity in different beach zones (water, wet sand and dry sand) applying and comparing two colorimetric screening methods based on fluorescein and tetrazolium salt biotransformation, using as a reference the classical method of membrane filtration employing Colilert® as a chromogenic substrate. To achieve this goal, 40 sites were tested with the colorimetric and classical methods. It was ensured that these sites had different characteristics: 20 had passed and 20 had failed recent government microbial contamination tests to determine bathing water quality. Each group of 20 sites was subdivided into two groups of 10 sites according to the level of frequency by visitors. A rapid and low-cost colorimetric method was developed, which could help beach managers to evaluate microbial contamination levels as well as to make decisions regarding the need to undertake remedial actions.

2. Material and methods

2.1. Beach locations and water/sand microbiological classification

Water, wet sand (intertidal beach zone) and dry sand (supratidal beach zone) samples were collected from 40 sites located on 36 beaches along the Santa Catarina coast. Table 1 shows the georeferenced data (WGS84 *datum*) and the bathing quality according to the Brazilian Resolution CONAMA 274/2000. The sand samples from the 36 beaches showed similar granulometric characteristics, being comprised of fine sand without rocks, stones or agglomerated organic matter. Of the 40 sites, 20 had failed recent government microbial contamination tests carried out to determine bathing water quality. Of these, 10 sites were not frequented by visitors (sites 1 to 10, denominated CNF) and 10 sites received a low frequency of visitors (sites 11 to 20, denominated CLF). At the other 20 sites, the water had passed recent microbial contamination tests and of these 10 received a low frequency of visitors (sites 21 to 30, denominated ULF) and 10 sites were highly frequented by visitors (sites 31 to 40, denominated UHF). The same sample mass (solid or water) was used in all analysis to ensure an appropriate comparison. Recent beach monitoring data published by the State Environmental Protection Agency (FATMA; http://www.fatma.sc.gov.br/laboratorio/dlg_balneabilidade2.php) was used to select the sites used in this study based on whether they had passed or failed bathing water quality tests.

Water quality is generally classified according to the nature of the water usage. Brazilian legislation, such as CONAMA Resolution 274/2000, determines that seawater is appropriate for recreational use if 80% or more of the samples collected at the same location for the five consecutive weeks contain <1000 thermotolerant coliforms on average, or <800 *E. coli* per mL, or <100 *Enterococcus* per 100 mL. Seawater is considered inappropriate (representing a health risk) if this requirement is not met or if the last sample collected contains over 2500 thermotolerant coliforms, or >2000 *E. coli* per mL, or >400 *Enterococcus* per 100 mL (CONAMA, 2000). The reasoning behind the setting of particular guideline levels by Brazilian regulators has a historical origin. Prior to the creation of Brazilian environmental agencies, marine pollution projects considered the “California standard” (1940s) of 1000 total coliforms per 100 mL. Later, Brazilian regulators adopted this guideline value in the CONAMA Resolutions (including 274/2000). It seems that this value originated from strictly esthetic considerations. Subsequent studies in the USA on *Enterococci* and *E. coli* bacteria present in recreational waters led CONAMA to adopt these parameters in their resolutions as additional indicators of recreational water quality.

With respect to the microbiological classification of beach sands, to the best of our knowledge, despite proposals put forward by researchers (e.g., Mendes et al., 1993; ABAE, 2011), Rio de Janeiro is the only Brazilian city which has a Municipal Resolution (SMAC Resolution 468/2010) related to this issue (SMAC, 2010). Table 2 shows the numeric values for the microbiological classification of beach sand. The guideline values established by this Resolution are based on a study on the sanitary quality of beach sands in Portugal (Azores archipelago), which was carried out in 1993/1995 by Mendes et al. (1993), and also on a pilot project carried out previously by SMAC, which established the colimetric standards based on the analysis of sand on beaches subjected to low levels of anthropogenic impact. It should be noted that the values proposed by Mendes were also adopted by the European Blue Flag Association (ABAE, 2011). In addition, this Municipal Resolution recommends no primary contact with sand that has signs of pollution (perceived via odors or visually).

2.2. Water and sand sampling

Water samples were collected according to the standard procedure described in APHA et al. (2012). Sand samples, both wet and dry, were collected according to the SMAC (2010) protocol, which is summarized

Table 1
Beach localization and classification (in relation to the contamination) according to the water quality criteria of Brazilian Resolution CONAMA 274/2000. Frequentation criteria was estimated according to the authors survey.

Locality identification	Georeference (WGS84 *datum*)	Beach classification[a]	Locality identification	Georeference (WGS84 *datum*)	Beach classification[a]
1 José Mendes	−27.61293287423; −48.54752607809	CNF	21 Solidão	−27.7952671645009; −48.5342612003906	ULF
2 Beira Mar	−27.5846157095629; −48.5494123880114	CNF	22 Brava	−27.393086; −48.414084	ULF
3 Bom Abrigo	−27.6121387401247; −48.597005293926	CNF	23 Forte	−27.433946767139; −48.519843372842	ULF
4 Praia do Meio	−27.61363788356; −48.5851311167437	CNF	24 Sambaqui	−27.489345791033; −48.537968107323	ULF
5 Praia da Saudade	−27.6109142956139; −48.5795184931059	CNF	25 Cacupé Norte	−27.53248835197; −48.52543766534	ULF
6 Do Meio	−27.61363788356; −48.5851311167437	CNF	26 Estaleiro	−27.03307368188784; −48.58220380621382	ULF
7 Matadouro	−27.5869590098687; −48.5764904152094	CNF	27 Navegantes 03	−26.8398232432361; −48.63051626205563	ULF
8 Jardim Atlântico	−27.5738221075775; −48.5973044209087	CNF	28 Palmas	−27.324055996788; −48.53835758628	ULF
9 Ribeirão da Ilha	−27.71925291568; −48.56449568815	CNF	29 Magalhães	−27.402447663146; −48.562341862298	ULF
10 Cacupé	−27.5424225552425; −48.524921429456	CNF	30 São Miguel	−27.4522581915289; −48.6330515894704	ULF
11 Ponta das Canas	−27.413658; −48.428132	CLF	31 Camboriú Sul	−27.00510823103653; −48.60512689178738	UHF
12 Ingleses	−27.4258945060097; −48.3984399927141	CLF	32 Cabeçudas	−26.927564876419; −48.632902803689	UHF
13 Canasvieiras	−27.42598510565; −48.45018893125	CLF	33 Jurerê	−27.436608832162; −48.501681664501	UHF
14 Taquaras	−27.0099882064489; −48.5791615914880	CLF	34 Cachoeira Bom Jesus	−27.4203670004763; −48.4351209963068	UHF
15 Camboriú Norte	−26.9724484643764; −48.6327700443469	CLF	35 Barra da Lagoa	−27.5739682074886; −48.4233343338740	UHF
16 Itapema Sul	−27.116662; −48.607453	CLF	36 Mole	−27.6026308929178; −48.432467521022	UHF
17 Guararema	−27.637542; −48.631057	CLF	37 Joaquina	−27.6290784648312; −48.4482309951289	UHF
18 Do Balneário	−27.5777896170202; −48.5784540287464	CLF	38 Campeche	−27.687002; −48.4801459	UHF
19 Da Armação	−27.7501898642938; −48.5026413153606	CLF	39 Jurerê Internacional	−27.436608832162; −48.501681664501	UHF
20 Campeche Sul	−27.6901707000176; −48.481243339719	CLF	40 Itapema	−27.10684101223904; −48.61267500266039	UHF

[a] CNF = contaminated and not frequented; CLF = contaminated and low frequentation; ULF = uncontaminated and low frequentation; UHF = uncontaminated and high frequentation.

herein. For each site, three rectangular areas (1 m × 0.5 m) located 2 m from a selected central point (20 m distant from each other) were delimited and five sub-samples (the center point and the four corners of the rectangle) were collected from the surface layer to a depth of 15 cm, with the aid of a PVC cylindrical sampler with a diameter of 3.6 cm. The five sub-samples were mixed in a labeled sterile bag to compose a single sample, which was keep at around 4 °C in an airtight container until analysis. Nine samples were generated for each site (3 for dry sand, 3 for wet sand, and 3 for water), totaling 360 samples. All samples were collected at the end of the day in the 2016 summer season, when the beach sand had significant levels of waste material in the case of highly frequented beaches. It was noted that the last rain event occurred one week prior to the sand collections.

Table 2
Rio de Janeiro city beach sand microbiological classification according to the Municipal Resolution 468/2010.

Beach sand classification	Total coliforms (MPN 100 g^{-1} of sand)	*Escherichia coli* (MPN 100 g^{-1} of sand)
Optimum	≤10000	≤40
Good	>10000–20000	>40–400
Regular	>20000–30000	>400–3,800
Contact Not Recommended	>30000	>3,800

2.2.1. Microbiological analysis

The reference method used for the detection and quantitation of total coliforms and *E. coli* was the membrane filtration (MF) test using the chromogenic substrates 4-methylumbelliferyl-β-d-glucuronide (MUG) and ortho-nitrophenyl-β-d-galactopyranoside (ONPG) approved by APHA et al. (2012). A yellow color (reaction of enzyme β-galactosidase with ONPG) indicates the presence of total coliforms. The reaction of the enzyme β-glucuronidase with MUG will result in a blue fluorescence (under illumination by longwave UV) indicating the presence of *E. coli*, while a clear medium indicates the no contamination was detected. Prior to the analysis, sterile autoclaved seawater (10 mL) was added to each water or sand sample in a 1:1 ratio (w/w). The sand sample solutions were well mixed in a continuous agitation table before filtration through a cellulose acetate membrane filter with a pore size of 100 μm. After this initial filtration of the sand samples, all samples were filtered through a cellulose nitrate membrane filter with a pore size of 0.45 μm. The membranes were then placed in Petri dishes on an absorbent pad containing a nutritional broth and incubated at 37 °C in an incubator for 24 h. The quantitative growth results were expressed in colony forming units (CFU 100 mL^{-1}). The samples were classified as appropriate or inappropriate for bathing, according to the results for total coliforms and *E. coli*, as indicated in the CONAMA Resolution 274/2000 for water samples or SMAC Resolution 468/2010 for sand samples (see Section 2.1, above).

2.2.2. Colorimetric sample analysis

2.2.2.1. Hydrolytic enzyme activity measured with FDA. Total hydrolytic enzyme activity was assessed by the fluorescein diacetate (FDA, Sigma-Aldrich, F7378) hydrolysis method of Schnürer and Rosswall (1982), with minor modifications. FDA is a nonspecific and sensitive assay suitable for measuring total microbial activity applying a short incubation period. Culture-dependent methods do not provide an insight into the entire microbial community, so methods based on total microbial activity can be applied in studies on the microbial communities of environmental samples. FDA is hydrolyzed by a number of different enzymes, such as proteases, lipases, and esterases, and the product of the enzymatic conversion is fluorescein, which can be visualized in cells by fluorescence microscopy or quantified by fluorometry or spectrophotometry (Schnürer and Rosswall (1982). In this procedure, water (10 mL) or sand (10 g) samples were placed in 20 mL polycarbonate centrifuge tubes and 10 mL of 30 mM phosphate buffer (adjusted to pH 7.6) was added. The tubes were shaken gently for 10 min, after which 300 μL of a 2 mg L^{-1} FDA solution in acetone (10%) was added. A further 6-h period of shaking was then applied before terminating the reaction. The test tubes with solid samples were then centrifuged at 3400 rpm for 5 min followed by filtration of the supernatant (Whatman 0.45 μm). The filtrate was analyzed for FDA hydrolysis through the measurement of the absorbance at 495 nm. Hydrolytic enzyme activity analysis was conducted in triplicate for each sample. A calibration curve obtained from the serial dilution of a slurry sewage sample was used to relate the FDA absorbance to the number of bacteria in Petri dishes (CFU 100 mL^{-1}), allowing the number of bacteria in the samples to be estimated. The samples were then classified as appropriate or inappropriate for bathing, according to the results for total coliforms and *E. coli*, as indicated in the CONAMA Resolution 274/2000 for water samples or SMAC Resolution 468/2010 for sand samples (see Section 2.1 above).

2.2.2.2. Hydrolytic enzyme activity measured with tetrazolium salt (TTC). Hydrolytic enzyme activity analysis based on the reduction of triphenyl tetrazolium chloride (TTC, Sigma-Aldrich T8877) was conducted, as described above for the FDA hydrolysis method, in triplicate for each sample. However, in this case, 300 μL of an 8 mg L^{-1} TTC solution was added to the sample and phosphate buffer. A further 12-h period of shaking was then applied before terminating the reaction. After filtration, TTC reduction was measured at an absorbance of 620 nm. A calibration curve was obtained from the serial dilution of a slurry sewage sample was used to relate the TTC absorbance to the number of bacteria in Petri dishes (CFU 100 mL^{-1}), allowing the number of bacteria in the samples to be estimated. The samples were then classified as appropriate or inappropriate for bathing, as described above for the FDA measurement.

2.3. Statistical analysis

Analysis of the variance (ANOVA test) was used to identify differences between the microbiological analysis methods, as well as between the beach compartments. The ANOVA test included three factors: compartment (water, dry sand and wet sand), detection method (FDA, TTC and MF), and site (40 sampling sites), and all factors were considered orthogonal and fixed. Principal component analysis (PCA) and determination of the Pearson correlation coefficient were applied to data generated with the three analytical methods for the three compartments. The statistical variables were previously standardized and centralized (Clarke and Warwick, 1994). The selected significance level was 5%. The statistical package used for the analysis was the Plymouth Routines in Multivariate Ecological Research (PRIMER).

3. Results

Figs. 1 to 3 show the results for the microbiological analysis of the beach samples (sand and water) obtained applying the three analytical methods used to assess the microbiological quality of the beach sites.

The results in Fig. 1 confirm the previous beach water quality classifications obtained in the beach quality monitoring program of the Santa Catarina State Environmental Protection Agency (FATMA). Thus, the beaches which FATMA classified as showing water contamination (sites CNF and CLF) were found to be contaminated (that is, the beach water quality was unacceptable for bathing according to the CONAMA Resolution 274/2000 criteria) applying the three different methods tested in this study. Similarly, the beaches which FATMA considered did not show water contamination (sites ULF and UHF) were found to be uncontaminated in this study (that is, the beach water quality was acceptable for bathing, according to the CONAMA Resolution 274/2000 criteria).

It can be observed in Fig. 2 that the quality profile for the wet beach sand samples is similar to that observed for the beach water samples in

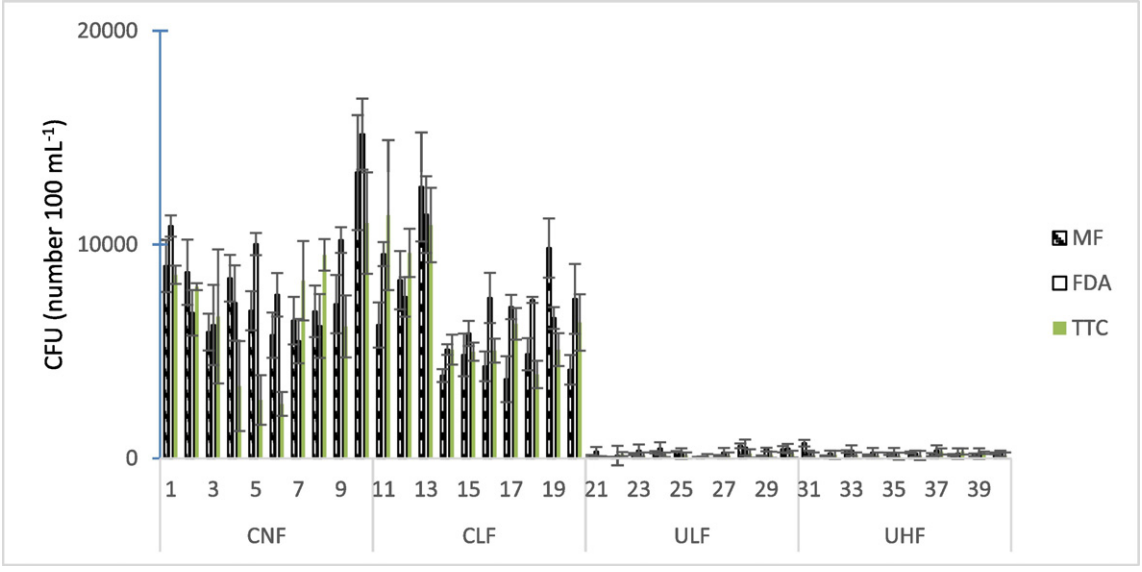

Fig. 1. Beach waters microbiological quantitative analysis (CFU 100 mL^{-1}) of 40 sampling points using 3 different analytical methods. Mean and standard deviation are showed ($n = 3$). MF = Membrane filtration (total coliforms and *E. coli*); FDA = Fluorescein diacetate (all microbes); TTC = Tetrazolium salt (all microbes).

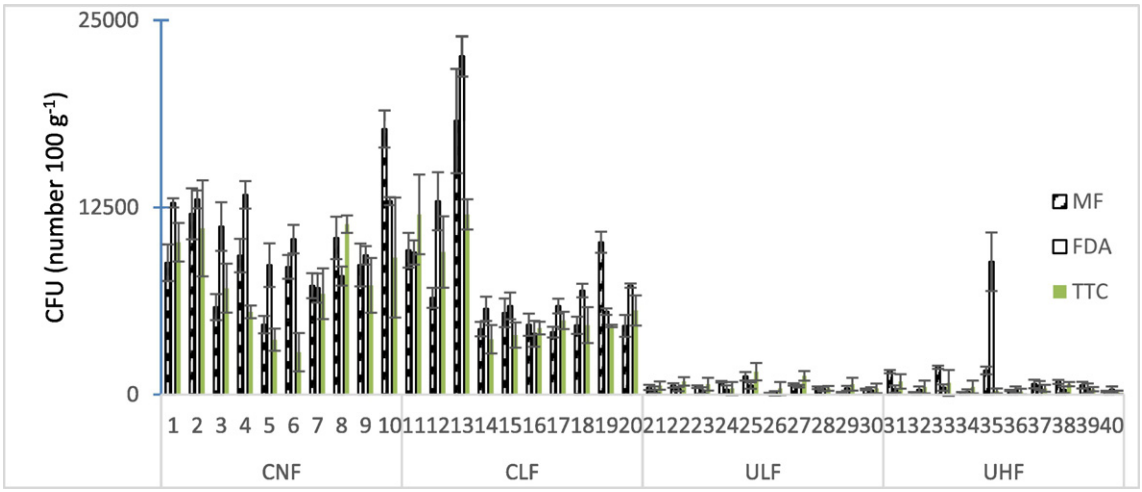

Fig. 2. Beach wet sand microbiological quantitative analysis (CFU 100 g^{-1}) of 40 sampling points using 3 different analytical methods. Mean and standard deviation are showed ($n = 3$). MF = Membrane filtration (total coliforms and *E. coli*); FDA = Fluorescein diacetate (all microbes); TTC = Tetrazolium salt (all microbes).

Fig. 1. Considering the beaches in Fig. 2 which FATMA classified as contaminated (sites CNF and CLF), 9 were classed as presenting optimum wet sand quality, while 11 presented good wet sand quality, according to at least one of three methods tested. In this respect, it should be noted that the MF method is specific to total coliforms and *E. coli*, while the FDA and TCC are not organism-specific methods. In relation to the beaches of Fig. 2 which FATMA classified as uncontaminated (sites ULF and UHF), all of them presented optimum wet sand quality. When the profiles of Figs. 1 and 2 are compared, it is clear that water contamination can influence the wet sand quality of a beach, independent of frequency of beachgoers.

The microbiological contamination results for the dry beach sand measured by the three methods are shown in Fig. 3. For this compartment, it can be observed that the values obtained for the beaches which previously failed the government bathing water quality test (sites CNF and CLF) differ considerably from those observed for the ULF beach values. However, for some of the beaches in the UHF group the results were similar to those of the CNF and CLF beaches. Fig. 3 shows that 5 dry beach sand samples were classified as being of good quality, while all others were classified as being of optimum quality. Interestingly, among the former 5 beaches, 2 belong to the UHF group of sites and thus the beaches that presented good dry sand quality include the beaches most frequented by people. However, it should be noted that none of the dry beach sand samples collected from the 40 beaches can be considered contaminated (i.e., Contact Not Recommended) according to the limits established by the SMAC Resolution 468/2010.

A direct comparison between Figs. 2 and 3 shows that wet and dry sand quality differs in terms of contamination intensity, which affects the beach quality, since 11 wet beach sands of the CNF and CLF groups were considered as being of optimum quality, while 17 dry beach sands of the same groups obtained the optimum quality classification. Based on Figs. 1 to 3, a ranking of the beach contamination intensity could be established for the samples analyzed: wet sand > seawater > dry sand.

The results of the PCA analysis (Fig. 4) show two factorial axes that explain 96.0% of the total variation. Axis 1 (PC1), related to the negative coordinates (higher contamination values), is responsible for 91.4% of the observed variation. In Fig. 4, it can be observed that for the dry sand compartment, microbiological contamination increased in the following order for the beach classes: ULF < CNF < CLF < UHF. In the case of wet sand, increasing microbiological contamination showed the

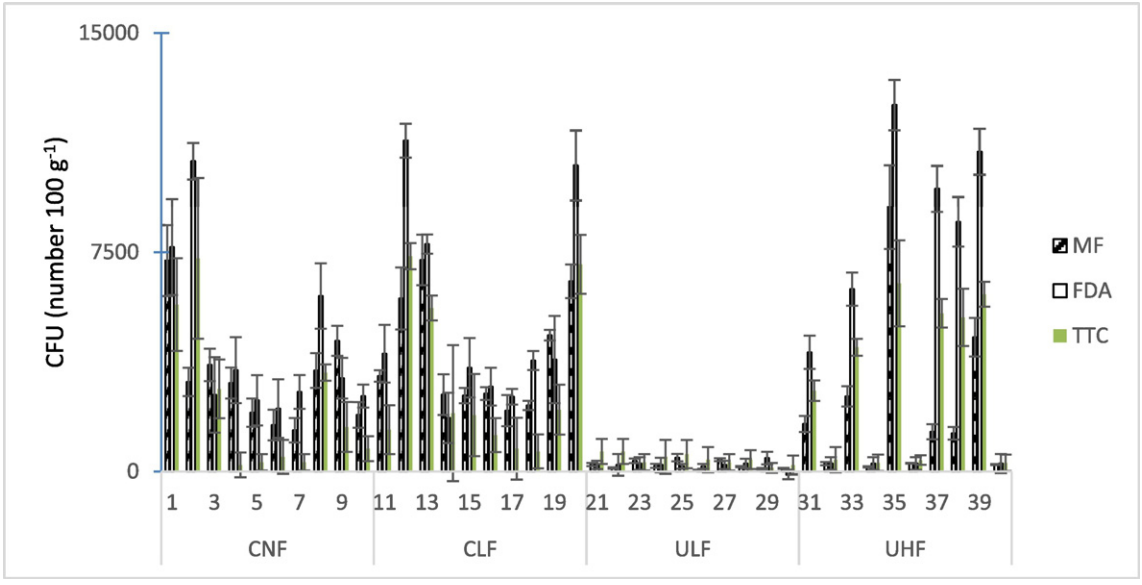

Fig. 3. Beach dry sand microbiological quantitative analysis (CFU 100 g^{-1}) of 40 sampling points using 3 different analytical methods. Mean and standard deviation are showed ($n = 3$). MF = Membrane filtration (total coliforms and *E. coli*); FDA = Fluorescein diacetate (all microbes); TTC = Tetrazolium salt (all microbes).

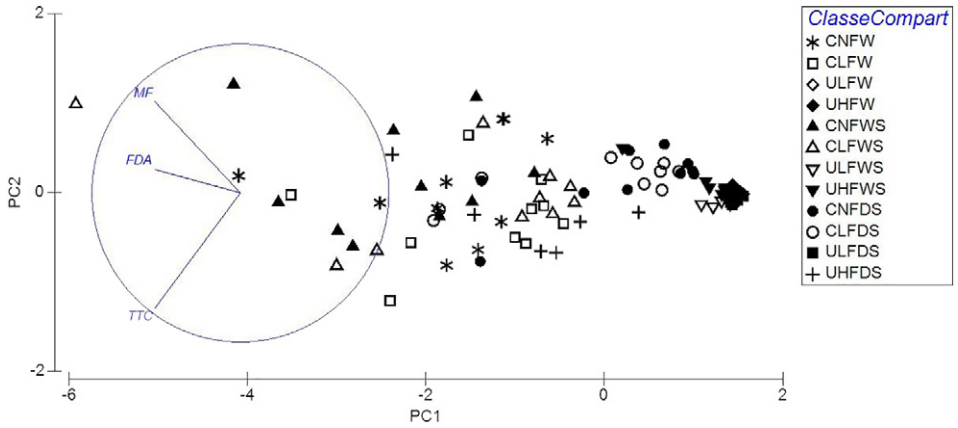

Fig. 4. Principal component analysis (PCA) for microbiological contamination results from three different methods (i.e., variables) considering the different beach compartments of different beach classes.

following order: ULF < UHF < CNF < CLF. Axis 2 (PC2) is responsible for 4.6% of the observed variation and is associated with the inverse relation between the MF and TTC methods, the former with a positive coordinate (0.614) and the latter with a negative coordinate (−0.774). In addition, a Pearson correlation was determined to better understand the data distribution, where R^2-values of 0.876 were obtained for the correlation between MF and FDA and 0.863 for that between MF and TTC.

4. Discussion

Historically, in Brazil, beach managers use visual inspection as a technical criterion to assess beaches quality in terms of the risk to human health, but this evaluation simply represents an esthetic evaluation. Clearly, this criterion focuses on solid waste or color variations and does not assess the microbiological quality of the sand. Although the microbiological quality of beach sand has recently become a public health issue, coastal managers and environmental agencies in developing countries have to deal with institutional and economic limitations (Wagener, 2005; Solo-Gabriele et al., 2016). The first methodological approach to this evaluation is the determination of total fecal coliforms and *E. coli*, the results being used as indicators of water quality, which can currently be considered as a minimum criterion in beach sand quality assessments. However, standardized methods do not provide results fast enough to determine whether the sand requires remediation in order for the beach to be ready to receive visitors the next morning. Figs. 1 to 4 shows that the three analytical methods were able to assess beach sand and water quality, since the values obtained for the beach samples were similar for all methods. Furthermore, data from this study demonstrate the microbiological contamination of dry sand collected from some of the highly frequented beaches where the seawater had passed the government bathing water quality test. According to the MF method, fecal contamination (determined from the data on total coliforms and *E. coli*) was predominant in some of the samples analyzed. However, in reports in the literature, dry beach sand samples presented higher yeast and *Streptococci* contamination levels than wet beach sand (Vieira et al., 2001; Sato et al., 2005). Since this beach zone is not under the influence of the tides, it is probable that this contamination originated from illegal diffuse sewage discharges and rain water runoff from residences located near the beach. In general, there is no difference between the microbiological contamination profiles obtained for seawater that had failed the government bathing water quality test and wet sand. In this regard, seawater can contaminate or dilute the wet sand, depending on the degree of water contamination and/or other factors (e.g., presence of suspended organic matter undergoing decomposition). This leads to the following question: for quality classification purposes, should wet beach sand limits be the same as those used for the seawater classification?

In addressing this question, we must bear in mind that it is very difficult to establish a direct relationship between the contamination levels of liquid and solid samples, since the two matrices have different physico-chemical properties that can influence the microbial refuge/development. In a mass by mass comparison, wet beach sand appears as the principal reservoir for microbes. Furthermore, from the toxicological point of view, the levels of exposure of beachgoers to these matrices are quite different. During swimming the body is completely submerged in the potentially contaminated water, while the degree of exposure to wet beach sand is lower, being generally limited to the soles of the feet for adults and the hands, feet and legs for children. This difference in relation to the surface contact may explain the different acceptable contamination levels established by the environmental agencies for these two compartments. Based in our results, we suggest that wet beach sand could be submitted to the same classification used for seawater contamination. Further studies need to be conducted to clarify this question of the consequences of exposure. It seems that the present classification of beach sand quality proposed by SMAC needs to be improved in terms of sensitivity, since none of the beach sands were classified as 'Regular' or 'Contact Not Recommended' despite the high levels of contamination verified in some of the seawater samples.

With regard to the sources of beach sand contamination, contamination originating from urban runoff (especially on urban beaches) has been reported, and also sewage inputs could arrive in groundwater, in areas where adequate collection and treatment systems are not in place. The amount of pore water flowing from terrestrial areas towards the beach can be surprisingly large and, according to some studies, a significant proportion of nutrients, organic matter and microorganisms in the surf zone can originate from this source (Santos et al., 2012; Solo-Gabriele et al., 2016; Vogel et al., 2016).

In relation to the PCA carried out with all our results, two axes explain almost all of the observed variation, where axis 1 (PC1) was responsible for >96% of the variation in the results. On this axis, the variables, i.e., the three analytical methods, assumed high negative loadings (−0.579 for FDA, −0.576 for TTC, and −0.577 for MF), samples showing a contamination gradient (the more contaminated the sample the more negative the loading will be). Axis 2 (PC2), with 4.6% of the variation, revealed an inverse relationship between MF and TTC, the first coordinate being positive and the second coordinate being negative. The marked decline in the percentage of variation along this axis suggests that this axis can be treated as noise and not as a factor. Briefly, the PCA indicated that the FDA and TTC results were very similar to the microbiological results. However, the FDA method provided results after only 6 h, while the TTC and MF methods require more time to show measurable values (12 h and 24 h, respectively).

Regarding the sensitivity of the different methods, the bacterial detection procedure selected could have a strong impact on the classification

of seawater quality (Sousa et al., 2010). However, no study has shown, to the best of our knowledge, that the microbiological classification of beach sand samples is influenced by the microbiological measurement method. According to our results, it appears that the use of the FDA colorimetric method to quantify microorganisms in beach samples represents a sensitive, low cost and time-saving approach to the screening of beach microbiological quality. Furthermore, the detection of beach contamination by this screening method could confirm the need for subcultures and further biochemical or genomic testing to identify the presence of specific microorganisms with human pathogenic potential in beach samples. Clearly, the three analytical methods do not address parasitological contamination of the sand by, for instance, the cysts, eggs and larvae of different parasites (WHO, 2003; Sato et al., 2005; Wagener, 2005; Solo-Gabriele et al., 2016). Thus, the criteria used to classification the quality of beach water and sand for recreational purposes could be also improved by considering the parasitological aspect, which can be very important in some specific regions of the world. In this context, analysis for specific pathogens and unconventional indicators should be conducted at high-risk sites to protect human health (WHO, 2003; Solo-Gabriele et al., 2016).

5. Conclusions and perspectives

The three tested methods (MF, FDA and TTC) classified the seawater of 40 beaches in accordance with monitoring results published by FATMA (the environmental agency of Santa Catarina State, Brazil). The profile of wet beach sand contamination (in terms of location and contamination intensity) is similar to the results obtained for seawater contamination. However, since the contamination level required to change the sand quality status is high, the beaches are classified as acceptable for recreational purpose. Our results suggest that more studies must be performed in order to evaluate whether seawater contamination values could be used to classify wet beach sand. Dry beach sand contamination could be subjected to the beachgoers (and/or animals) intensity frequentation, and a ranking of beach contamination intensity could be established among samples analyzed: wet sand ≥ seawater > dry sand.

The establishment of new criteria to measure beach sand quality will lead to new demands for remedial action at some sites. The FDA screening method is a rapid and low-cost colorimetric approach that could help beach managers to evaluate the degree of microbiological contamination in small areas or large regions. It could also allow them to make rapid decisions regarding the need for remedial action and to evaluate the effectiveness of treatment applied to contaminated sand. Mechanical cleaning (e.g., sweeping and aeration by sand tilling), for instance, commonly used to eliminate visible debris in highly frequented beaches, can reduce the amount of organic matter and therefore reduce the development of microorganisms, although this practice will not eliminate them. Disinfection products could be applied to beach sand. But in this case, the effectiveness and (eco)toxicological effects must be evaluated, as well as the residual microbiological status, detecting the presence of microbes by rapid FDA analysis. Also, cultural behavior like good personal hygiene and correct waste disposal, as well as the prohibition of animals on beaches, can help in the management of the microbiological quality of beaches.

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