Estudos de
BIOLOGIA Ambiente e Diversidade

ORIGINAL PAPER

Microbiological monitoring of seawater and sand of beaches Matinhos, Caiobá e Guaratuba-PR, Brazil

Monitoramento microbiológico da água e areia das praias de

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Abstract
This research aimed to verify the health conditions of seawater and beach sand in the beaches of Matinhos, Caiobá and Guaratuba in Paraná, summer 2004/2005. Total Coliforms (TC), Escherichia coli (EC) and Enterococci (ENT) parameters were evaluated. TC, EC, ENT densities were determined by the Multiple Tube Method and estimated by the most probable number (MPN) using, in that order, chromogenic substrate (X-GAL), fluorogenic substrate MUG, and chromogenic substrate (X-GLU). Only the beaches of Matinhos (sampling point MS5) and Guaratuba (sampling point GA6) have shown bathing water quality. Dry sand presented higher contamination than wet sand, 69.4% and 54.2% respectively. Among the indicators, Enterococci was the most restrictive where 51.4% of the water and 100% of the sand (wet and dry) samples were disqualifieda
Keywords: Coliforms. Enterococci. Escherichi

Resumo
O objetivo da pesquisa foi verificar as condições sanitárias das águas do mar e das areias das praias de Matinhos, Caiobá e Guaratuba do litoral do Paraná, no verão 2004/2005. Foram avaliados os parâmetros: Coliformes totais (CT), Escherichia coli (EC) e Enterococos (ENT). A densidade de CT, EC e ENT foi determinada pela técnica de tubos múltiplos e com estimativa pelo número mais provável (NMP), respectivamente, sistema enzimático substrato cromogênico X-GAL, substrato fluorogênico MUG e substrato cromogênico X-GLU. Apenas as praias de Matinhos (MS5) e Guaratuba (GA6) apresentaram condições de balneabilidade. A areia seca apresentou contaminação superior a areia úmida, 69,4% e 54,2%, respectivamente. O parâmetro Enterococos foi o mais restritivo dos indicadores, desclassificando 51,4% e 100% das amostras de água e areias (úmida e seca), respectivamente.
***Palavras-chave**: Coliformes. Enterococos. Escherichia.*

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Introduction

Sea water that receives sewage can contaminate the beach sand by introducing bacteria, virus, and parasites (Companhia de Tecnologia de Saneamento Ambiental – CETESB, 2004).

Bathing water quality control of freshwater and seawater aim mainly to its microbiological quality. However, the concern about beach sand contamination has increased in the last few years due to improper garbage disposal, animal waste, untreated domestic sewage and pollution that are brought by rain water.

Historically, Faecal Coliform group has been used as microbiological indicator of recreational quality of water and sand. However, CONAMA (Brazilian National Environmental Council) regulation n. 274/00 (CONAMA, 2000) establishes and determines Faecal Coliforms, *Escherichia coli*, and Enterococci as main microbiological indicators of bathing water quality.

Bacteria survival in the sand is affected by numerous elements such as organic matter, humidity, temperature, sunlight, and water retention ability, among others (Wheeler Alm, Burke, & Spain, 2003) highlight that organic matter is important to water retention, aggregate formation and stability, and also to create microhabitats. These are important factors to microorganisms survival.

According to the Blue Flag Programee (2002) it is sufficient to use only dry sand analysis when monitoring microbiological quality of beach sand since the water analysis already gives information that makes wet sand analysis unnecessary.

In Portugal, there are limit values established to the microbiological quality of the sand they were elaborated by Mendes *et al.* (1993) and adopted by the European Community which use total coliforms, faecal coliforms, faecal streptococci, and *Candida* sp. as indicators.

The state of São Paulo, through CETESB (1998) (State Basic Sanitation Engineering Company) and the state of Rio de Janeiro, through its "Secretaria Municipal de Meio Ambiente" (2000) (Municipal Department of Environment Protection) (SMMA, 2000) have already analyzed the sand on the beaches. For Rio de Janeiro (SMMA, 2000) some measures were taken such as mechanical revolving of the sand resulting in an excellent sanitary quality, and also the publication of the "Resolução Municipal n. 81/2000" (Municipal regulation) establishing a maximum limit accepted of colicount for classifying sand to recreational contact.

Due to the inexistence of a survey about sanitary conditions in the beaches of Paraná state, and to the epidemiological importance of the sand designed to recreational contact, this research aimed to evaluate seawater and sand conditions, directing to generate information for establishing a national standard of the quality of the sand.

Methodology

Area description

The municipalities of Matinhos (Matinhos and Caiobá beaches) and Guaratuba, both in the coast of the state of Parana, were chosen to this research for having high inflow of people and for presenting higher potential risk to the public owing to the use of areas that are close to channels and rivers with sewage discharges.

There were selected four sampling points at Matinhos, two at Caiobá, and three at the Guaratuba beach (Table 1). In 2004, sampling dates were: 1st (Feb/29th/04), 2nd (Dec/14th/04) and 3rd (Jan/18th/05). Samples of seawater, and wet and dry sand were collected from the nine

points previously defined according to the Bulletin bathing water quality IAP (2004), which had the highest rates of contamination.

During the sampling period climatic data were monitored in two weather stations located on Antonina (Matinhos and Caiobá) and Guaratuba.

Sampling

Sampling methods of seawater collection followed the recommendation of "Instituto Ambiental do Parana-IAP" (Parana Environmental Institute) (IAP, 2004). Collecting sand was carried out in a 2 m^2 area (1 m x 1 m), in a depth of 10 cm, five subsamples with approximately 100 g of superficial wet sand and dry sand were collected and transferred to a sterilized plastic container (CETESB, 1989/L5.550) (CETESB, 1989).

Dry sand samples were collected in areas which are not reached by the tide but are visited by bathers. Wet sand samples were collected in the intermediate area between dry sand and seawater.

Table 1. Georeferencing of water and sand sampling points in Parana coast – Brazil (Instituto Ambiental do Paraná 2009)

			GPS Coordinates (m)	
Beach	Local	Point	North	East
Matinhos	Left side of Matinhos river (100 m)	MS-1	747.503	7.142,899
	Left side of Matinhos river (150 m)	MS-9	747.525	7.142,949
	Right side of Matinhos river (100 m)	MS-2	747.438	7.142,737
	Left side of the "Morro" (hill) (100 m)	MS-5	747.487	7.142,222
Caiobá	Left side of the channel of Caiobá (200 m)	CA-2	747.035	7.140,703
	Right side of the channel of Caiobá (100 m)	CA-1	746.961	7.140,491
Guaratuba	Left side of Brejatuba river (200 m)	GA-4	743.732	7.134,533
	Right side of Brejatuba river (50 m)	GA-10	743.822	7.134,426
	Left side of the "Morro do Cristo"(hill) (200 m)	GA-6	744.033	7.134,315

Microbiological and chemical analyses

Parameters of microbiological analysis of the water and the sand were performed according to APHA/AWWA/WPCF (1998). Total coliforms, *Escherichia coli* and Enterococci densities were determined by the multiple tube technique enzymatic system using chromogenic (X-GAL), fluorogenic (MUG), and chromogenic (X-GLU) substrates, respectively. Fluorocult LMX broth Merck (ref.1.106620) was used for determining total coliforms and *E. coli*, and Chromocult broth Merck (ref.11102940) for Enterococci. Chemical parameters were defined in the sand: pH, Na, P and organic carbon content following (Marques & Motta, 2003).

Data of microbiological parameters were submited to Pearson's correlation using the statistical assistance of ASSISTAT software, version 7.5 beta (Silva, 2011).

Results and discussions

Microbiological analysis of water

On the beach of Matinhos, for Total Coliforms (TC) parameter, points MS1 and MS9 showed indices higher than the standards established by CONAMA regulation n. 20/86 (5.000 NMP/100 mL) (CONAMA, 1986), Figure 1. These two sampling points are located 150 m on the left side of Matinhos river, which receives high inflow of sanitary sewage. The river discharges in the ocean, and probably influenced by the tide or current, tends to carry contaminants to the left bank of the river. Evanson e Ambrose (2006), monitoring indicators of water (TC, EC and ENT) in the Southeast coast of California (USA), reinforce that the increase

of water contaminants can be associated to the grow and/or survival of microbial population due to a change in the environmental parameters such as salinity, humidity, and nutrients increase.

In the same way, on the beach of Caiobá, the sampled points (CA1 and CA2) were within bathing water quality standards for TC. In Guaratuba, the point GA4 showed an index higher than the standards established by CONAMA regulation n. 20/86 (CONAMA, 1986), both in Feb./04 and Jan/04 data collection, whereas point GA10 showed it only in Feb./04.

According to CONAMA regulation n. 274/00 (CONAMA, 2000), regarding sea water, *E. coli* index (EC) is 800 NMP/100 mL. On the beach of Matinhos, it was verified that at points MS1 and MS9 counts were higher than the indices in all data collection dates, whereas at MS2 only in Jan/05.

At the point CA2 on the beach of Caiobá, regarding bathing water quality, values were higher than standards in all data collection dates. This point is located 200 m to the left of Caiobá channel been influenced by it. The point GA4 on the beach of Guaratuba presented higher counts than standard indices in all samplings, whereas point GA10 presented it only on Feb./04.

Enterococci (ENT) index established by CONAMA regulation n. 274/00 (CONAMA, 2000) is 100NMP/100 mL. Thus, while comparing with the data that was founded, it was observed on the beach of Matinhos (MS1 and MS9 points) that it presented a similar situation to the other indicators in all sampling, that is to say, out of bathing water quality standard, compromising seawater quality during summer season.

Similarly to the beach of Matinhos, Guaratuba showed high contamination, as the GA4 point was above standard in all samplings. The presence of contamination, mostly human caused, is explained by the proximity of the collecting points to Matinhos and Brejatuba rivers (Figure 1).

On the beach of Caiobá, the CA2 point showed indices higher than standards (CONAMA, 2000) in all evaluations, consequently, with bathing water quality restrictions. The CA1 point was within the index only on Jan/05 sampling date, as it is located 100 m to the right of Caiobá channel, not been influenced by its contamination, as it happens with the CA2 point.

Samples that exceeded the established limit on CONAMA regulation n. 20/86 (CONAMA, 1986) and 274/00 (CONAMA, 2000), regardless indicator, added to 51.4% (Figure 1). This last regulation establishes that using only one indicator (TC, EC or ENT) is enough when monitoring bathing water quality on beaches. Thereby, it was verified that from all nine monitored points, only MS5 (Matinhos) and GA6 (Guaratuba) satisfied the standard of bathing water quality over the summer of 2004/2005; moreover, on the beach of Caiobá, bathing water conditions was not verified. These points presented the highest contamination indices, therefore, mainly due to the high occurrence of precipitation in Matinhos and Caiobá which was 322 (Feb/04); 417.6 mm (Dec/04) and 345.6 mm (Jan/05) and the air temperature was 23.4°C(Feb/04), 22,6 (Dec/04)and 23.7°C (Jan/05). In Guaratuba, precipitation was 257.8 (Feb./04); 314.6 mm (Dec./04) and 304.6 mm (Jan/05) and the air temperature was 24.4°C (Feb/04), 22,6 (Dec/04) and 24.0°C (Jan/05) according with SIMEPAR (2009). Krogh and Scanes (1996) had also observed that the rain was the most influencing factor on faecal coliforms and faecal streptococci levels in seawater on beaches in Sydney, Australia.

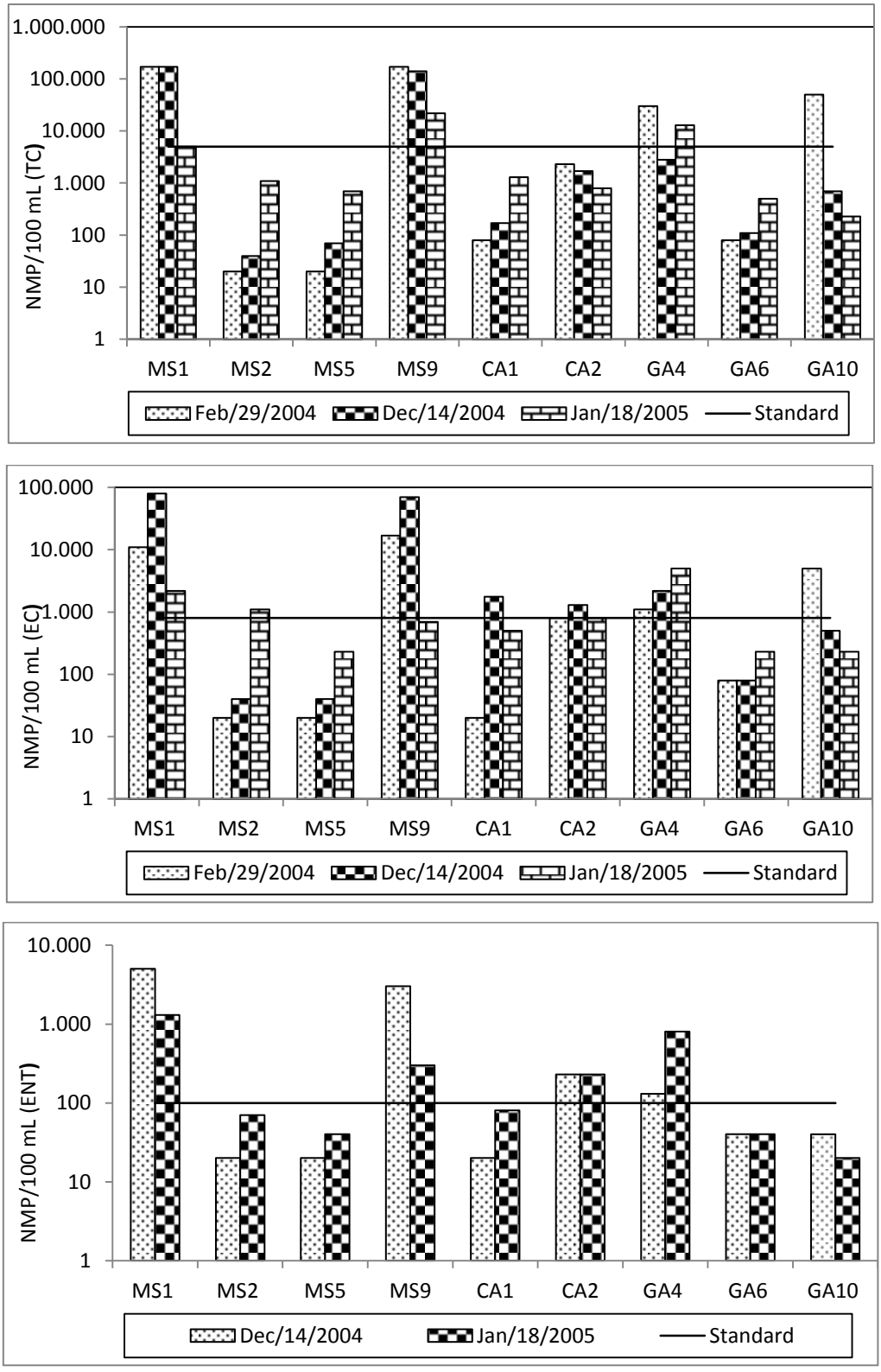


Figure 1. Total Coliforms (TC), Escherichia coli (EC) and Enterococci (ENT) densities in the seawater, wet sand and dry sand of the beache of Matinhos (MS1, MS2, MS5, and MS9) summer 2004/2005. Source: survey data

The obtained data in this study are in support of the results of Oliveira and Pinhata (2008) in Santos (town in São Paulo state), where they observed that the impact of organic load

discharged in receiving waterbodies (Sato et al., 2005) is aggravated by the amount of rain-fall during the summer.

In the seawater environment, Pearson's correlation coefficients showed significance (p <0.05) in the TC x EC (r = 0.85*), EC x ENT (r = 0.93*), and TC x ENT (r = 0.94*) parameters.

The TC x EC correlation was also observed by Noble, Moore, Leecaster, McGee and Weisberg (2003) on two beaches of California-USA, when they obtained (r = 0.83-0.86). Correlations were expected for all parameters, since TC group includes *E. coli* specie. On the other hand, Shibata, Solo-Gabriele, Fleming and Elmir (2004) didn't find significant variation among indicators while evaluating the relation between indicators and possible sources of contamination, except for total coliforms, even with significant change in the physical-chemical parameters in the monitored period.

Microbiological analyses of the sand

Considering that the type and the intensity of the contact between man and microorganisms is higher in the seawater than it is in the sand (wet and dry), with man presenting greater risks, the use of the same indices applied for seawater would represent an excessive restriction. However, according to the values obtained from the microbiological parameters in the sand and to the lack of an epidemiologic survey correlating to the risks to human health, it was opt to elaborate a proposal, called *proposta do Paraná* (Parana proposal), which considers two times the established indices for water (Table 2).

Wet sand

In relation to Parana proposal (Table 2) at the point MS1 (in Feb./04) and in all data collection dates at point MS9, TC parameter on Matinhos beach (Figure 3) presented higher values, whereas only point CA1 (in Feb/04 and Jan/05) presented it on Caiobá beach. The monitored points on the beach of Guaratuba showed the lowest indices in comparison to the other beaches. Higher indices were found in the data collection date of Dec./04 and Feb./04 at points GA4 and GA10.

A municipal regulation of Rio de Janeiro (RMRJ) n. 81/2000 (SMMA, 2000) establishes the maximum limit for total Coliforms of 30.000 NMP/100g of sand. In this sense, points MS1 and MS9 on the beach of Matinhos exceeded the established values only in Feb/04 and Guaratuba in Dec/04. Point GA6 was within limits for Parana proposal and RMRJ n. 81/2000 (SMMA, 2000).

Table 2. Tolerance limit proposal for the microbiological quality of the sand for recreational contact on the beaches of Parana state (Parana Proposal) Suggested for been at Conama regulation 274/00; ** Data transformed into +1; ***Sampling can be made monthly during Summer and bimonthly in the other seasons

Parameter	Maximum limit (NMP/100 g)	Maximum limit transformed** (NMP/100 g)	Sampling frequency***
Total Coliforms	10.000	101	monthly
Faecal Coliforms*	2.000	46	monthly
Escherichia coli	1600	41	monthly
Enterococci	200	15	monthly

Source: survey data

On the beach of Matinhos at the points MS1 and MS9, in the evaluation of *E. coli* (EC) density, values were higher than those on Parana proposal (Table 1) in all data collection dates. The point MS2 (Figure 1) was above limit in Dec/04 and Jan/05, although at point MS5 densities founded were lower than the proposed limit. The points of Caiobá beach (CA1 and CA2) haven't presented contamination only in the Dec/04 data collection date, in the others areas they were considered inappropriate for recreational purposes. On the beach of Guaratuba, points GA4 (Dec/04 and Jan/05) and GA 10 (Dec/04) presented superior densities, however point GA6 was appropriate for recreation.

In all beaches and seasons, for Enterococci (ENT) parameter the counts were above the proposed limit for the state of Parana (Table 2). These results are in accordance with a publication of Wheeler Alm et al. (2003) that observed a high presence of ENT in six beaches of the American coast, in the wave-washed zone between the sand and recreational water, yet verified higher densities of this indicator in wet sand when comparing to *E. coli.*

Correlation analysis between water vs wet sand environment shows that TC vs TC (r = 0.52*) and TC vs EC (r = 0.69*) interactions were significant (p <0.05), which was expected, since TC population in the water is the one going to originate the population in the wet sand. It is important to emphasize that *E. coli* dissemination might occur in the other way around, promoted by the wind, that is to say, from the sand to the seawater, increasing its population in this environment (Williamson et al., 2004).

On the research carried out by Shibata et al. (2004) working with wet sand, it was observed that indicator concentration (Enterococci, *Escherichia coli*, Faecal coliforms, Total coliforms and *C. perfringens*) varied significantly till the beach boarder and that higher concentrations were observed in the high tide, when the waved-washed zone was submersed. The results suggest that this zone can serve as inoculum source of microorganisms animals, and possibly for including human being, animals, and possibly the survival and growth of these microorganisms, due to the unique environmental conditions found on this area.

While working with faecal indicators, Wheeler Alm et al. (2003) found a correlation (r = 0. 60) between *E. coli* count in the water and in the 5 cm of superficial sand. In general, Enterococci were more abundant in the 5-10 cm layer and *E. coli* in 0-5 cm. These data showed that wet sand constitutes a reservoir of faecal indicator bacteria. Evaluating the survival of *E.coli* and Enterococci in the sand of the beach in relation to seawater, Hartz et al. (2008) highlight that the sand can be an important reservoir of faecal microorganisms metabolically active.

Dry sand

On the beach of Matinhos, regarding TC parameter, the point MS9 (Figure 3) was within the proposed standard (Table 2) and the RMRJ n. 81/2000 regulation (SMMA, 2000), whereas the points MS5 (Feb/04 and Dec/04 data collection dates) and MS1 (Jan/05) reached higher indices. On the beach of Caiobá, at the point CA1, indices were higher in all data collecting dates while at point CA2 it happened only in Feb/04 and Jan/04. In Guaratuba, points GA4, GA6, and GA10 exceeded the established limits.

At the points MS5, MS1, MS2 and MS9, the parameter EC was higher in all data collection dates, although the last three presented it only in Jan/05. On the beach of Caiobá (points CA1 and CA2) on the data collection of Feb/04 and Jan/05, and on Guaratuba beach at the point GA4, the values founded surpassed the limits at the points GA6 and GA10 in Dec/04 and Jan/05.

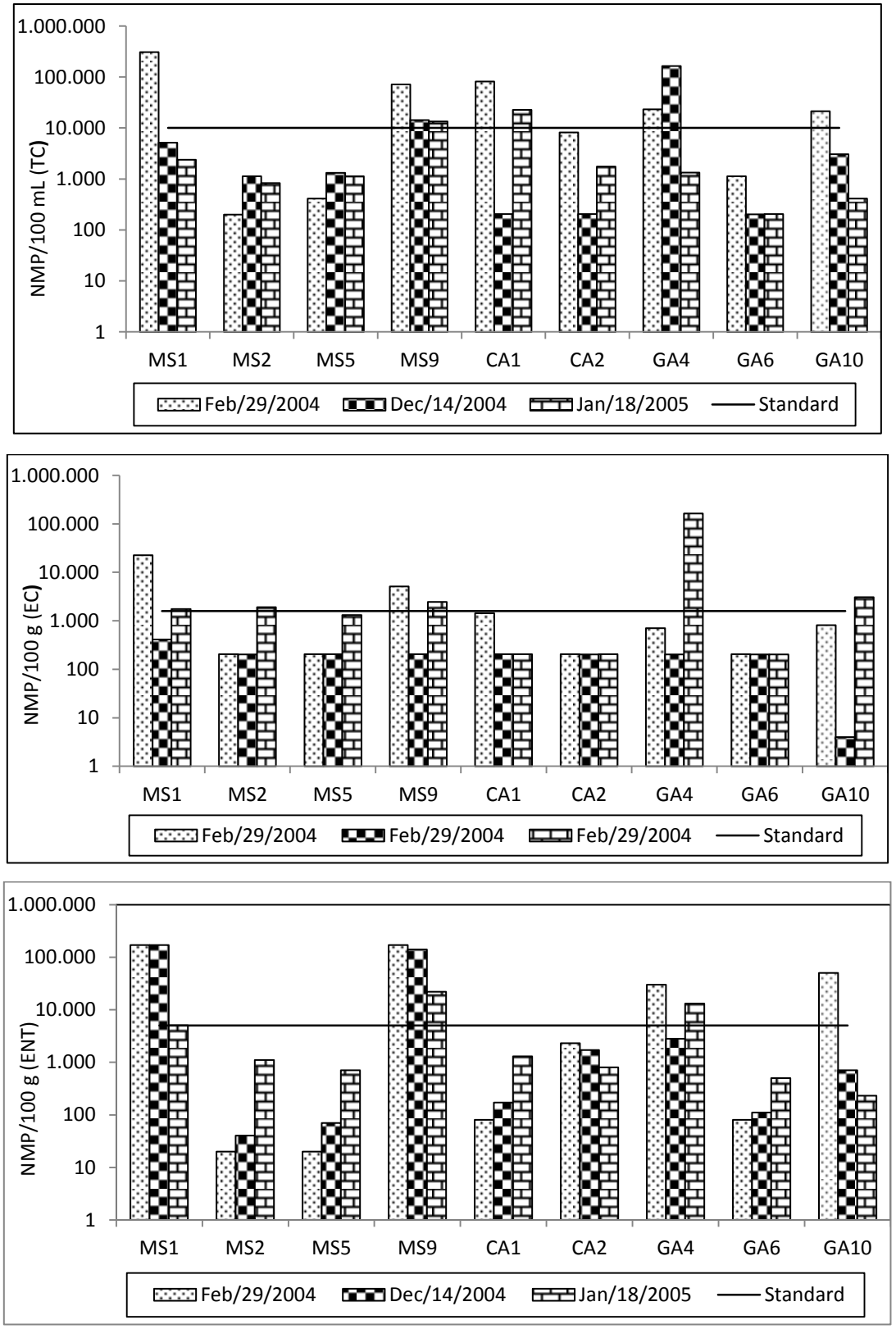
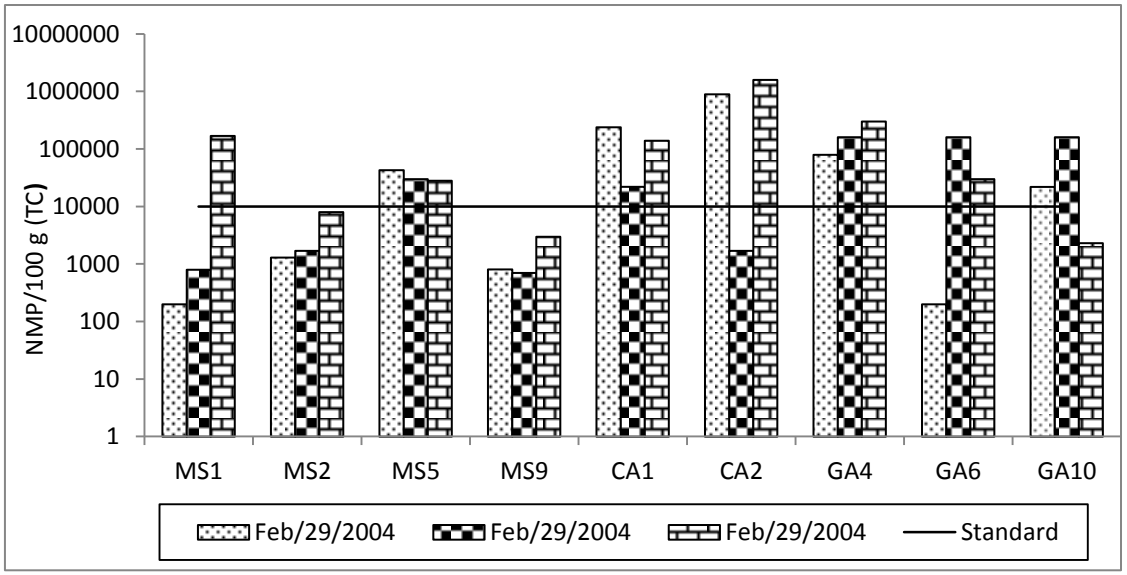


Figure 2. Total Coliforms (TC), Escherichia coli (EC) and Enterococci (ENT) densities in the seawater, wet sand and dry sand of the beache of Caiobá (CA1 and CA2) summer 2004/2005. Source: survey data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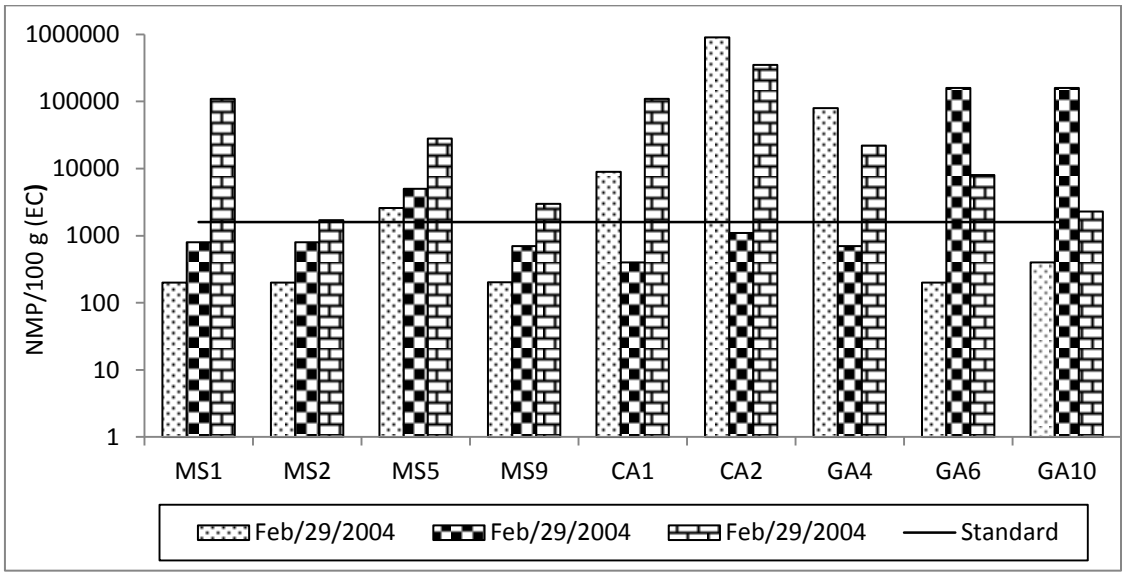
Standard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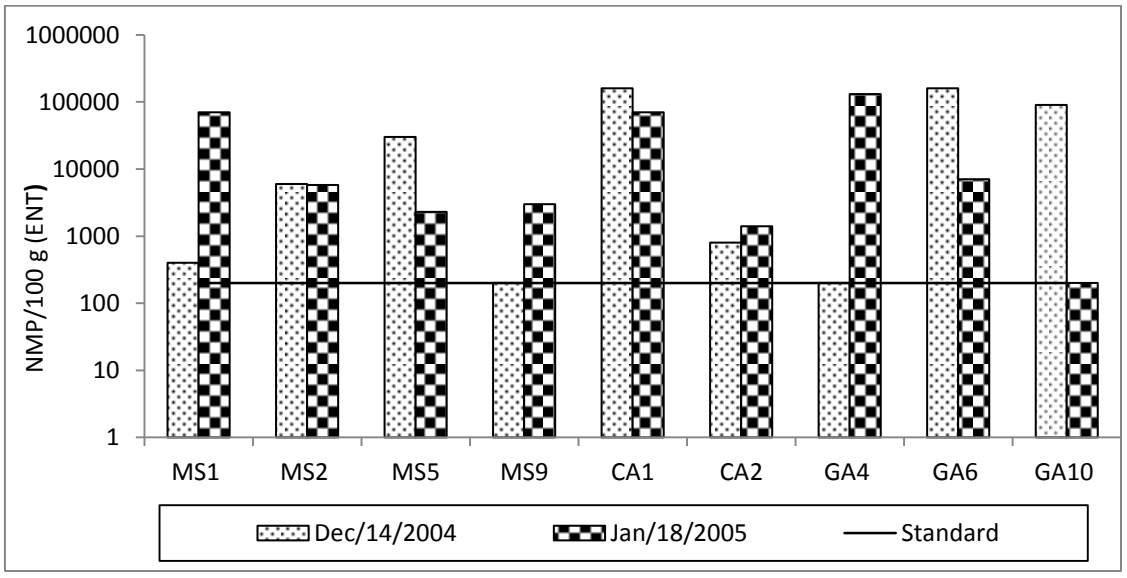
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GA4
GA6
GA10
Dec/14/2004
Jan/18/2005
Standard

Figure 3. Total Coliforms (TC), Escherichia coli (EC) and Enterococci (ENT) densities in the seawater, wet sand and dry sand of the beache of Guaratuba (GA4, GA6 and GA10), summer 2004/2005. Source: survey data

Regarding Enterococci (ENT), on the beach of Matinhos (points MS1, MS2, MS5, and MS9) and Caiobá (points CA1 and CA2), indices were higher than those established by Parana proposal. Similarly to what was observed on Matinhos beach, two points (CA1 and CA2) in Caiobá were also higher than the index established by Parana proposal. It is important to highlight that within all sampled points on the beach of Guaratuba, the highest gross value was 160.000 NMP/100g of dry sand, which represents a value 800 times greater than the required limit.

The application of the required limit (Table 2) showed that all monitored beaches presented ENT indices higher, that is to say, contamination, which makes them inappropriate to recreational contact. In the environment of wet and dry sand, it was also verified that pH conditions (5.9 – 6.7) were favorable for the survival of analyzed bacteria, since extremes of acidity and alkalinity (6 < pH $CaCl_2$> 8) are unfavorable (Estrada, Aller, Aller, Gómez, & Morán, 2004).

The results showed that this group of bacteria survives and grows on the dry sand environment different sources of pollution (Oliveira & Pinhata, 2008). Moreover, favorable abiotic factors such as temperature, humidity, pH, and organic matter among others, indicate the phenomenon of bioaccumulation, as it was established by Mancini et al. (2005) on the beaches of Italy; Elmanama, Fahd, Afifi, Abdallah and Bahr (2005) on the beach of Gaza in Israel; and Oliveira and Pinhata (2008) on the beach of Gonzaguinha – Santos, state of São Paulo, Brazil.

It was verified significant correlation ($p < 0.05$) between water x dry sand environment among EC x TC parameters ($r = 0.71$*), and on wet sand x dry sand environment between EC x TC ($r = 0.69$*). Data obtained in this research are contrary to "Associação Bandeira Azul da Europa" (2002) (Blue Flag Programee), which didn't find correlation among the environment previously mentioned, for TC, EC and ENT parameters on the beaches of Portugal. No significant correlation was noted among microbiological (TC, EC and ENT) and chemical (pH, P, Na, C) parameters in the sand, wet and dry, showing that the higher bacterial survival is not exclusively associated to the availability of nutrients, especially on dry sand. This fact is confirmed for the incidence of samples with values that exceed the limits of the indicators (65.3%) determined at Parana proposal (Figure 3). Nevertheless, factors as anthropogenic influence such as remains of food, fruits, vegetables, and the presence of domestic animal excrement must be evaluated.

Conclusion

Only the beaches of Matinhos (point MS5) and Guaratuba (point GA6) showed bathing water quality, whereas the beach of Caiobá (points CA1 and CA2) showed to be inappropriate. Dry sand showed contamination higher than wet sand. Enterococci parameter (NMP/100 mL of water) or (NMP/100 g of sand) presented to be the most restrictive indicator. It is possible to make a preliminary proposal to the environmental agencies to evaluate the microbiological conditions of the dry sand on the beaches of Parana with the data obtained.

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