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BACTERIAL INDICATORS OF BATHING WATER QUALITY IN THE DOURO ESTUARY AND PORTO COASTAL FRONT

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Dissertação de Mestrado em Ciências do Mar – Recursos Marinhos

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Dissertação de Candidatura ao grau de Mestre em Ciências da Ciências do Mar – Recursos Marinhos, submetida ao Instituto de Ciências Biomédicas de Abel Salazar da Universidade do Porto.

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Resumo

A qualidade microbiológica das águas balneares surge como um dos principais requisitos para a utilização das zonas balneares. Apesar da nova Directiva relativa à qualidade das águas balneares (2006/7/EC) pressupor uma alteração drástica no que diz respeito à monitorização, métodos e práticas de análise, várias questões permanecem por responder. A variabilidade horária e de maré foi investigada em quatro praias urbanas do Porto (Noroeste de Portugal), de Junho a Agosto de 2007. Amostras horárias foram recolhidas e os parâmetros físico-químicos foram medidos. Durante o período de estudo, verificaram-se variações mensais nos indicadores fecais apenas nas praias mais poluídas. Apesar de se ter observado uma tendência para a existência de uma maior concentração de indicadores fecais durante o período matinal, apenas Gondarém, a praia menos poluída, apresentou uma contaminação significativamente maior durante a manhã ($p<0.01$). O estado da maré também não influenciou a concentração horária das bactérias fecais. Para melhor compreender a distribuição espacial dos indicadores bacterianos na frente marítima das praias, foram realizadas campanhas de amostragem durante os Verões de 2008 e 2009, cobrindo uma área de aproximadamente 10 km^2. Os dados relativos aos indicadores bacterianos e parâmetros físico-químicos foram mapeados recorrendo a um Sistema de Informação Geográfica, ressaltando a influência do Rio Douro. Os resultados demonstraram a contribuição negativa dos rios (Douro e Leça) e pequenas ribeiras poluídas na qualidade da água das praias, enfatizando a necessidade de optimização dos protocolos de amostragem para a monitorização da qualidade das águas balneares.

Através de um método de análise estatística multivariada foi estabelecida uma relação entre os indicadores fecais e os parâmetros físico-químicos, de maneira a prever as concentrações bacterianas em cada praia. O coeficiente de determinação máximo obtido foi observado nas duas praias mais poluídas, onde 65% e 70% da variabilidade dos coliformes fecais poderia ser definida utilizando 48.3% e 64.9% da variabilidade dos parâmetros abióticos.

Abstract

The microbiological quality of bathing water arises as a major issue for the use of bathing areas. Although the present Bathing Directive (2006/7/EC) broke new ground in what monitoring, harmonized methods and practices of analysis are concerned, several questions remained unanswered. In this vein, diel and tidal variability was investigated in four urban beaches of Porto (NW Portugal), from June until August of the 2007. Hourly samples for microbiological analyses were collected, as well as measurements of water physical-chemical characteristics. During the study period, monthly variations of fecal indicators were only observed in the most polluted beaches. Despite the general trend for higher fecal bacteria abundance during the morning period in all four beaches, only the less polluted beach exhibited significantly higher contamination during the morning ($p<0.01$). Moreover, tidal phase did not influence the diel pattern of bacteria. In order to ascertain the spatial distribution of indicator bacteria of the urban beachfront, surveys were organized during the 2008 and 2009 covering a 10 km^2 area. Bacteriological and physical and chemical data were mapped using GIS techniques. The influence of the river Douro, located at one of the end-members of the studied area was evident, as well as the contribution of another polluted river (Leça) and small streams on the water quality of urban beaches, calling the attention to the optimization of sampling protocols for monitoring the water quality of bathing waters.

A relationship between fecal bacteria and water physical-chemical characteristics was established using a multivariate statistical method approach in order to predict bacteria concentrations in each beach. The maximum predictive success (R^2) was observed in the most polluted beaches, were 65% and 70% of fecal coliforms variation could be defined, using 48.3% and 64.9% of the variation in the abiotic parameters studied.

Contents

List of Figures

List of Tables

Abbreviations

DL 74/90 – Decreto-Lei nº 74/90

DL 236/98 – Decreto-Lei nº 236/98

DL 135/2009 - Decreto-Lei nº 135/2009

EU – European Union

FC – Fecal coliforms

FE – Fecal enterococci

FEE - Foundation for Education and Environment

GIS – Geographic Information System

GV – Guideline values

MV - Mandatory values

NW - Northwest

PCA – Principal component analysis

PC – Principal component

SE - Southeast

1. Introduction

A bathing water is any element of surface water, namely inland, transitional and coastal waters, where the competent authority expects a large number of people to bathe and has not imposed a permanent bathing prohibition, or issued permanent advice against bathing (Directive 2006/7/EC).

Coastal beaches are located in the interface land/sea and the land activities are major sources of pollution in the marine environment. The coastline is a major element in the geographic, recreational, commercial and ecological fabric of many countries and provides major destinations for local, national and international tourists. About 60 percent (3.6 billion) of the world population lives within 60 kilometers of the coast. According to the United Nations Environment Program (UNEP), this proportion will rise to 75 percent (6.4 billion) within three decades - nearly a billion more people than the current global population (UN, 2010). Urban beaches are, therefore, of particular concern once they face a wide variety of stressors affecting both the ecosystem and human health and, once they tend to be very popular among the urban beachgoers due the proximity and often the lack of other alternatives.

According to the United Nations Convention on the Law of the Sea marine pollution is the “introduction of man, directly or indirectly, of substances or energy into the marine environment (including estuaries) resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fishing, impairment of quality for use of sea water, and reduction of amenities". Water quality should, therefore, be protected, defended, managed and treated in order to preserve and improve the quality of the environment and to protect human health (Directive 2006/7/CE). The most important sources of pollution are the inputs of domestic, industrial and agricultural effluents, being the domestic sewage discharges the ones that cause most health concern (Bartram and Rees, 2000). Discharges may occur regularly or exceptionally, through unregulated private discharges or storm water. The latter, negatively affects water quality since excess water drags animal feces and other bacterial sources that have been deposited on land between storms and infiltrates on the sewage transmission infrastructure causing leaks (Ackerman and Weisberg, 2003). The diffuse outputs of pollution sources are more difficult to predict and the effect of the overall discharges is dependent on both quantity and composition of the effluent and on the receiving capacity of waters to accept that effluent (Bartram and Rees, 2000).

1.1. Microbiological quality of bathing waters

The new Directive 2006/7/EC concerning the management of bathing water quality, defines pollution as “the presence of microbiological contamination or other organisms or waste affecting bathing water quality and presenting a risk to bathers' health”. Therefore, the majority of research in the field of water quality and health has focused on microbiologic hazards (Pond, 2005).

One of the most important aspects of aquatic microbiology is the fact that human diseases can be transmitted through water. Bathing water generally contains pathogenic and non-pathogenic microorganisms that are ubiquitous populations of the water and free living pathogens that derive from sewage effluents, farming activities and livestock, industrial processes, domestic animals and wildlife and populations using water for recreation (WHO, 2003).

The number of waterborne pathogenic microorganisms present on water and the physical conditions of the exposed individuals determines if diseases are caused, although exposure does not always lead to infection nor does infection result in clinical illness (Pond, 2005). Epidemiological studies have shown a number of adverse outcomes to human health associated with fecal polluted bathing water, despite the fact that infections and illness originated from bathing water contact are difficult to detect through routine surveillance systems because they are generally mild and even when illness is more severe, it still may be difficult to attribute to water exposure (WHO, 2003). The total yearly global impact of infectious human diseases related to pathogenic microorganisms from land- based sea pollution was estimated to lead to an economic loss of 12 thousand million US dollars (Shuval, 2003).

Although most illnesses contracted trough bathing water contact are mild, hazard of more severe diseases exists, since bacteria may cause typhoid, cholera and leptospirosis; viruses can cause aseptic meningitis, encephalitis, poliomyelitis, hepatitis, myocarditis and diabetes; and protozoa can cause primary amoebic meningoencephalitis and Schistosomiasis (Pond, 2005).

Available studies suggest that the more frequent adverse health effects associated to contaminated water are enteric illness, such as gastroenteritis. Transmission of pathogens that can cause gastroenteritis is biologically plausible and is analogous to waterborne disease transmission in drinking water. The association has been repeatedly reported in epidemiological studies, including studies demonstrating a dose–response relationship (Prüss, 1998).

The cause effect relationship between fecal pollution and acute febrile respiratory illness (AFRI) and respiratory diseases in general is biological plausible, since significant dose response relationship are well documented (Fleisher *et al*., 1996). However, when compared to gastroenteritis, the probability of contracting acute febrile respiratory illness is smaller (WHO, 2003).

Associations between ear infections and microbiological fecal pollution were also observed (Fleisher *et al*., 1996), and the cause-effect relationship between these has biological plausibility, since, ear problems is greatly elevated in bathers over non-bathers even after exposure to water with few fecal index organisms (van Asperen *et al*., 1995). Eye contact with bathing waters, regardless of water quality, compromises the immune defenses of the eyes, but there are no studies that evidence an increase of eye diseases associated with water pollution (Prüss, 1998).

Relation between pollution and skin symptoms is unclear, since some studies associate microbiological water quality to skin symptoms (Ferley et al., 1989), and in controlled studies that association is not reported (WHO, 2003).

The more severe health outcomes may occur among bathers swimming in polluted water who are short-term visitors from regions with low endemic disease incidence (WHO, 2003). Children are a special group since they have greater opportunities for exposure: they tend to be more frequent users of bathing waters and for longer periods of time compared to older age groups increasing the risk of accidental ingestion (Pond, 2005). The decline in microbiological water quality may also pose increased risks to health of the elderly and immuno-compromised being these more susceptible to pathogenic organisms that occur in the environment (WHO, 2003).

There are many unanswered questions regarding the severity and frequency of illness associated with bathing water use. The difficulties associated with attributing an infection to bathing water use are numerous and the majority of research in this field has focused on the minor symptoms, and other more serious illnesses that could result from that use, have not yet been investigated to any great extent. However, the acute diseases attributable to waterborne pathogens and their epidemiology have been well described, but the sequelae that can result from these diseases have not. Assessing potential sequelae of waterborne infections is a critical part of microbial risk assessment and the formulation of public policy (Pond, 2005).

1.2. Assessing microbiological bathing water quality

Testing directly for the presence of a large variety of water pathogens is very difficult and time-consuming, therefore fecal contamination indicators, like fecal coliforms and fecal enterococci, are used.

Fecal coliforms are bacteria capable of producing gas from lactose at high temperatures (44.5ºC). They were defined as a group in the early of the 60 years (Clark and Kabler, 1964). In the sanitary point of view they are more reliable indicator than total coliforms, which are a heterogeneous group widespread in nature with members from other sources than fecal (Bordalo, 1991). On the other hand, about 80% of fecal coliforms are *Escherichia coli* (Bordalo, 1994).

Fecal coliforms include genera like Klebsiella and Escherichia (Dufour, 1977). *E. coli* is the only biotype of the family Enterobacteriaceae that is always of fecal origin (Bonde, 1977; Hardina and Fujioka, 1991). On the other hand, several studies denote limitations to fecal coliforms and *E. coli* as ideal fecal indicators or pathogenic organism indicators. Their main disadvantages are their presence in environments without fecal contamination (Hazen and Toranzos, 1990; Hardina and Fujioka, 1991). Also, their low survival when compared with other fecal pathogenic are main disadvantages (Borrego *et al*., 1983; Cornax *et al*., 1990)

Fecal enterococci (streptococci) have been also used as an index of fecal pollution in bathing water. The group contains species of two genera—Enterococcus and Streptococcus (Holt *et al*., 1993) and, although several species of both genera are included under the term enterococci (Leclerc *et al*., 1996), the species most predominant in the polluted aquatic environments are *Enterococcus faecalis*, *E. faecium* and *E. durans* (Volterra *et al*., 1986; Sinton and Donnison, 1994; Audicana *et al*., 1995). The term includes all the species described as members of the genus *Enterococcus* that fulfill the following criteria: growth at 10 °C and 45 °C, resistance at 60 °C for 30 min, growth at pH 9.6 and at 6.5% NaCl, and the ability to reduce 0.1% methylene blue. Since the most common environmental species fulfill these criteria, in practice the terms fecal streptococci, enterococci, intestinal enterococci and *Enterococcus* group may refer to the same microorganisms. In order to allow standardization, the International Organization for Standardization has defined the intestinal enterococci as the appropriate subgroup of the fecal streptococci to monitor (WHO, 2003).

When comparing both fecal indicators, enterococci present higher resistance to environmental conditions. The differential die-off of fecal enterococci in marine and freshwater environments is not as great as fecal coliforms die-off (Hanes and Fragala, 1967; Chamberlain and Mitchell, 1978), and the rate of die-off of enterococci does not increase as the intensity of sunlight increases, as it happens with fecal coliforms. Enterococci survival rate is higher even when their initial number is smaller than fecal coliforms, sugesting that fecal enterococci are better indicators (Bordalo *et al*., 2002).

1.3. Legislation

The main concern of water quality standards has been the control and minimization of the pollution, either accidental or chronic that negatively affects bathing waters and though public health. Portuguese quality management of bathing waters was performed, until the end of 2009 bathing season, according to the requirements of the Council Directive of 8 December 1975 concerning the quality of bathing water (76/160/EEC), transposed in 1990 into the Portuguese legal criteria (DL 74/90) and revised in 1998 (DL 236/98). The above mentioned Directive was one of the first pieces of European Union (EU) environmental legislation and established the standards applicable to the classification of bathing waters, as well as the standards concerning the compliance of inland or coastal bathing water quality and their sanitary inspection. It reflected the knowledge and experience of the 70s and defined minimum quality standards that bathing waters should comply: physical-chemical and microbiological parameters, mandatory values (MV) and guideline values (GV) for those parameters, sampling minimum frequency and analysis methods for water inspection (EEC, 1976). Physical parameters consisted in pH, color, transparency, dissolved oxygen, ammonia, nitrogen Kjeldahl, mineral oils, surface-active substances reacting with methylene blue, phenols, pesticides, heavy metals, nitrates and phosphates, cyanides, residues and floating materials (EEC, 1976). Microbiological parameters included total coliforms, fecal coliforms, fecal streptococci, salmonella, and entero viruses. Competent authorities of each Member State were responsible by the implementation and management of monitoring programs in the designated bathing areas. Sampling started two weeks before the beginning of the bathing season and proceeded during the bathing season at least every two weeks. Classification of bathing water, obtained from the results of parametric control, was divided in three levels (Table 1).

Table 1. Quality levels for bathing water quality assessement.

Good	If 80% of the samples do not exceed the GV
Sufficient	If 95% of the samples do not exceed the MV.
Bad	If more than 5% of the samples exceed the MV.

In general, bathing water quality has improved since the implementation of the first EU legislation, over thirty years ago. Notwithstanding, science, technology and management evolved since the 70s and in 2002 the EU promoted the revision of the Directive concerning the quality of bathing water, one of the drivers for the focused implementation of the Water Framework Directive. One of the main criticisms to the old Directive was that the issue of bathing water quality was just a matter of ‘product control’, when it should be real quality management and quality assurance (COM, 2000). Besides this critique, others were pointed: parameters were outdated and others were no longer relevant, water monitoring was done only for compliance checking and not in order to gain a better understanding of bathing waters, the Directive did not specify analysis methods, so laboratories have used a variety of methods and the results were not fully comparable, microbiological analysis required considerable time which meant that, in case the water sample was confirmed to be non-compliant, any (re)action to address that non-compliance would be too late and people might have been exposed to pollution (COM, 2000).

The new Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC, implied a drastic change in bathing water management. One of the most important aspects of this new approach is the execution of a bathing water profile, which consists in a description of all the physical, geographical and hidrological characteristics of the bathing water, in the identification and assessment of causes of pollution and in the risk of contamination associated with the objective of establishing management measures to prevent, reduce or eliminate pollution causes. The new Directive establishes the analysis of two parameters only (fecal enteroccoci and *E. coli*) to meet the requirements of monitoring and assessing the quality of bathing waters, as well as their classifications, instead of the previously nineteen parameters required. Cyanobacteria, macro-algae and phytoplankton can be included in the monitored parameters if the water profile indicates a potential for proliferation of the latter (EC, 2006).

Bathing water quality assessments are carried out after the end of each bathing season on the basis of the set of bathing water quality data compiled in relation to that bathing season and the three preceding bathing seasons. As a result, bathing water shall be classified in accordance with specific criteria in four quality levels: poor, sufficient, good, or excellent (Table 2). Until the end of 2015, all EU State Members must achieve the “sufficient” classification. For each bathing water classified as ‘poor’, management measures should be taken, including bathing prohibition or advice against bathing, with a view to preventing bathers' exposure to pollution, and adequate measures to prevent, reduce or eliminate the pollution causes (EC, 2006).

Table 2. Quality levels for bathing water classification according to Directive 2006/7/EC.

	Coastal and transitional waters	
	Fecal enteroccoci (cfu 100 ml^{-1})	*Escherichia coli* (cfu 100 ml^{-1})
Excellent	MV = 100	MV = 250
	If 95% of the samples do not exceed the mandatory values (MV)	
Good	MV = 200	MV = 500
	If 95% of the samples do not exceed the mandatory values (MV)	
Sufficient	MV = 185	MV = 500
	If 90% of the samples do not exceed the mandatory values (MV)	
Poor	If in 90% of the samples, values exceed MV referred in “Sufficient”	

Information related to bathing water classification, description of the bathing water and occasional pollution should be disseminated and promptly made available to the public during bathing season in an easily accessible place in the near vicinity of each bathing water by appropriate media and technologies, including the Internet (EC, 2006).

Directive 2006/7/EC brought, therefore, a new insight of bathing water quality management beyond just monitoring: it requires pro-active measures in order to reduce pollution (including short-term incidents) and associated risks, which, in turn, implies a full understanding of the bathing area, and public information in near real-time. It was transposed to Portuguese law framework by Decreto-Lei nº 135/2009 of 3 June (DL 135/2009), to be applied in the 2010 bathing season.

1.3.1. Beach grading/award schemes

One important tool used by associations and governments to enhance the public capacity for informed personal choice is beach grading or award schemes. In Europe, the Foundation for Education and Environment (FEE), a private institution, has attributed a quality label (“Blue Flag”) to beaches and marinas. Blue Flag works towards sustainable development through publicly awarding sites that meet strict criteria dealing with water quality, environmental education and information, environmental management, and safety and other services (FEE, 2010). Besides international programs, many countries have their own equivalent programs, since this award schemes can have a large influence on tourism and, as a result, are generally seen as desirable by local authorities and agencies responsible for tourism (WHO, 2003). Their main objectives are giving consumers information about water quality so that they can make informed choices and assess risks when bathing in coastal waters, advise businesses that operate nearby and that want to reduce risks caused by adverse publicity about poor water quality; and help resort managers and local authorities that wish to ensure that there are common standards and a common system for measuring those standards (Nelson et al., 1999).

1.4. Objectives

Although the new legislation establishes water bathing profiles creation in order to indentify all potential sources of pollution that may affect bathing waters and the health of the bather, there are still several problems, mainly related to the sampling process that not contemplates dial and tidal variability and, thus, are often left out when developing coastal water monitoring programs. Therefore, the main goal of the present work was to ascertain and assess the influence of the end part of urban water cycle on coastal bathing water quality of a highly urbanized area located between two estuaries. In order to accomplish this goal, there were specific objectives:

- To optimize sampling protocols for monitoring water quality, investigating diel and tidal variability in four beaches during the bathing season most crowded months.
- Evaluate spatial and temporal distribution patterns of indicator bacteria on the urban beach front during two bathing seasons and to assess polluted rivers and small streams contribution and influence on bathing water quality employing a geographic information system (GIS).
- Establish a relationship between fecal bacteria and water physical-chemical characteristics using a multivariate statistical method approach in order to predict bacteria concentrations.

2. Material and Methods

2.1. Study area

This study was conducted in Porto and Matosinhos, two neighbor cities located in the northwest (NW) Portugal that are highly dense urban areas and therefore more likely to increase the microbial contamination entering their costal front, but also very popular among the locals.

The study area is approximately 4.5 km long and is delimited by the mouth of two rivers, the Douro River in the south, which watershed is the largest in the Iberian Peninsula, and the Leça River, in the north. Besides these two major rivers, four small streams and sixteen-rain water outlets drained directly to the coastal front during the studied period. As a result of the lack of proper sewage infrastructures in several parts of both cities, untreated domestic wastewater reaches the coastal zone through the different waterways. Since 2007, several of these streams and channels have been intercepted and diverted, during bathing season, to a wastewater treatment plant in order to improve bathing water quality.

2.2. Sampling Strategy

During a three years period, two sampling efforts were conducted in Porto/Matosinhos coastal front.

Beaches

In a first phase, diel and tidal variability was evaluated in four beaches of the two cities (Pastoras, Gondarém, Castelo do Queijo and Matosinhos beach, from south to north, respectively) from June to August 2007 (Figure 1). Once a month, on each beach, samples were collected hourly during eleven hours in similar tidal conditions and always at the same location. Samples were taken in a location with one meter deep water column and at 30 centimeters below the water surface, to avoid the upper layer affected by ultraviolet radiation. Samples were retrieved against the current in order to prevent accidental contamination by the operator. Immediately after collection, they were stored in cool boxes to conserve them at low temperatures and protect from exposure to direct sunlight (CE, 2006). Water quality analysis was carried out in the middle and the end of the day (no later than 6 hours after collection).

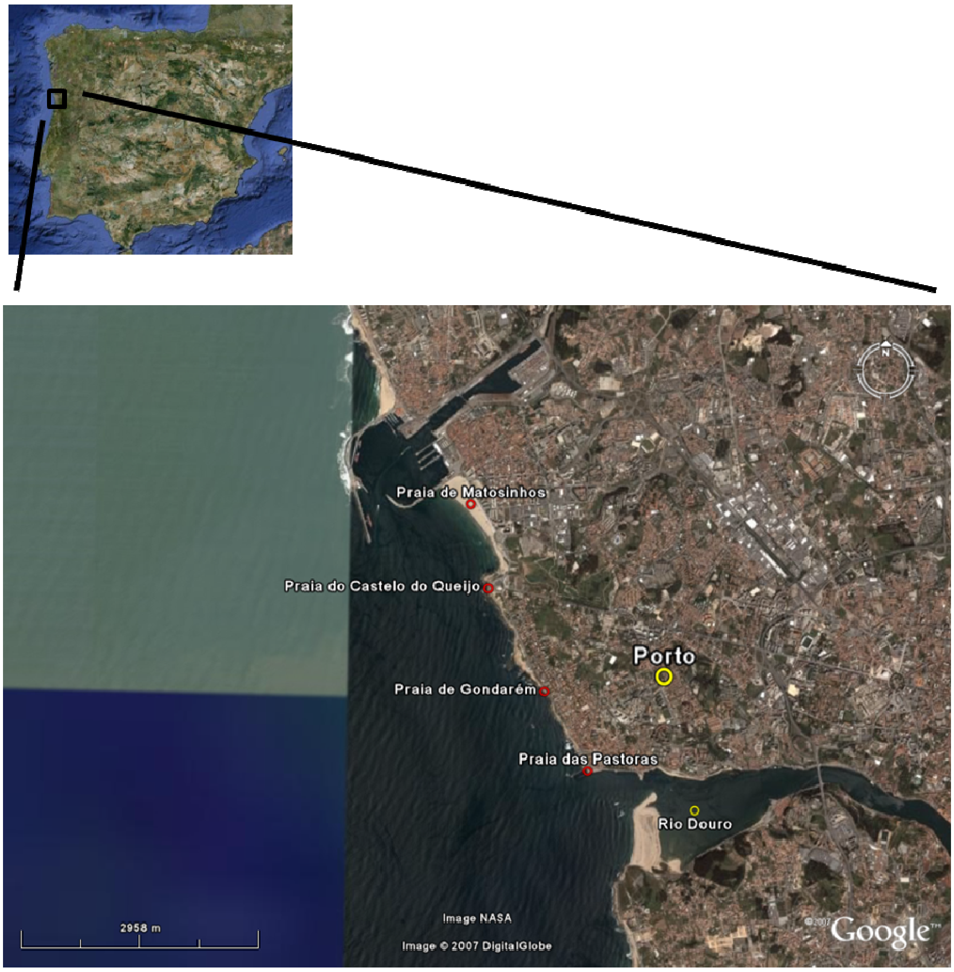

Figure 1. Location of the sampled beaches: Pastoras, Gondarém, Castelo do Queijo and Matosinhos in the NW Portugal.

Coastal front

In order to explain the tidal, temporal and spatial variability of the water quality, in the second sampling effort, sampling area was extended to the adjacent coastal area, covering Porto/Matosinhos coastal front (Figure 2). Five surveys were conducted in the bathing season of 2008 and 2009, covering an area of 10 km^2 approximately. Two different sampling crews were involved to facilitate sample collection, one in the shoreline and the other offshore on a boat. Collection, storage and transport of samples were conducted as described before.

Figure 2. Sampling area grid on coastal front of Porto/Matosinhos.

2.3. Microbiological analysis

All samples were evaluated for microbial indicators, fecal coliforms (FC) and fecal enterococci (FE) and processed within 6 hours maximum from collection. Samples were concentrated onto a sterile membrane of 0.45 µm pore size and 47 mm diameter (Schleicher Schull ME 25/21 ST). Fecal coliforms were assayed in FC-agar (Difco 0883-01) (Table 3). Since the expected number of non-lactose fermenting bacteria was insignificant, addition of rosolic acid was not necessary. Typical blue colonies were counted after a 24h incubation at 44.5 ºC.

Table 3. Composition of the selective media mFC.

Composition (g L^{-1}):	
Pancreatic Digest of Caseine	6.0
Protease Peptone No. 3	9.0
Yeast extract	3.0
Lactose	12.5
Bile salts No. 3	1.5
Sodium chloride	5.0
Agar	15.0
Aniline Blue	0.1

pH = 7,4 ± 0,2 at 25 ºC

Fecal enterococci were assayed in Slanetz & Bartley agar (Oxid CM0377) (Table 4) and typical redish-brown colonies were counted after a 48h incubation at 44.5 ºC.

Table 4. Composition of the selective media Slanetz & Bartley.

Composition (g L^{-1}):	
Tryptose	20.0
Yeast extract	5.0
Glucose	2.0
$K_2HPO_4 \cdot 2H_2O$	4.0
Sodium azide	0.4
Tetrazolium chloride	0.1
Agar	10.0

pH = 7,2 ± 0,2 at 25 ºC

2.3. Physical-chemical analysis

At each collection point, physical-chemical parameters, namely temperature, salinity, dissolved oxygen (%) pH and turbidity, were also measured with a multiparameter probe (YSI 6000), previously calibrated. Wind speed and direction were also recorded during the first sampling effort, at each beach, with a portable meteorological station (Oregon Scientific).

2.4. River Flow

Douro River has about 50 large hydroelectric power dams along its catchment. The last dam (Crestuma-Lever) is located 21.6 km upstream of the river mouth. Discharge data used in this study were provided by INAG (Instituto da Água, I.P.)

2.5. Data analysis

Data mapping

To assess Porto/Matosinhos coastal front spatial patterns, mapping of the recorded coastal front parameters was performed with a GIS, ArcGIS 9.3, using kriging as the interpolation method. Kriging has been used to characterize spatial patterns of many environmental variables such as rainfall (Sarangi *et al*., 2005), soil moisture (Bardossy and Lehmann, 1998), groundwater level (Desbarats *et al*., 2002), concentration of groundwater contaminants (Grunwald *et al*., 2004) and water quality (Yang and Jin, 2010).

Statistical treatment of the data

For data analysis, the non-parametric test Kruskal-Walis was used to detect differences in the bacteria concentrations and in environmental parameters, since variables failed normality and homoscedasticity assumptions. Post-hoc comparisons were applied to detect differences between pairs of groups.

Principal component analysis (PCA) was used to reduce the dimensionality of the environmental data and retain as much as possible of the variability present in the data, which could be achieved by transforming the data into a new set of variables, the *principal components* (PC). PCs are ordered so that the first few retain the most of the variation in all of the original variables, and the original data is transformed to new coordinate systems on the principle of variance maximization. The first PC shows the direction of highest variance of the data and the second is orthogonal to the first PC and in the direction of the second highest variance (Gurmessa and Bárdossy, 2009). The loadings on each PC correspond to its eigenvectors. The eigenvalues represent the variance of the data in the corresponding PC.

Variables were log-transformed to reduce skewness and kurtosis and standardized (mean was scaled to zero and variance to one), to minimize problems arising from different measurement scales (Peré-Trepat *et al*, 2006; Zhou *et al*, 2007). Missing data were completed using the expectation-maximisation (EM) algorithm. The number of principal components considered for analysis was chosen because the correspondent eigenvalues were greater than unity. PC scores of the parameters were used as independent variables in multiple linear regression analysis (Çamdevýren et al., 2005) to identify the best predictors of FC and FE concentrations. Determination coefficient (R^2) was used as the standard criterion of predictive success.

Primer 6 was used to perfom PCA and STATISTICA 7 was used to perform multiple regressions, and the Kruskal-Walis tests.

3. Results

3.1. Beaches

The four studied beaches presented differences in the FC concentrations, with Matosinhos recording the highest values ($p<0.05$) when compared with the other three beaches. The lowest concentrations were observed in Gondarém. FE concentrations were also higher in Matosinhos, which presented significant differences with Pastoras and Gondarém ($p<0.001$). The latter recorded the lowest values and had significant differences with Castelo do Queijo ($p<0.05$).

Changes in the water quality were observed along the day in the four beaches and a general trend for a higher fecal contamination during the morning was verified (see below).

Pastoras beach

Pastoras beach exhibited significant differences in FC concentrations between months ($p<0.01$), with August presenting the higher values (Figure 3). The dominant wind direction was northwest (NW) in June and July and southeast (SE) in August. FE concentrations also exhibited significant differences between months and July recorded the lowest concentrations ($p<0.01$) (Figure 3). In Table 5 the statistical descriptive of all parameters is presented.

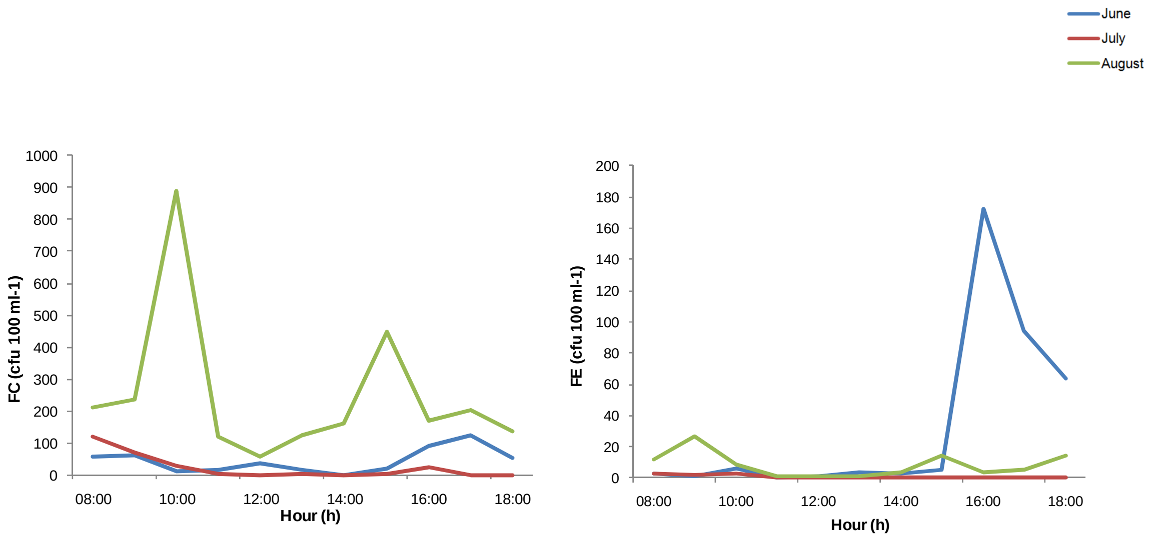

Figure 3. Diel and monthly variation of fecal bacteria in Pastoras beach.

Table 5. Statistical descriptive (mean, minimum (Min), maximum (Max) and standard error (StE)) of water variables of Pastoras beach in June (Jn), July (Jl) and August (Ag).

	Temperature (ºC)			Salinity			Dissolved Oxygen (%O_2)			pH			Turbidity (NTU)		
	Jn	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**
Mean	17.5	17.7	16.6	30.4	34.7	35.2	145.7	161.8	114.2	8.1	8.5	7.9	0.7	0.4	13.2
Min	15.5	14.7	15	25.9	33.9	34.7	107.2	91	93.2	7.8	7.9	7.8	0.3	0	3
Max	19	21.6	17.8	32.1	35.4	35.7	197.1	236.9	154.9	8.3	8.9	8.1	1.9	2.4	23.8
StE	0.4	0.7	0.3	0.5	0.1	0.1	7.5	16.4	5.8	0.1	0.1	0	0.2	0.2	2.3

	FC (ufc 100ml^{-1})			FE(ufc 100ml^{-1})			Wind (km/h)			Wind Direction			River Flow (m^{3}s^{-1})		
	Jn	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**
Mean	46	25	252	32	1	8	13.9	16.9	8.8	NW	NW	SE	580.2	242.2	291.5
Min	2	1	59	1	0	1	0.0	0.0	2.5				235.8	0	0
Max	126	121	890	173	3	27	25.7	29.5	15.6				759.2	584.5	737.5
StE	11	12	71	17	0	2	2.9	2.9	1.3				57.3	79.9	88.9

Gondarém

Bacteria concentrations observed in July were significantly higher than concentrations in June ($p<0.05$). Gondarém presented significantly different concentrations of FC, between the morning and the afternoon in the month of July, with lower values in the afternoon ($p<0.05$) (Figure 4). In Table 6 the statistical descriptive of all parameters is presented.

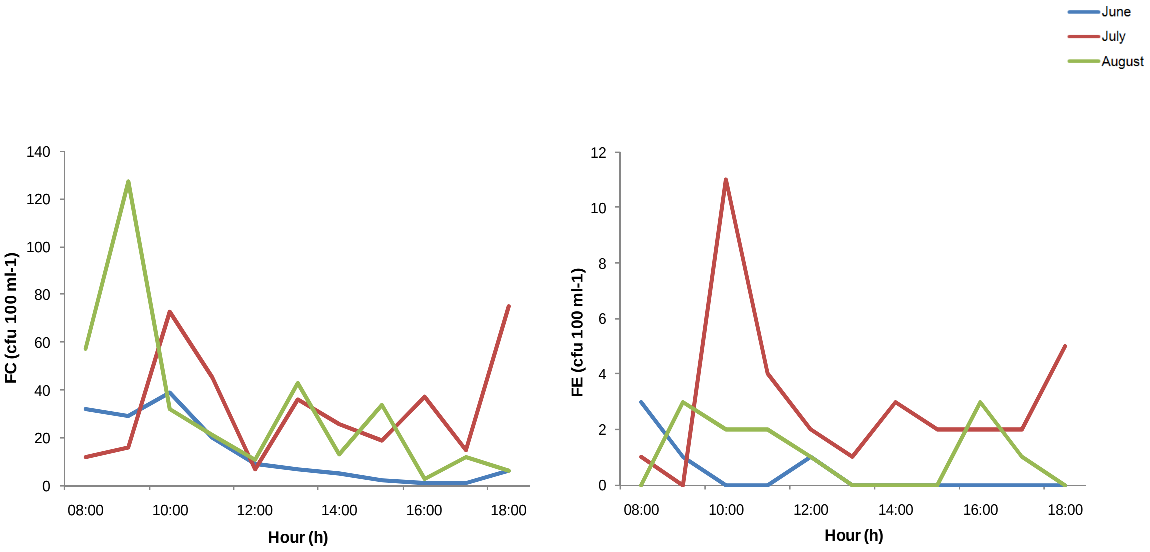

Figure 4. Diel and monthly variation of fecal bacteria in Gondarém beach.

Table 6. Statistical descriptive (mean, minimum(Min), maximum(Max) and standard error (StE)) of water variables of Gondarém beach in June (Jn), July (Jl) and August (Ag).

	Temperature (ºC)			Salinity			Dissolved Oxygen (%O_2)			pH			Turbidity (NTU)		
	Jn	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**
Mean	15.3	18.4	16.0	35.1	34.4	34.3	112.3	115.0	113.5	7.8	8.3	7.8	3.5	1.3	1.5
Min	14.3	16.3	14.6	34.7	32.9	34.1	107.9	104.5	97.8	7.6	8.1	7.6	1.2	0.2	0.2
Max	15.9	20.2	17.2	35.3	35.0	34.6	123.7	132.8	133.2	8.0	8.5	8.0	7.9	2.9	3.4
StE	0.2	0.4	0.3	0.1	0.2	0.0	1.4	2.9	3.8	0.0	0.0	0.0	0.5	0.3	0.3

	FC (ufc 100ml^{-1})			FE(ufc 100ml^{-1})			Wind (km/h)			Wind Direction			River Flow (m^{3}s^{-1})		
	Jn	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**
Mean	14	33	33	0	3	1	22.6	6.8	8.1	NW	NW	NW	476.5	307.4	446.5
Min	1	7	3	0	0	0	6.5	0.0	0.0				285.8	0.0	0.0
Max	39	75	127	3	11	3	39.5	13.0	15.5				562.0	676.2	797.4
StE	4	7	11	0	1	0	3.4	1.5	1.7				25.0	88.9	101.0

Castelo do Queijo

Castelo do Queijo did not recorded any significant temporal variations although numbers tended to increase in the morning. Adjacent to this beach was located a small stream that was eventually closed after the first survey in June, which can explain why in this month FC guide values were exceeded (Figure 5). In Table 7 the statistical descriptive of all parameters is presented.

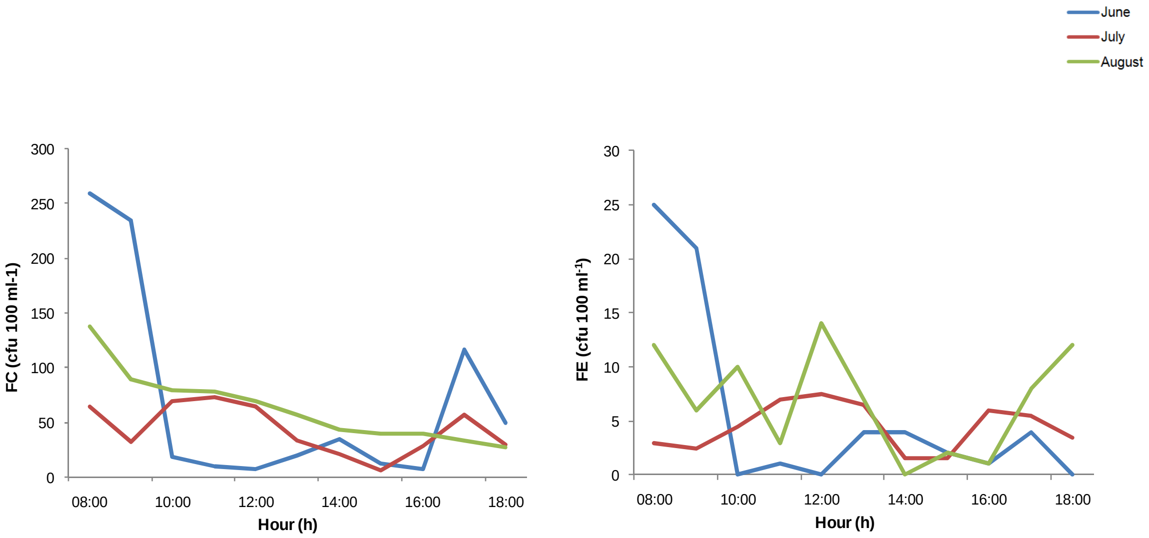

Figure 5. Diel and monthly variation of fecal bacteria in Castelo do Queijo beach.

Table 7. Statistical descriptive (mean, minimum(Min), maximum(Max) and standard error(StE)) of water variables of Castelo do Queijo beach in June (Jn), July (Jl) and August (Ag).

	Temperature (ºC)			Salinity			Dissolved Oxygen (%O_2)			pH			Turbidity (NTU)		
	Jn	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**
Mean	15.8	17.5	16.0	34.9	35.2	35.8	144.0	127.9	114.8	8.2	8.4	7.9	3.2	0.3	3.6
Min	13.9	15.0	13.5	34.7	34.2	35.5	118.2	99.9	96.3	8.0	8.1	7.6	1.1	0.0	2.0
Max	16.8	19.4	17.7	35.2	36.3	36.1	165.1	174.6	146.3	8.3	8.6	8.1	8.3	2.2	6.8
StE	0.3	0.5	0.4	0.0	0.2	0.1	3.6	7.1	5.5	0.0	0.1	0.0	0.8	0.2	0.5

	FC (ufc 100ml^{-1})			FE(ufc 100ml^{-1})			Wind (km/h)			Wind Direction			River Flow (m^{3}s^{-1})		
	Jn	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**
Mean	70	44	63	6	4	7	22.5	13.8	15.1	NW	NW	NW	472.2	411.5	499.7
Min	8	7	27	0	2	0	7.6	5.4	5.0				0.0	0.0	0.0
Max	259	73	138	25	8	14	33.2	21.5	21.6				751.7	741.4	894.3
StE	28	7	10	3	1	1	2.7	1.6	1.7				64.5	93.9	102.9

Matosinhos

Matosinhos beach exhibited significant differences in FC and FE values between months ($p<0.05$), with August presenting the higher concentrations (Figure 6). A small stream drains directly to the beach probably causing the high values observed (FC concentrations exceeded the mandatory values in the three months). In Table 8, the statistical descriptive of all parameters is presented. Although overall differences between morning and afternoon were not statistically significant, the obtained results show high variation in bacterial concentrations during the day: in June, for example, minimum FC concentration was 21 cfu 100ml^{-1}, at 15:00, below the guide values, while maximum was 5100 cfu 100ml^{-1} (well above the mandatory values) a few hours earlier, at 8:00.

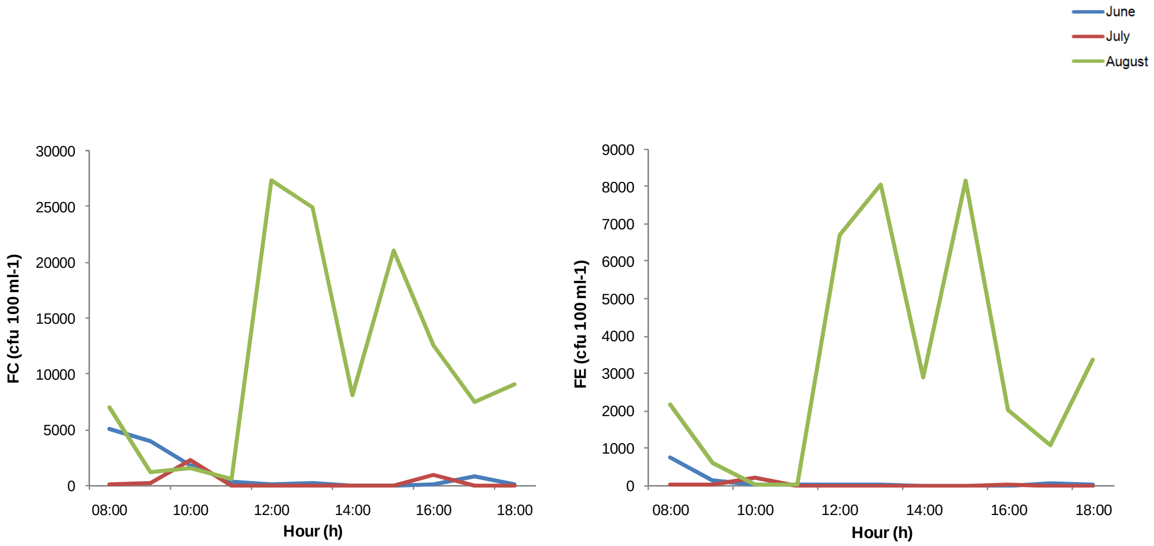

Figure 6. Diel and monthly variation of fecal bacteria in Matosinhos beach.

Table 8. Statistical descriptive (mean, minimum(Min), maximum(Max) and standard error (StE)) of water variables of Matosinhos beach in June (Jn), July (Jl) and August (Ag).

	Temperature (ºC)			Salinity			Dissolved Oxygen (%O_2)			pH			Turbidity (NTU)		
	Jn	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**
Mean	17.0	18.4	16.3	30.9	34.6	31.5	117.3	106.2	100.7	8.0	8.3	7.8	1.7	1.0	1.6
Min	13.9	16.1	15.5	29.3	33.7	30.7	107.5	102.6	97.0	7.8	8.2	7.2	0.7	0.3	0.2
Max	19.3	21.1	16.8	33.8	35.1	33.4	125.7	113.6	108.3	8.0	8.7	8.0	4.2	2.9	4.0
StE	0.54	0.5	0.1	0.42	0.1	0.3	1.43	1.2	1.1	0.0	0.0	0.1	0.3	0.3	0.4

	FC (ufc 100ml^{-1})			FE(ufc 100ml^{-1})			Wind (km/h)			Wind Direction			River Flow (m^{3}s^{-1})		
	Jn	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**	**Jn**	**Jl**	**Ag**
Mean	1141	326	10983	98	23	3190	18.5	15.7	12.8	NW	NW	SE	527.5	411.8	163.5
Min	21	3	544	3	0	7	0.0	3.3	7.7				200.7	0.0	0.0
Max	5100	2234	27360	760	210	8160	31.9	22.3	24.1				766.5	686.0	732.3
StE	534	209	2868	67	19	926	3.4	2.0	1.5				63.5	72.4	78.1

3.2. Coastal front

Tidal variation

The two 2008 surveys (August 12 and August 22) corresponded to flood and ebb tides respectively, where compared for tidal variation and no significant differences were observed. Although the ebb tide survey had registered a higher river flow mean (203.1 m^3s^{-1} during the ebb tide and 27.6 m^3s^{-1} during the flood tide), mean concentrations of fecal bacteria were higher in the flood tide. Tidal stage does not seem to play any role in affecting the microbial levels in this area, since results of each beach and coastal front did not revealed any significant differences between fecal bacteria concentrations. Spatial distribution of fecal bacteria in the two surveys is presented in Figures 11 and 15.

Temporal variation

For assessing water quality within a two-year period, results from July and August of 2008 and 2009 (ebb tides) were compared, and differences in fecal bacteria concentrations at the coastal front were not significant ($p<0.05$). However, differences between months were observed, being FC concentrations in July of 2009 significantly higher than August 2009 ($p<0.01$); and FE concentrations in August 2009 were significantly higher than concentrations in July 2008 ($p<0.05$).

Spatial Variation

Temporal variations observed were considered when evaluating spatial variations, so separate spatial analysis for each survey was carried out. From Figures 7 to 26, variables spatial patterns of fecal bacteria and abiotic parameters are presented.

Overall, beaches like Castelo do Queijo and Gondarém seemed to be less affected by the most significant pollution sources, Douro River and Matosinhos beach small stream. Douro and Leça rivers drain two different sized catchments and Douro River influence can clearly be observed with the plume of fecal bacteria visible in all maps, whereas Leça River influence on the sampled area was small and not visible, probably due to dilution and the much lower river discharge (data not shown). The poor water quality verified in Matosinhos beach during the first sampling effort was therefore confirmed to be caused by the small stream draining to that beach, which fecal bacteria signal is visible in all map surveys (Figures 7, 11, 15, 19 and 23). On the 12 August 2008 survey, it is clearly visible an additional pollution source outlet – a small stream draining to a beach located between Gondarém and Castelo do Queijo that, due to the malfunction of the interception devise was discharging into the beach.

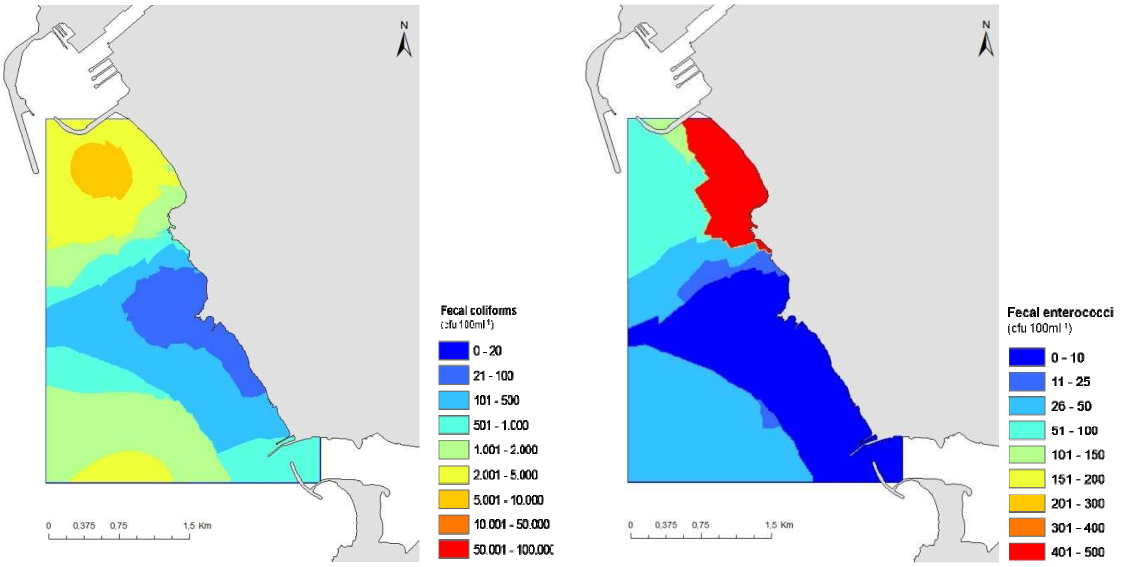

Figure 7. Spatial variations of fecal coliforms and enterococci on 7 of July 2008.

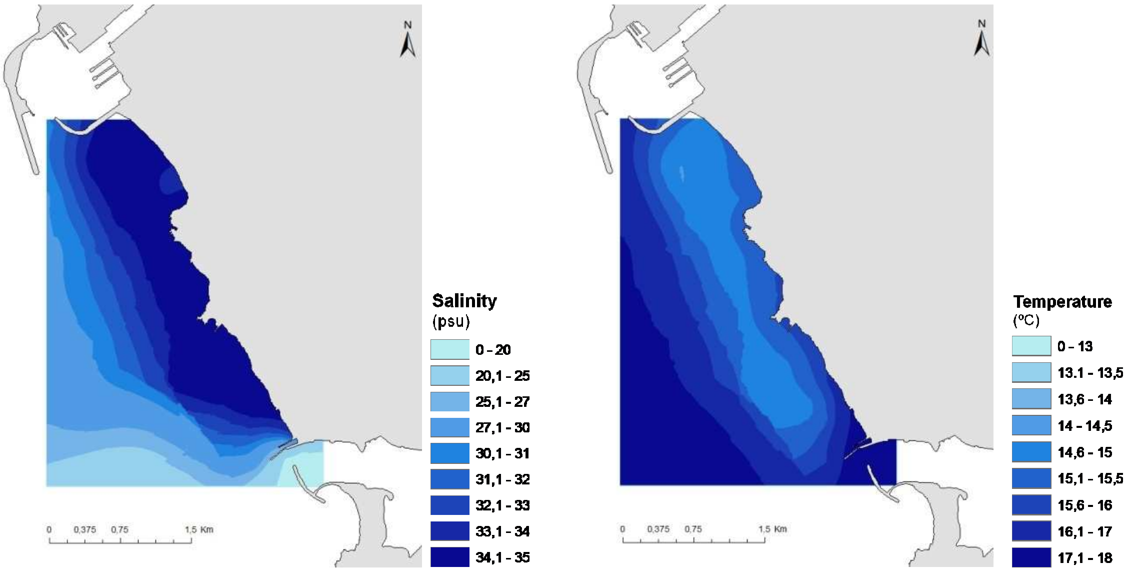

Figure 8. Spatial variations of fecal salinity and temperature on 7 of July 2008.

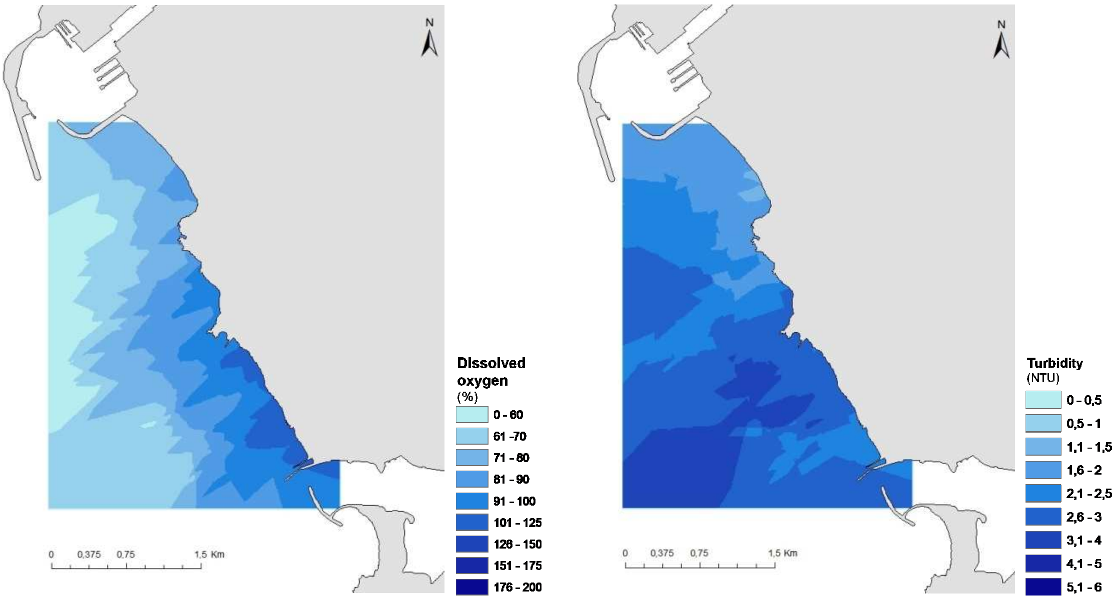

Figure 9. Spatial variations of dissolved oxygen and turbidity on 7 of July 2008.

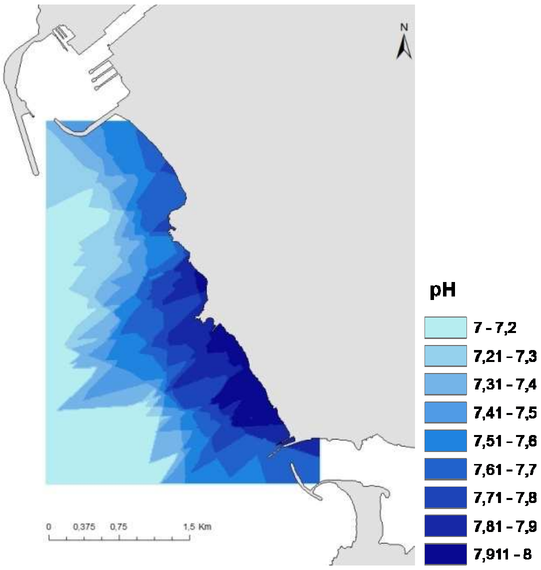

Figure 10. Spatial variations of pH on 7 of July 2008.

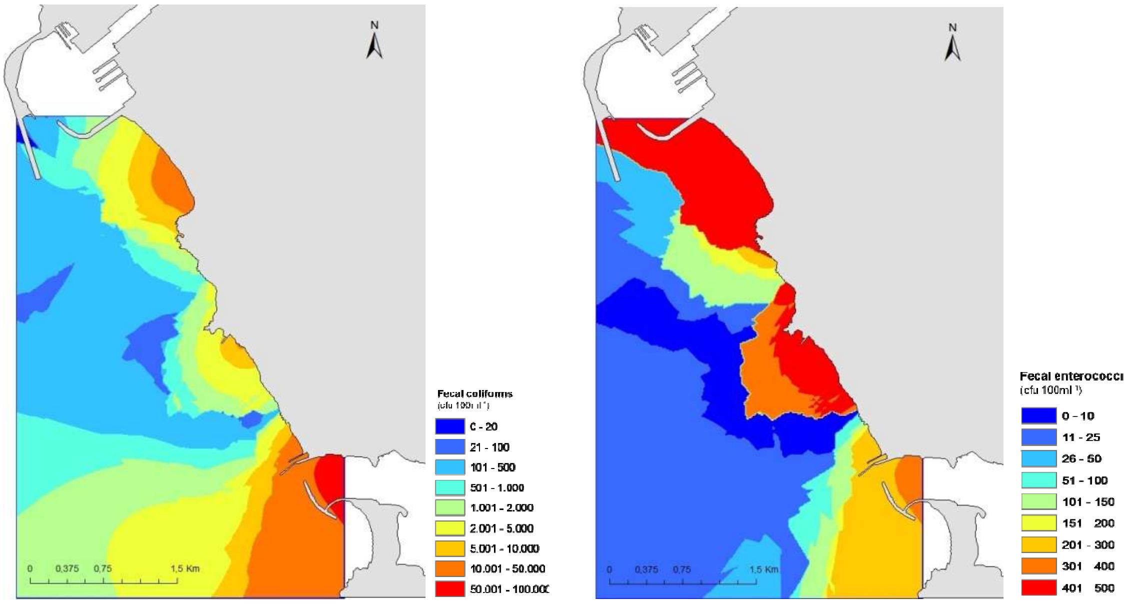

Figure 11. Spatial variations of fecal coliforms and enterococci on 12 of August 2008.

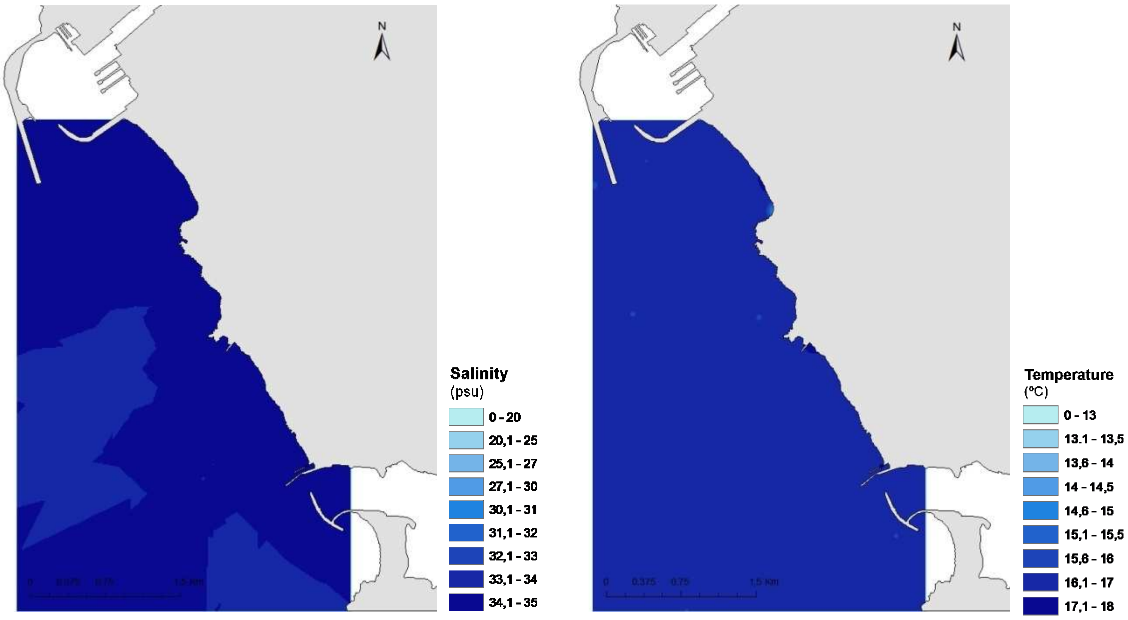

Figure 12. Spatial variations of salinity and temperature 12 of August 2008.

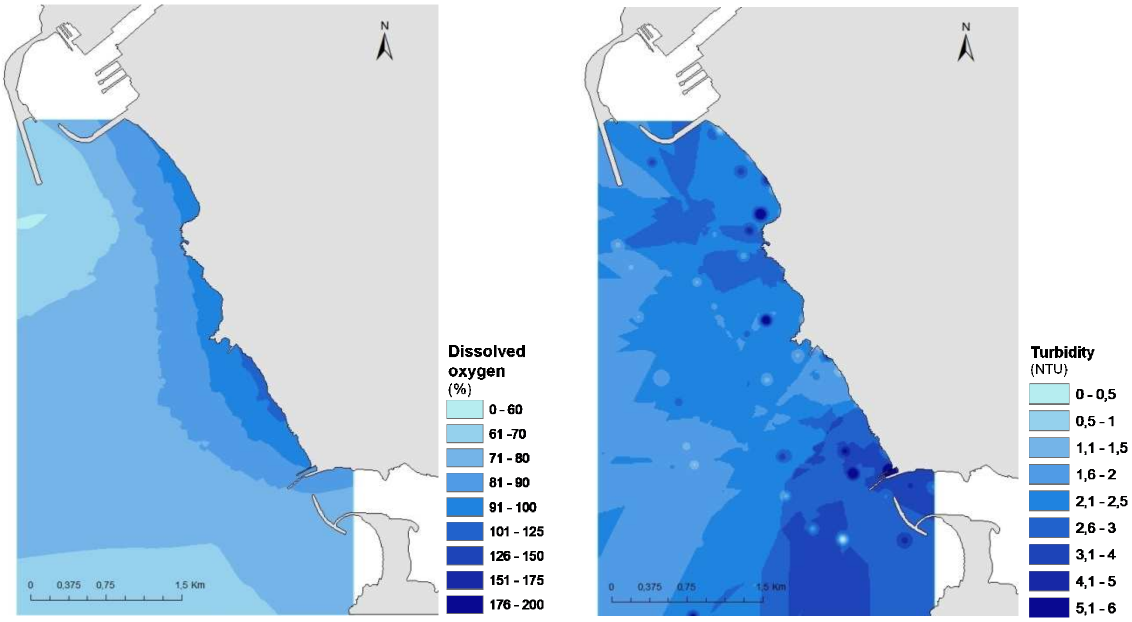

Figure 13. Spatial variations of dissolved oxygen and turbidity on 12 of August 2008.

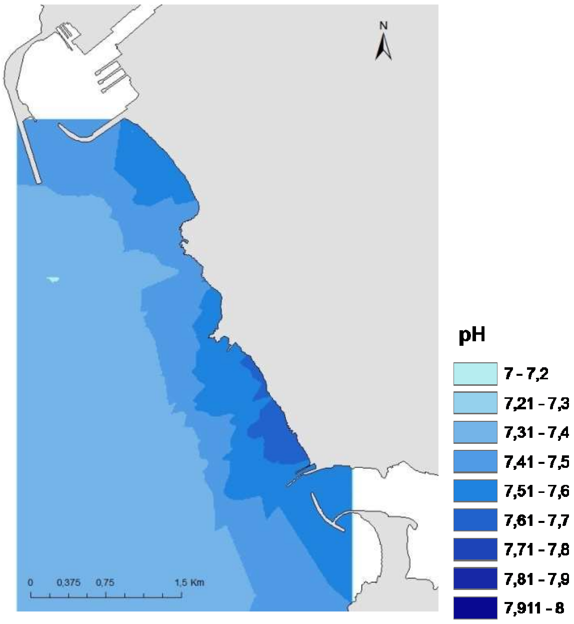

Figure 14. Spatial variations of pH on 12 of August 2008.

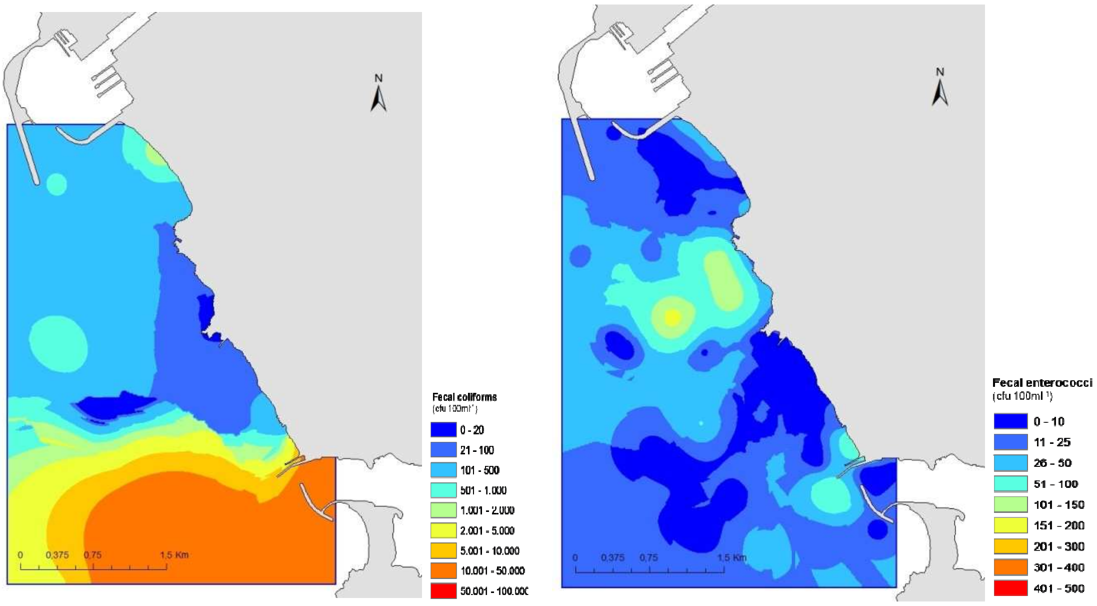

Figure 15. Spatial variations of fecal coliforms and enterococci on 22 of August 2008.

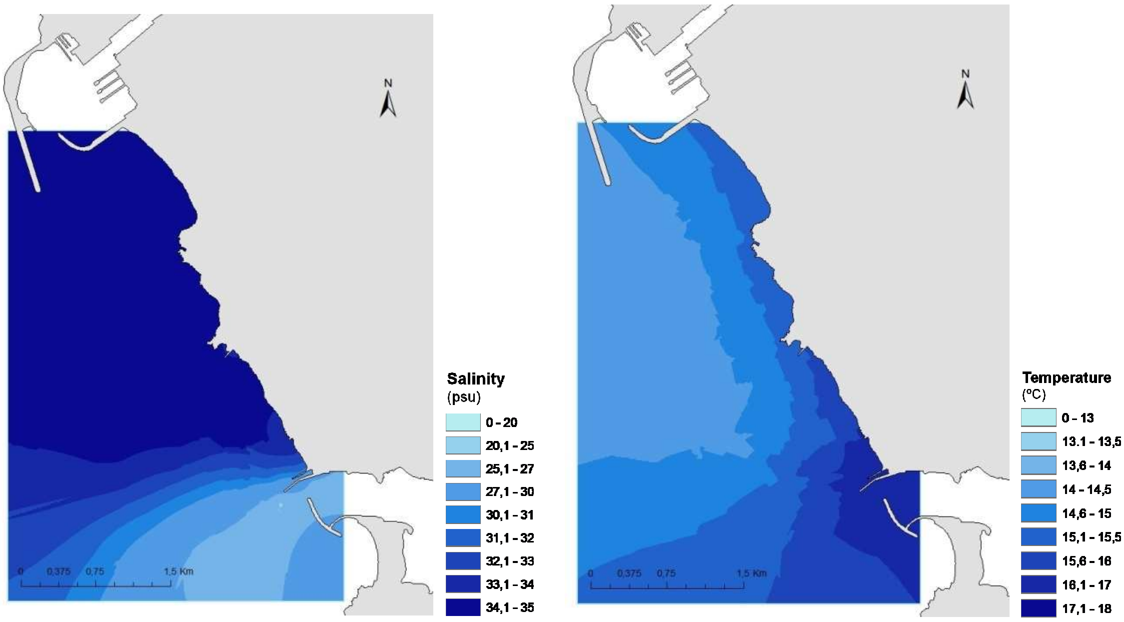

Figure 16. Spatial variations of fecal salinity and temperature on 22 of August 2008.

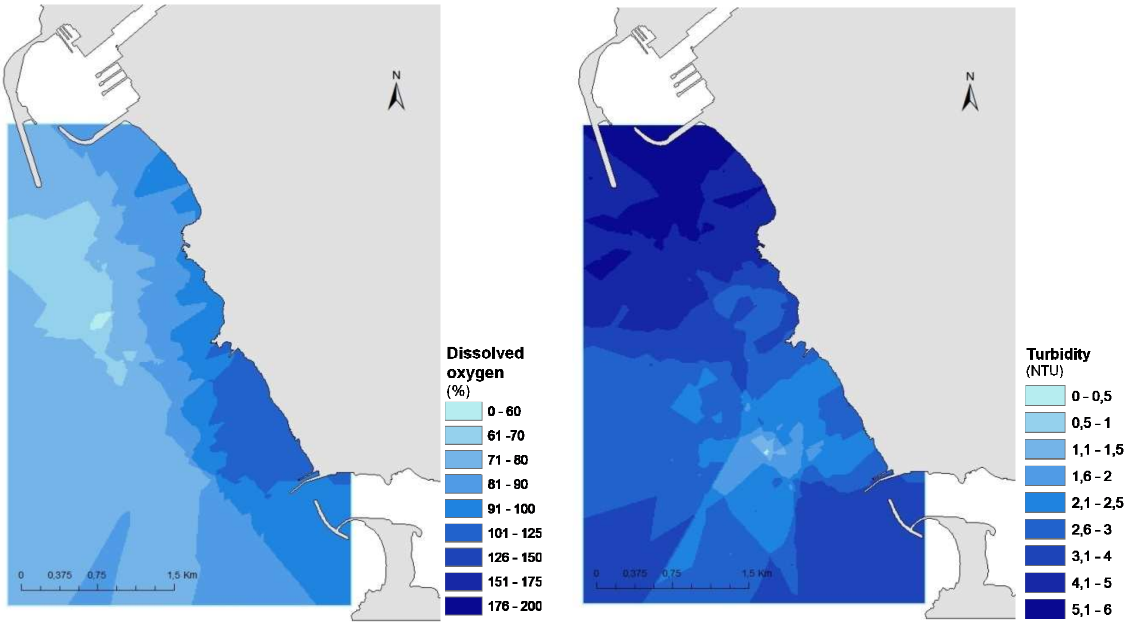

Figure 17. Spatial variations of dissolved oxygen and turbidity on 22 of August 2008.

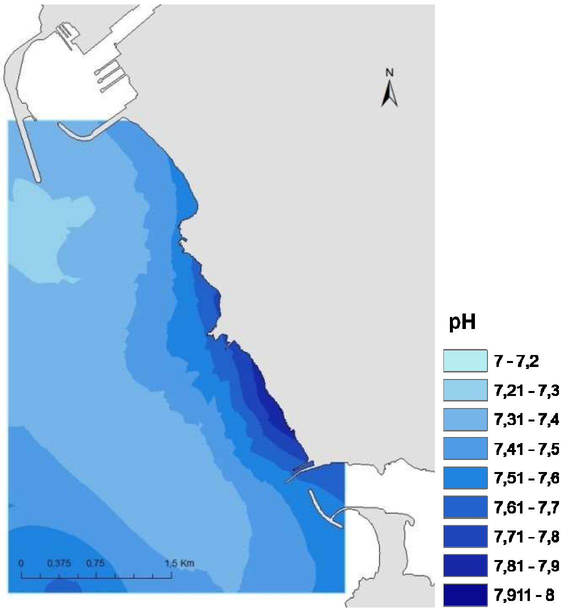

Figure 18. Spatial variations of pH on 22 of August 2008.

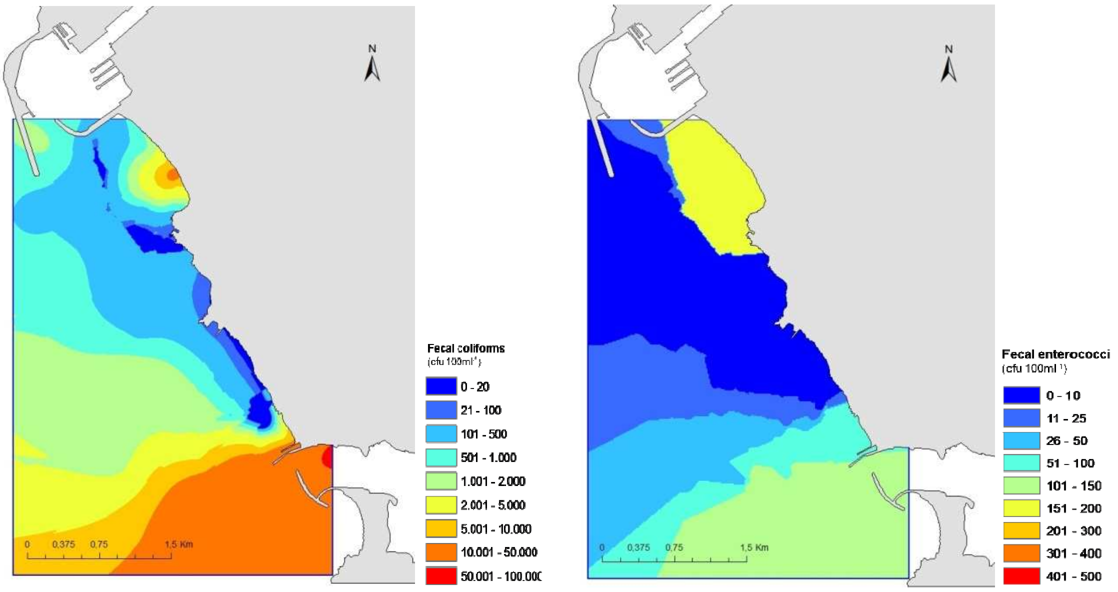

Figure 19. Spatial variations of fecal coliforms and enterococci on 10 of July 2009.

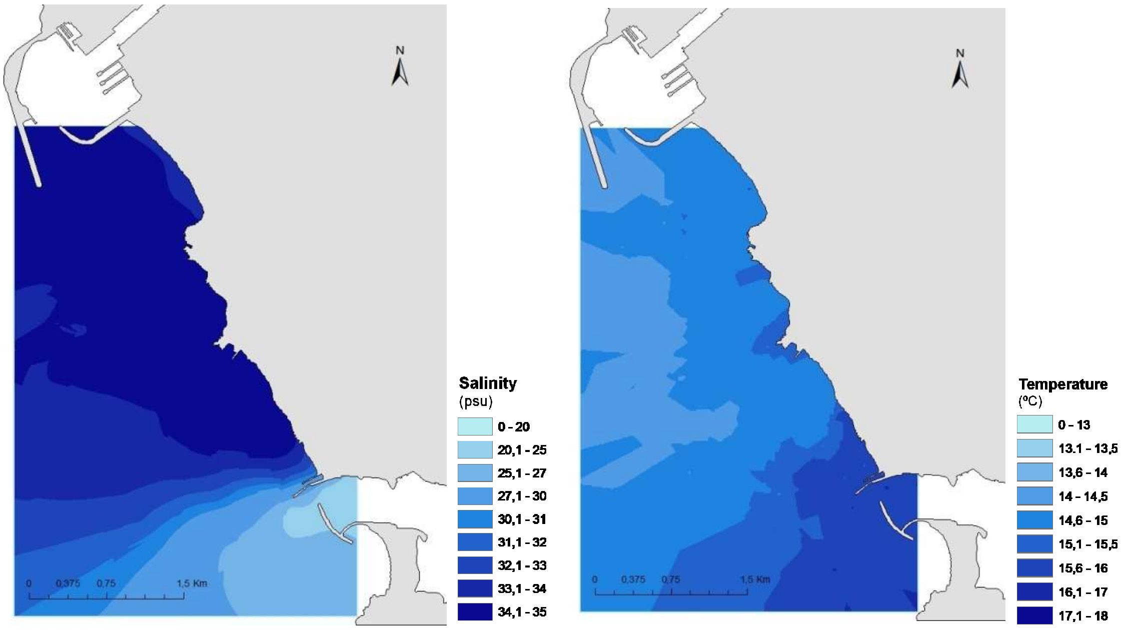

Figure 20. Spatial variations of salinity and temperature on 10 of July 2009.

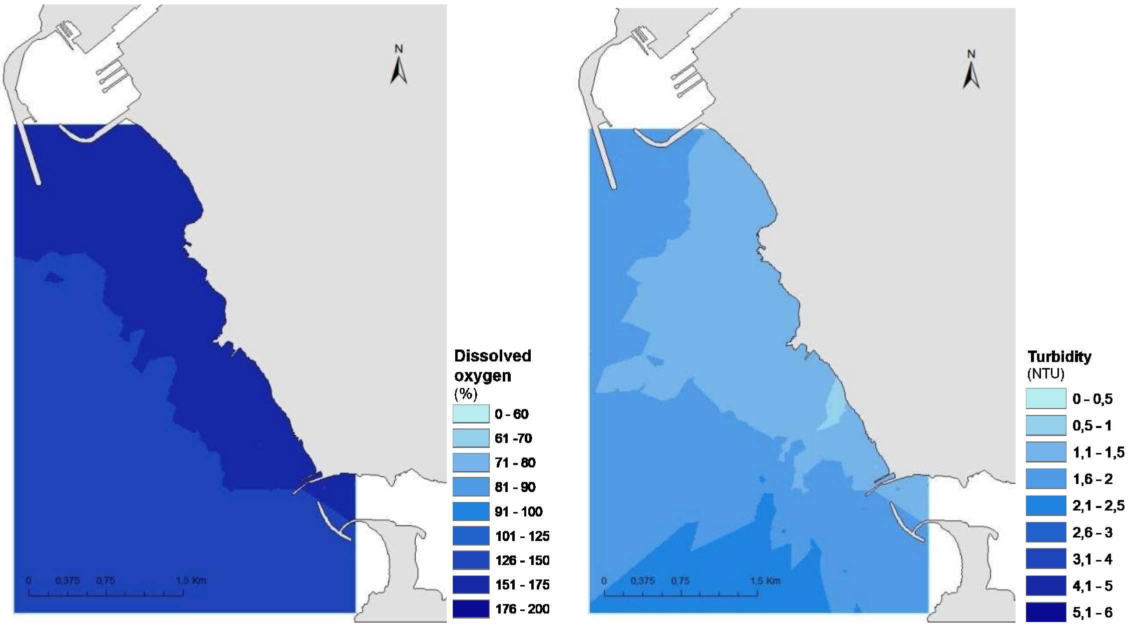

Figure 21. Spatial variations of dissolved oxygen turbidity on 10 of July 2009.

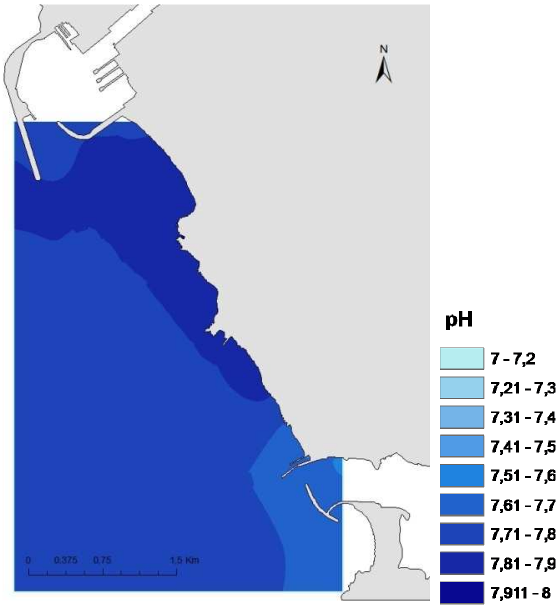

Figure 22. Spatial variations of pH on 10 of July 2009.

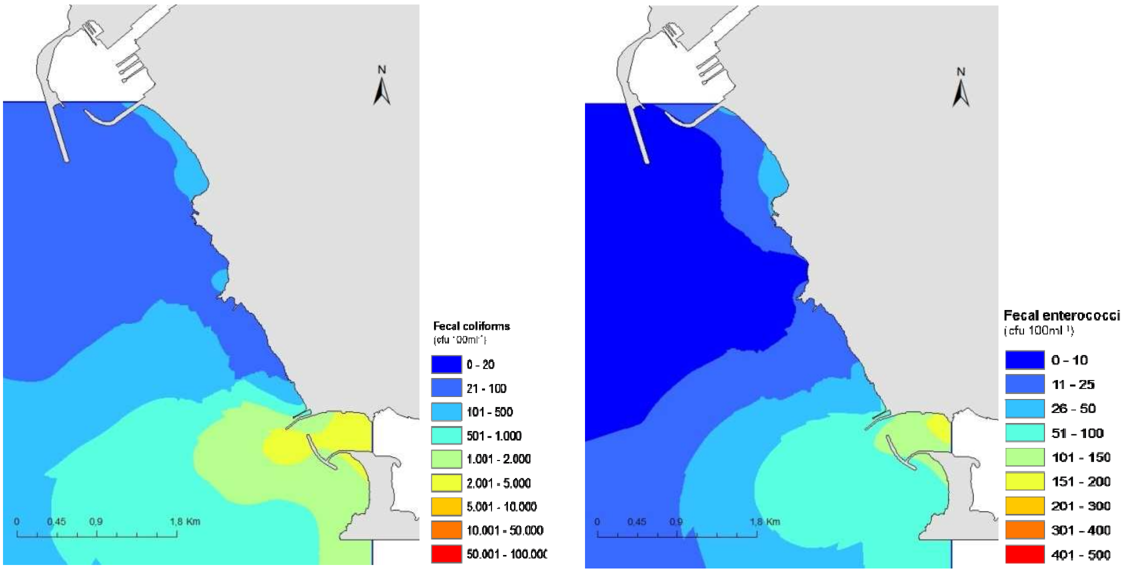

Figure 23. Spatial variations of fecal coliforms and enterococci on 12 of August 2009.

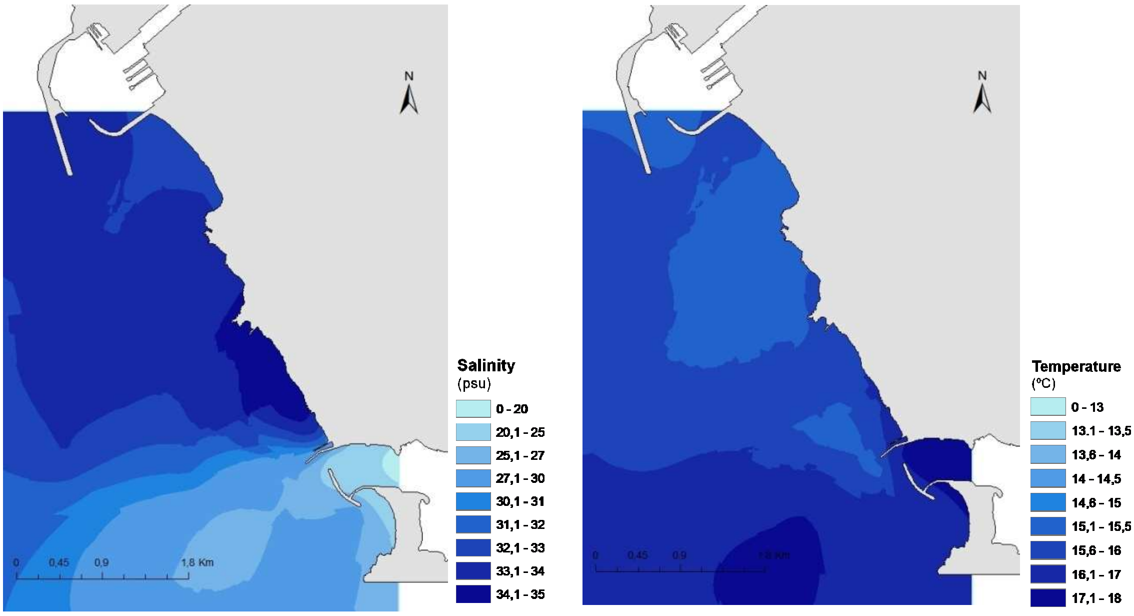

Figure 24. Spatial variations of fecal salinity and temperature on 12 of August 2009.

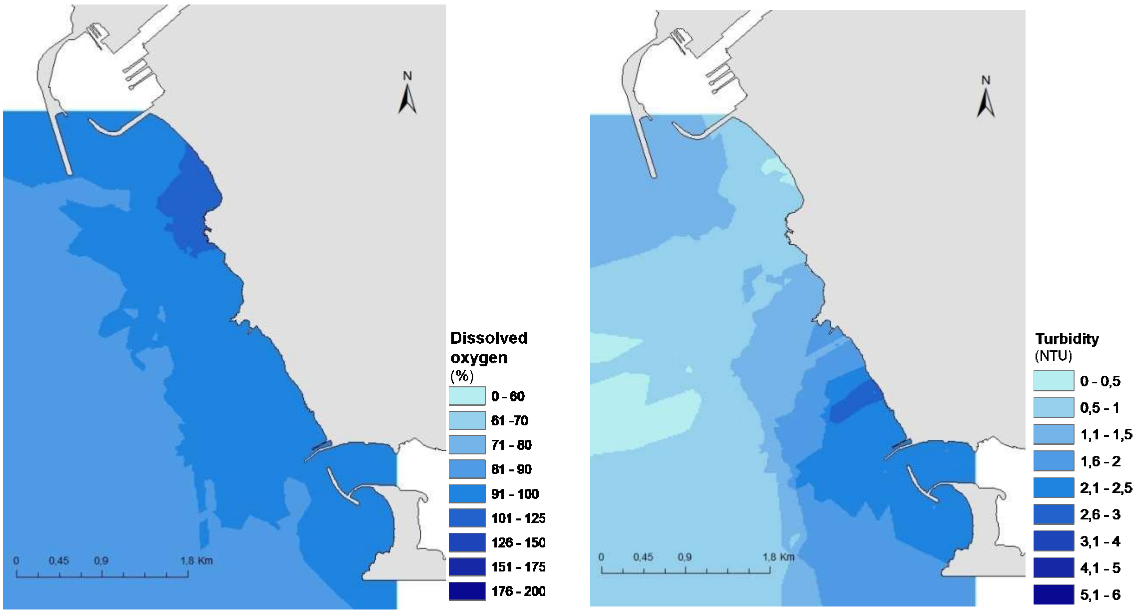

Figure 25. Spatial variations of dissolved oxygen and turbidity on 12 of August 2009.

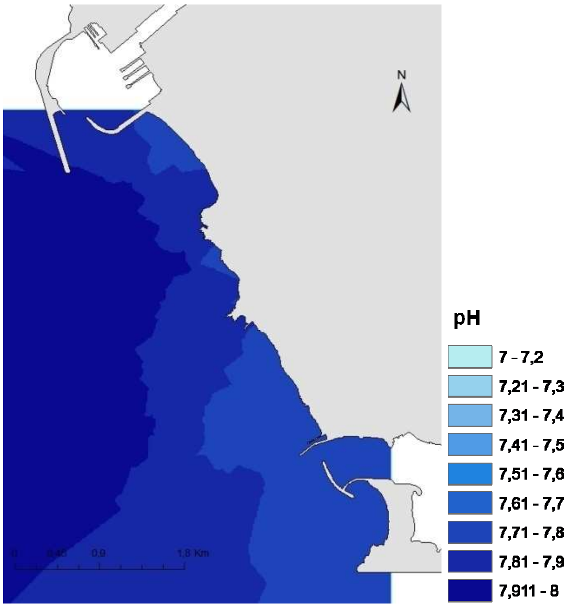

Figure 26. Spatial variations of pH on 12 of August 2009.

3.3 Environmental control

Significant differences in salinity values were observed in all beaches ($p<0.05$). Matosinhos had the lower salinities, which can be explained by the fact that the sampling point was near a small stream draining to the beach, or due to the influence of Leça. Pastoras beach presented the second-to-last salinity values because it was located close to the mouth of Douro River. Castelo do Queijo and Gondarém presented the higher salinities. These aspects can be observed in the map surveys for spatial variation (Figures 8 12, 16, 20 and 24), denoting the different influences of freshwater inputs on the beaches.

Pastoras

PCA allowed the reduction of the eight environmental variables to three significant PCs. Pastoras beach environmental PCA variables explained 83% of the variance of the original data set (Table 9 and 10). PC1 explained 48.3% of total variance and was mostly contributed by temperature, dissolved oxygen, pH and NW wind, all positively correlated with the principal component. PC2 accounted for 17.8% of the variability and was positively contributed by salinity, turbidity and SE wind, and negatively contributed by tide height. PC3 was negatively contributed by river flow, which accounted for 17% of total variance. The plot of scores 1 and 2 (Figure 27) show a sample pattern distributed along the temperature, pH and dissolved oxygen vectors. August month samples were clearly influenced by wind, which direction was SE, and turbidity, significantly higher ($p<0.01$) in this survey. The separation was evident, when fecal bacteria bubbles were superimposed.

Table 9. Loadings of the eight environmental variables on the three significant principal components of Pastoras beach.

Variable	PC1	PC2	PC3
Temperature	0.391	0.309	-0.288
Salinity	-0.113	0.485	0.376
Dissolved Oxygen	0.425	0.269	0.01
pH	0.426	0.237	0.132
Turbidity	-0.344	0.393	-0.288
Tide Height	-0.066	-0.465	-0.31
River Flow	0.231	0.041	-0.69
SE wind (SE)	-0.355	0.395	-0.315
NW wind (NW)	0.413	-0.111	0.054

Table 10. Eigenvalues, percent variation and cumulative percent variation of the first 3 principal components

PC	Eigenvalues	%Variation	Cum.%Variation
1	4.34	48.3	48.3
2	1.6	17.8	66.1
3	1.53	17	83

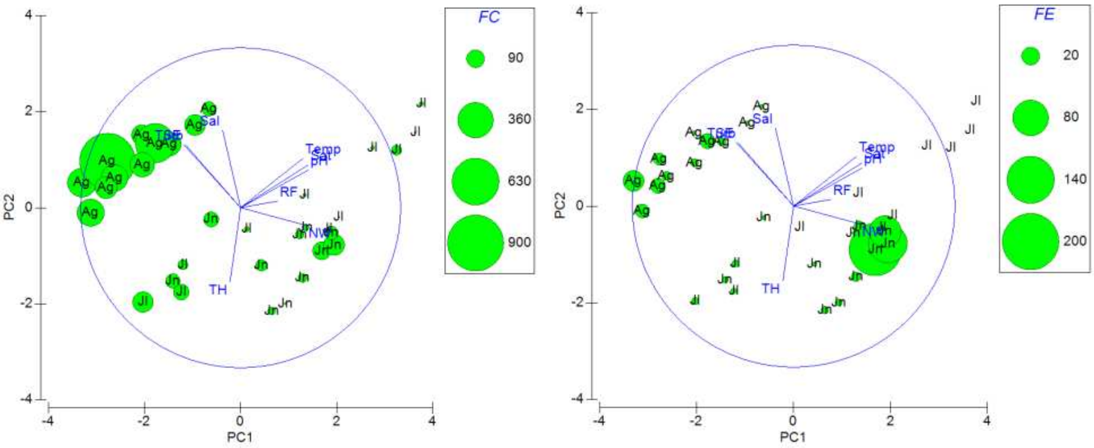

Figure 27. Bivariate plots of the scores of PC 1 and 2of Pastoras beach with superimposed vectors (circle and lines) of the environmental variables and superimposed bubbles of fecal bacteria. Bubble size is related to the magnitude of the variable represented.

Multiple regression analysis of FC and FE on the score values obtained from PCA was performed and score 1 was found to have significantly linear relationship with FC (Table 11). Therefore, score 1, which defined a variation of 48,3% in environmental water parameters, implied that 65% could be estimated by this modeling approach. FE did not had a relation with any of the three scores. Although PC1 was mostly participated by temperature, dissolved oxygen, pH and NW wind, the other variables were partially incorporated in the model, once they are also included in the PC. Predicted values of Log(FC) values in Pastoras beach could be obtained by the following model:

$$\text{Log(FC)} = 1.6 - 0.26(\text{score } 1)$$

Table 11. Results of regression analysis of fecal bacteria on Pastoras beach PC scores

	FC						FE					
	Beta	B	SE	t	p	R^2	Beta	B	SE	t	p	R^2
Pastoras												
Intercept		1.60	0.08	20.81	0.000	0.65		0.63	0.10	6.56	0.000	
S1	*-0.77*	*-0.26*	*0.04*	*-7.04*	*0.000*		-0.27	-0.08	0.05	-1.67	0.106	
S2	0.15	0.08	0.06	1.34	0.191		-0.22	-0.11	0.08	-1.36	0.183	
S3	-0.20	-0.12	0.06	-1.85	0.074		-0.31	-0.15	0.08	-1.91	0.066	

Beta - Standardized regression coefficient.
B - Regression coefficient.
SE – Standard error of B

Gondarém

The eingenvalues of the first four principal components of Gondarém beach PCA explained 83.5% of the total variance of the original data set (Table 12 and 13). The variables that contributed the most to PC1, which explained 29.5% of total variance, were temperature and pH (negatively correlated with PC), and salinity and turbidity (positively correlated with PC). PC2 accounted for 24.9% of the variability and was negatively participated by tide height and NW wind. PC3 was positively contributed by river flow and accounted for 17.3% of the variability. Dissolved oxygen and SE wind (the latter negatively correlated with PC) contributed to PC4, explaining 11.8% of total variance. The plots of scores 1 and 2 (Figure 28) showed samples corresponding to June month on the right of the PC1 axis, confirming the significantly higher salinity ($p<0.01$) verified in these samples. July, with significantly higher pH ($p<0.01$) was located on the left of the PC1 axis. No clear trend was observed on the fecal bacteria superimposed bubles, except that concentrations in June were the lowest.

Table 12. Loadings of the eight environmental variables on the four significant PCs of Gondarém beach

Variable	PC1	PC2	PC3	PC4
Temperature	-0.496	-0.174	0.361	0.004
Salinity	0.411	0.282	0.221	-0.321
Dissolved Oxygen	-0.107	0.39	0.346	0.446
pH	-0.489	0.135	0.35	-0.04
Turbidity	0.416	0.341	0.219	0.077
Tide Height	0.051	-0.563	0.094	-0.297
River Flow	0.149	-0.103	0.655	-0.179
SE wind	-0.179	0.321	-0.029	-0.746
NW wind	0.323	-0.417	0.303	0.119

Table 13. Eigenvalues, percent variation and cumulative percent variation of the first four PCs.

PC	Eigenvalues	%Variation	Cum.%Variation
1	2.65	29.5	29.5
2	2.24	24.9	54.3
3	1.56	17.3	71.6
4	1.06	11.8	83.5

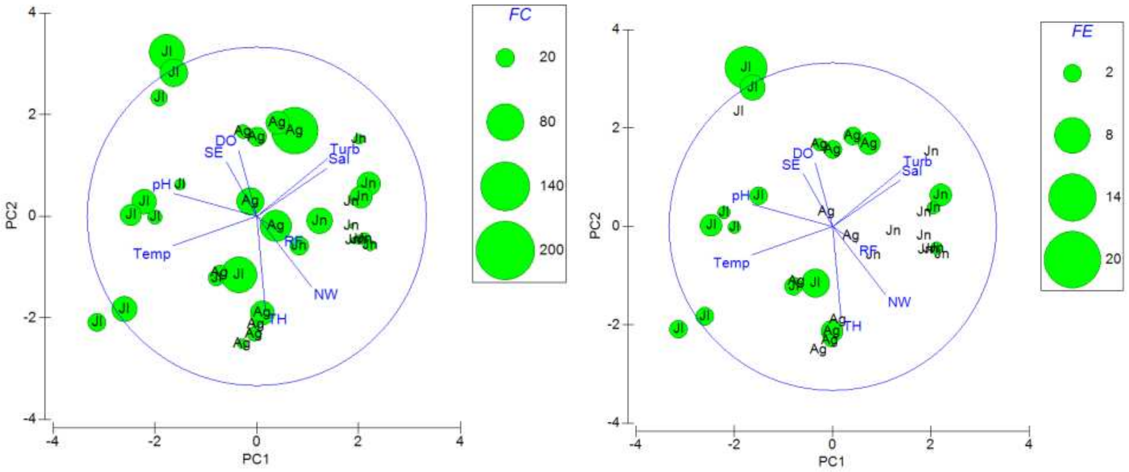

Figure 28. Bivariate plots of the scores of PC 1 and 2 of Gondarém beach with superimposed vectors (circle and lines) of the environmental variables and superimposed bubbles of fecal bacteria. Bubble size is related to the magnitude of the variable represented.

Scores 1 and 3, that defined a variability of 46.8% of the total environmental data set, were found to have significantly linear relationship with FC (R^2=0.34). Score 1 had a significantly linear relationship with FE (R^2=0.28) (Table 14). Predicted values of Log(FC) and Log(FE) values in Gondarém beach could be obtained by the following model:

$$\mathrm{Log(FC)} = 1.24 - 0.1\ (\mathrm{score1}) - 0.12(\mathrm{score3})$$

$$\mathrm{Log(FE)} = 0.29 - 0.08\ (\mathrm{score1})$$

Table 14. Results of regression analysis of fecal bacteria on Gondarém beach PC scores.

	FC							FE					
	Beta	B	SE	t	p	R^2		Beta	B	SE	t	p	R^2
Gondarém													
Intercept		1.24	0.07	18.24	0.000	0.34	Intercept		0.29	0.05	6.23	0.000	0.28
S1	*-0.37*	*-0.10*	*0.04*	*-2.39*	*0.024*		S1	*-0.44*	*-0.08*	*0.03*	*-2.74*	*0.010*	
S2	0.28	0.08	0.05	1.84	0.077		S2	0.29	0.06	0.03	1.78	0.086	
S3	*-0.34*	*-0.12*	*0.06*	*-2.25*	*0.033*		S3	0.02	0.00	0.04	0.11	0.910	
S4	0.11	0.05	0.07	0.69	0.494		S4	0.03	0.01	0.05	0.18	0.859	

Beta - Standardized regression coefficient.

B - Regression coefficient.

SE – Standard error of B

Castelo do Queijo

The first three Castelo do Queijo beach principal components explained 79.9% of the total variance contained in the original data set (Table 15 and 16). PC1 explained 46.6% of total variance and was negatively contributed by temperature, dissolved oxygen, pH, river flow and NW wind. PC2 accounted for 18.6% of the variability and was negatively contributed by turbidity. PC3 was negatively participated by salinity and positively participated by tide height, accounting for 14.7% of total variance (Figure 29).

Table 15. Loadings of the eight environmental variables on the three significant PCs of Castelo do Queijo beach

Variable	PC1	PC2	PC3
Temperature	-0.424	0.16	-0.082
Salinity	0.246	-0.335	-0.433
Dissolved Oxygen	-0.396	-0.278	-0.05
pH	-0.445	0.253	-0.259
Turbidity	0.181	-0.634	0.373
Tide Height	0.125	0.435	0.671
River Flow	-0.433	-0.222	0.167
NW wind (NW)	-0.411	-0.284	0.345

Table 16.. Eigenvalues, percent variation and cumulative percent variation of the first three PCs.

PC	Eigenvalues	%Variation	Cum.%Variation
1	3.73	46.6	46.6
2	1.49	18.6	65.2
3	1.17	14.7	79.9

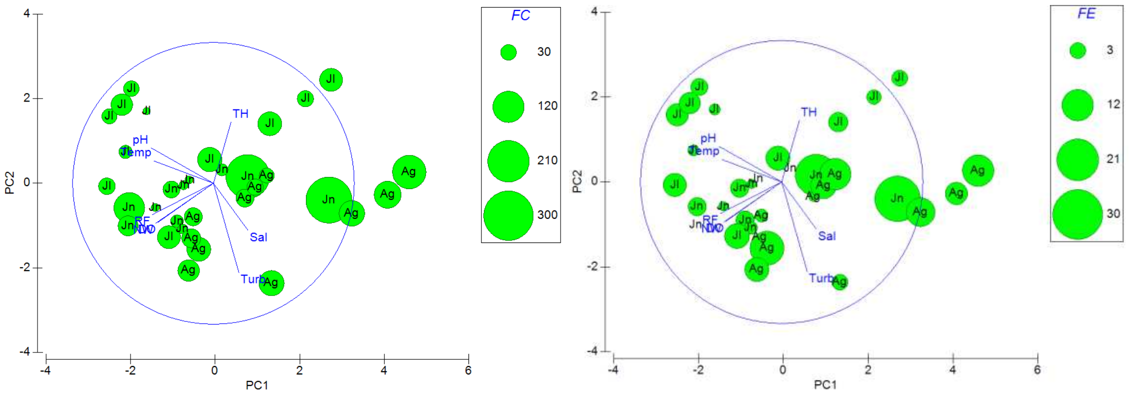

Figure 29. Bivariate plots of the scores of PC 1 and 2 of Castelo do Queijo beach with superimposed vectors (circle and lines) of the environmental variables and superimposed bubbles of fecal bacteria. Bubble size is related to the magnitude of the variable represented.

Score 1, that defined a variability of 46.6% of the total environmental data set, were found to have significantly linear relationship with FC (R^2=0.33) and FE (R^2=0.17) (Table 17). Predicted values of Log(FC) and Log(FE) values in Castelo do Queijo beach could be obtained by the following model:

$$\text{Log(FC)} = 1.62 + 0.1\ (\text{score1})$$

$$\text{Log(FE)} = 0.67 - 0.07\ (\text{score1})$$

Table 17. Results of regression analysis of fecal bacteria on Castelo do Queijo beach principal component scores

	FC							FE					
	Beta	B	SE	t	p	R^2		Beta	B	SE	t	p	R^2
Castelo do Queijo													
Intercept		1.62	0.06	28.44	0.00	0.33	Intercept		0.67	0.06	10.57	0.00	0.17
S1	*0.48*	*0.10*	*0.03*	*3.18*	*0.00*		S1	*0.37*	*0.07*	*0.03*	*2.18*	*0.04*	
S2	-0.15	-0.05	0.05	-0.99	0.33		S2	-0.01	0.00	0.05	-0.07	0.94	
S3	-0.27	-0.10	0.05	-1.80	0.08		S3	-0.17	-0.06	0.06	-1.02	0.32	

Beta - Standardized regression coefficient.

B - Regression coefficient.

SE – Standard error of B

Matosinhos

The eingenvalues of the first three principal components of Matosinhos beach PCA explained 81% of the total variance of the original data set (Table 18 and 19). The variables that contributed the most to PC1, which explained 41.4% of total variance, were pH, river flow, NW wind and SE wind, the latter negatively correlated with PC. PC2 accounted for 23.5% of the variability and was positively participated by salinity and negatively participated by turbidity and tide height. PC3 explained 16.1% of the total variance and was positively contributed by temperature and negatively contributed by dissolved oxygen. The plots of scores 1 and 2 (Figure 30) show that monthly samples were spread along the PC1 axis, with August samples on the right and June and July on the left of the plot area, denoting the strong influence of wind direction to water quality of the sampling point on this beach.

Table 18. Loadings of the eight environmental variables on the three significant PC of Matosinhos beach

Variable	PC1	PC2	PC3
Temperature	0.377	0.001	0.421
Salinity	0.18	0.526	0.355
Dissolved Oxygen	0.312	-0.314	-0.463
pH	0.376	0.198	0.287
Turbidity	0.056	-0.553	0.351
Tide Height	0.117	-0.497	0.375
River Flow	0.383	-0.135	-0.201
SE wind	-0.434	-0.109	0.295
NW wind	0.485	0.045	-0.092

Table 19. Eigenvalues, percent variation and cumulative percent variation of the first three PCs.

PC	Eigenvalues	%Variation	Cum.%Variation
1	3.73	41.4	41.4
2	2.11	23.5	64.9
3	1.45	16.1	81

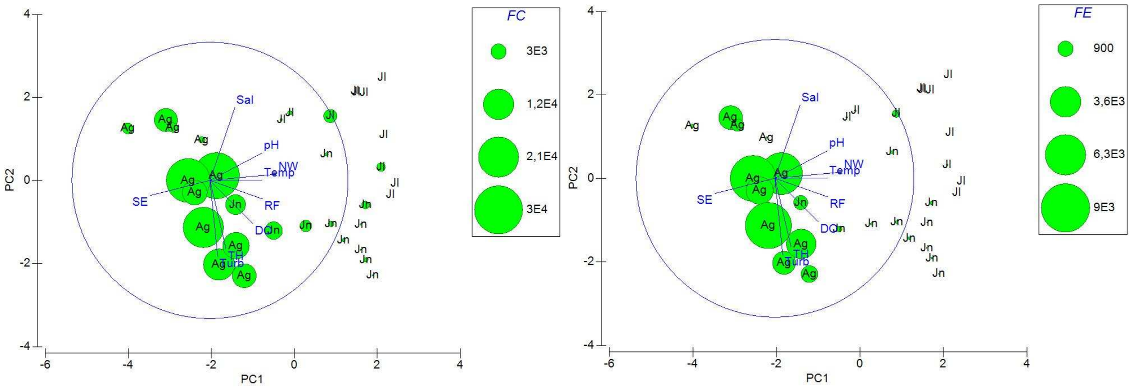

Figure 30. Bivariate plots of the scores of PC 1 and 2 of Matosinhos beach with superimposed vectors (circle and lines) of the environmental variables and superimposed bubbles of fecal bacteria. Bubble size is related to the magnitude of the variable represented.

Score 1 and 2, that defined a variability of 64.9% of the total environmental data set, were found to have significantly linear relationship with FC (R^2=0.70) and FE (R^2=0.72) (Table 19). Predicted values of Log(FC) and Log(FE) values in Matosinhos beach could be obtained by the following model:

$$\text{Log(FC)} = 2.62 - 0.49\ (\text{score1}) – 0.24\ (\text{score2})$$

$$\text{Log(FE)} = 1.69 – 0.53\ (\text{score1}) – 0.27\ (\text{score2})$$

Table 20. Results of regression analysis of fecal bacteria on Matosinhos beach principal component scores

	FC								FE					
	Beta	B	SE	t	p	R^2	B		B	SE	t	p	R^2
Matosinhos													
Intercept		2.62	0.12	22.18	0.00	0.70	Intercept		1.69	0.13	13.46	0.00	0.72
S1	*-0.79*	*-0.49*	*0.06*	*-7.81*	*0.00*		S1	*-0.78*	*-0.53*	*0.07*	*-7.96*	*0.00*	
S2	*-0.29*	*-0.24*	*0.08*	*-2.88*	*0.01*		S2	*-0.31*	*-0.27*	*0.09*	*-3.13*	*0.00*	
S3	-0.02	-0.02	0.10	-0.19	0.85		S3	0.10	0.10	0.11	0.97	0.34	

Beta - Standardized regression coefficient.
B - Regression coefficient.
SE – Standard error of B

Coastal front

The first three coastal front principal components explained 80.3% of the total variance contained in the original data set (Table 21 and 22). PC1 explained 38.1% of total variance and was contributed by dissolved oxygen and pH. PC2 accounted for 23.3% of the variability and was positively contributed by temperature and negatively contributed by salinity. PC3 was negatively participated by river flow and positively participated by turbidity, accounting for 18.9% of total variance.

Table 21. Loadings of the eight environmental variables on the three significant PC of coastal front.

Variable	PC1	PC2	PC3
Temp	-0.005	0.601	-0.084
Sal	0.099	-0.78	-0.235
DO	0.605	0.058	0.144
pH	0.515	0.13	-0.416
Turb	-0.394	-0.047	0.458
RF	-0.452	0.09	-0.731

Table 22. Eigenvalues, percent variation and cumulative percent variation of the first three PCs.

PC	Eigenvalues	%Variation	Cum.%Variation
1	2.42	38.1	38.1
2	1.48	23.3	61.4
3	1.2	18.9	80.3

Multiple regression analysis of FC and FE on the score values obtained from PCA were statistically significant ($p<0.05$) for all PC scores (Table 23). Therefore, a variability of 80.3% of the total environmental data set was found to have a significantly linear relationship with FC ($R^2=0.29$) and FE ($R^2=0.15$). Predicted values of Log(FC) and Log(FE) values of the coastal front could be obtained by the following model:

$$\text{Log(CF)}= 2.31 – 0.08(\text{score } 1) + 0.24(\text{score}2) + 0.39(\text{score } 3)$$

$$\text{Log(FE)}= 1.04 – 0.07(\text{score } 1) + 0.23(\text{score}2) + 0.09(\text{score } 3)$$

Table 23. Results of regression analysis of fecal bacteria on the coastal front principal component scores

	Fecal coliforms							Fecal enterococus					
	Beta	B	SE	t	p	R^2		Beta	B	SE	t	p	R^2
Coastal front													
Intercept		2.31	0.05	45.13	0.00	0.29	Intercept		1.04	0.05	22.83	0.00	0.15
S1	*-0.13*	*-0.08*	*0.03*	*-2.57*	*0.01*		S1	*-0.13*	*-0.07*	*0.03*	*-2.28*	*0.02*	
S2	*0.30*	*0.24*	*0.04*	*5.80*	*0.00*		S2	*0.34*	*0.23*	*0.04*	*6.04*	*0.00*	
S3	*0.43*	*0.39*	*0.05*	*8.38*	*0.00*		S3	*0.12*	*0.09*	*0.04*	*2.15*	*0.03*	

Beta - Standardized regression coefficient.

B - Regression coefficient.

SE – Standard error of B

4. Discussion

The four beaches in which this study has focused in the first phase are spread along an extension of 4.5 km, but despite this, large dissimilarities were found between them confirming the fact that the location and the physical constraints can influence the water quality of adjacent beaches (Bordalo, 2003) and that the fecal bacteria signal along the shoreline is highly variable in time and space.

In the past, Porto beaches were often “closed” during several bathing seasons for not complying with the mandatory values. On the 2007 bathing season, Castelo do Queijo was closed for not conforming to the values of the established parameters for water quality in the previous bathing season. Official results showed that fecal coliforms exceeded the guide values (100 ufc $100ml^{-1}$, according to DL 236/98) at least once in every beach. Fecal coliforms exceeded mandatory values (2000 ufc $100ml^{-1}$, according to DL 236/98) only in Matosinhos beach during the three surveys (June, July and August).

Diel variability trend in the morning can be explain by the higher loadings of raw sewage at that time (Bordalo, 2003) or the antibacterial effects of the UV light over the course of the day, that may contribute to the lower mean concentrations verified in the afternoon (Haack *et al*., 2003).

Tidal variability was not verified. Although several studies (Santoro and Boehm, 2007; Bordalo, 2003, Shibata *et al*., 2004) refer the eventual effect of tide level on the concentration of fecal bacteria, no significant differences were observed between ebb and flood tides, both in the first and second sampling efforts.

Gondarém and Castelo do Queijo were the less polluted beaches and did not registered any relevant temporal variations. Matosinhos and Pastoras, however, registered significant higher fecal coliforms bacteria in August which could be caused not only by a dilution factor, but also by wind direction, that was southeast during both sampling surveys. Pastoras beach is located by the mouth of Douro River, right above its northern jetty, and southeast wind drives the estuarine contaminated surface water north. In Matosinhos beach, since sampling point was located north of the stream, contaminated water was also driven north, were most of the beachgoers were concentrated.

Patterns of bacteria abundance were found to vary in response to weather patterns or vary systematically under different ambient conditions (e.g. morning vs. afternoon), so different monitoring schedules may yield different results in bathing water quality. Thus, in a single day, beach classification can alter from excellent to poor. Results presented indicate that to understand sources and environmental processes influencing bathing water quality consideration must be given to beach orientation related to regional weather patterns, regional and local hydrodynamics, nature, timing and magnitude of the various local source inputs, and interactions between these factors (Haack *et al*., 2003).

Spatial variability of the coastal front was successfully assessed with recourse to a Geographic Information System. Geodatabases allow the storage of spatial and attribute data within a single database management system and possesse many advantages such as supporting geometric networks and other topologically integrated features, maintaining data integrity, and centralizing information management (ESRI, 2002). Thus, integration of GIS approaches and extensive monitoring data can provide a base for underpinning environmental management at local, regional, national and international scales, since the results presented as geographic information display readily information to evaluate pollution sources and spatial patterns, facilitating the decision-making.

Spatial variability results confirm and reinforce the concept that coastal water quality is forced by a complex combination of local and external processes and raise questions about the efficacy of existing bathing water monitoring and reporting programs. The variability documented has, therefore, implications for the monitoring and mitigation of coastal pollution and decisions towards beach classification should not be based on a single sample concentration of indicator bacteria. In urban beaches, the design of bathing water profiles required by the new Bathing Water Directive (2006/7/EC) are a critical step that should reflect the diel and spatial variability of bathing waters in order to correct establish the frequency, time and sampling site of the sample collection, which should capture the overall microbiological quality of the water. The selection of sampling stations and time of sampling should take into consideration variables known to affect water quality such as the length of the bathing area presence and periodicity of point and non-point sources of fecal contamination, influences of local weather, the physical characteristics of the bathing area and the presence of bathers (Bartram and Rees, 2000). Once the nature and significance of the processes contributing to beach contamination is determined, possible monitoring approaches should be evaluated in the light of site-specific knowledge about sources and environmental variables.

Portuguese law criteria, DL 135/2009 of 3 June, that regulates bathing water identification, management, monitoring, classification and provision of information to the public on bathing water quality, establishes a minimum of four samples by bathing season in each designated bathing water. If the water profile fails to represent true variability and quality of bathing water, that will be reflected in less strict standards and competent authorities may not feel obligated to go beyond the minimum required by law. This may result in a high percentage of missing water quality exceedences. Acording to Leecaster and Weisberg (2001), who assessed five years of data from 24 Los Angeles area sites, sampling five times per week, resulted in observing 80% of the events in which standards were exceeded, and the frequency dropped to 55%, 25% and 5% for three times per week, weekly, and monthly sampling, respectively. They also found that nearly 70% of water quality exceedances were single-day events, even in the most frequently contaminated sites. This also poses a problem, since, according with the DL 135/2009, short-term pollution is not normally expected to affect bathing water quality for more than approximately 72 hours after the bathing water quality is first affected and in case of such event, the sample is not to be included in the bathing water quality data. Although the objective of this study was not to evaluate short time pollution, in Matosinhos beach, fecal coliforms concentrations were found to vary from poor to good, in few hours, so bathing water profile should reflect this, allowing timely and adequate management measures to be taken by authorities. Such measures should include information to the public and, if necessary, a temporary bathing prohibition

Improved monitoring will, therefore, ascertain trends in the quality of the environment and how quality is affected by anthropogenic activities (Bartram and Rees, 2000), allowing the mitigation of pollution events that pose any threat to public health and the achievement of the Bathing Water Directive(2006/7/EC) main goal: ‘good quality’ status in all European beaches.

The applied principal components regression approach aimed to estimate the values of the bacteria indicators at the basis of selected PCs of explanatory variables. Univariate simple regression models, can yield a high R^2 value, but may fail to define the complex nature of the ecosystem, whereas multivariate models are capable of assessing large number of variables and interrelations and are, therefore, more successful in defining and predicting biological processes (Çamdevýren et al., 2005). Multiple linear regression analyses has been used to investigate relationships between fecal indicator concentrations and environmental conditions (Crowther *et al*., 2001), but, when correlated predictors are used, multicollinearity can become the cause of statistical imprecision and unstable estimation of regression coefficients (Gurmessa and Bárdossy, 2009). The use of principal component scores in multiple linear regression models was successful in producing statistical significant models predicting mean fecal indicator concentrations. The maximum predictive success (R^2) was observed in Matosinhos and Pastoras beach, the most polluted beaches, were 65% and 70% of fecal coliforms variation could be defined, using 48.3% and 64.9% of the variation in the abiotic parameters studied.

Conclusion

Urban beaches are exposed to several environmental pressures, among them the discharges of non-treated or poorly municipal effluents to rivers or directly to coastal waters. The lack of treatment of those effluents and the lack of regulatory enforcement represents a health hazard to beachgoers, who, often, ignore the real state of the bathing area they are visiting. The present study demonstrated that spatial and temporal patterns of fecal indicator bacteria in urban coastal beaches are complex and highly variable, having immediate practical implications for the monitoring and mitigation of coastal pollution. Environmental conditions of the beach and adjacent areas, effluent discharges to rivers or beaches, as well as weather conditions and water hydrodynamics are essential to better understand quality evolution during the day and bathing season. These parameters are major influences to bathing water quality, therefore are extremely important when establishing the bathing water profile required by the new law critera.

In order to better establish management and monitoring plans, Geographical Information Systems are effective tools representing surface water quality and may play an important role improving assessment and decision-making.

Finally, the statistical model implemented could be a useful tool to eliminate multicolinearity problems and to reduce number of the variables in multiple regression models, yielding a reasonable predictivity for the abundance of fecal indicator bacteria.

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