Available online at www.sciencedirect.com
ScienceDirect
Int. J. Hyg. Environ. Health 211 (2008) 510–517
International Journal of Hygiene and Environmental Health
www.elsevier.de/ijheh

Microbial indicators of faecal contamination in waters and sediments of beach bathing zones

M.C. Garrido-Pérez*, E. Anfuso, A. Acevedo, J.A. Perales-Vargas-Machuca

Department of Chemical Engineering, Food Technologies and Environmental Technologies, Faculty of Marine and Environmental Sciences, University of Cadiz, Pol. Río San Pedro s/n, 11510 Puerto Real, Cadiz, Spain

Received 13 June 2006; received in revised form 15 January 2007; accepted 10 September 2007

Abstract

This study presents the results obtained of the microbial characterization of waters and sediments of 18 coastal bathing zones of the south-western coast of the Iberian Peninsula. To make this characterization, two indicators of faecal contamination have been selected: *faecal coliforms* (FC) and *Clostridium perfringens* (CP). The results show that low concentrations of FC and CP in water not necessarily implies that their concentration in sediment and elutriates has to be low as well. The highest concentrations were found in locations close to the mouth of rivers, and in beaches of low energy and hence low water renewal, and high accumulation of fine sediments. The concentrations of FC were lower than those obtained for CP in most of the sampling locations. Although quality standards for bathing waters do not take the parameter CP into account, it has been demonstrated that it should be a good indicator of faecal contamination.
© 2007 Elsevier GmbH. All rights reserved.

Keywords: Microbial pollution; Faecal coliforms; *Clostridium*; Bathing waters; Marine sediments; Elutriate; Cadiz (Spain)

Introduction

Coastal water pollution by urban wastewater is a fundamental environmental problem on a world scale (Steets and Holden, 2003; Wells, 2003). In the European Union, Directive 76/160/CEE (ECC, 1976) relating to the quality of bathing waters, regulates a total of five microbial indicators that are accepted indicators for sanitary control in bathing waters: total coliforms, faecal coliforms (FC), faecal streptococci, *Salmonella* and enteroviruses. Due to the difficulty and the cost represented by the analysis of *Salmonella* and enteroviruses, currently the routine microbial control of bathing waters is limited to the monitoring of coliforms and streptococci. This is one of the reasons that has led the European Commission to put forward, on various occasions during the last decade, the need to revise and modify the ruling Directive, with the objective of adapting the microbial quality criteria, to current scientific and technological progress (ECC, 2002). On February 2006 was approved the Directive 2006/7/CE concerning the management of bathing water quality and repealing Directive 76/160/EEC (ECC, 2006). One of the main aims of this Directive is to tighten but simplify the health standards for bathing water, limiting microbial control of waters to *Escherichia coli*, and *Enterococcus intestinalis*, bacterial groups included within the groups of "faecal coliforms" and "faecal streptococci", respectively. The new indicators selected provide the best correspondence available between

*Corresponding author. Tel.: +34 956 01 64 23; fax: +34 956 01 60 40.
E-mail address: carmen.garrido@uca.es (M.C. Garrido-Pérez).

1438-4639/$ - see front matter © 2007 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ijheh.2007.09.010

faecal contamination and the "short-term" health effects in recreational waters.

A significant deficiency in the previously cited normatives is that monitoring of bathing zones is limited to the waters, without taking into account the presence and concentration of microorganisms in the sediments or beds of aquatic systems. In general, very few studies have been published related to microbial quality of marine sediments. In some of these studies, it is concluded that, while the survival rate of microorganisms is slow in waters, in contrast, sediments are reservoirs of a many enteric organisms (Stephenson and Rychert, 1982; Stenstrom and Carlander, 2001; Wheeler et al., 2003; Shibata et al., 2004). This greatly increased rate of survival of these microorganisms in sediments is due to several reasons: the better nutritive conditions of sediment (Davies et al., 1995; Villar et al., 1999); sediment is a compartment that is more protected against the inactivation produced by solar radiation (Sinton et al., 1994; Davies-Colley et al., 1999); and it provides greater protection against depredation by protozoans (Davies and Bavor, 2000).

The genus *Clostridium* is notable within the group of more resistant microorganisms. *Clostridium* is a group of anaerobic bacteria whose natural habitat is the soil or animal and human intestines where they live as saprophytes. These microorganisms are characterized by forming endospores that enable them to survive in different habitats, both terrestrial and aquatic, waiting for favourable conditions for their growth. Thanks to these characteristics, they can be considered indicators of remote episodes of anthropogenic contamination, since they remain in a latent state while temperatures remain below 20 °C or in the presence of oxygen. In the last decade, the importance of this bacterial group in the study and control of coastal waters and sediments has been reflected in numerous papers and protocols (USEPA, 1986; Hill et al., 1996; Edwards et al., 1998; Lipp et al., 2001; Shibata et al., 2004; Skanavis and Yanko, 2001; Hughes and Thompson, 2004; Characklis et al., 2005; Dahlen et al., 2006).

In bathing zones, the continuous movement of the sediment could produce a transference of microorganisms to the water column (Crabill et al., 1999; An et al., 2002; Craig et al., 2004). Therefore, although the "first" and the "new" Directive do not include the microbial control of sediments, it is necessary to conduct studies that evaluate the possibility of including the analysis of sediments in these zones, and other groups of more resistant microorganisms that allow the identification of "long-term" faecal pollution.

In this paper, we present the results obtained from the microbial characterization of the waters, sediments and elutriates sampled from 18 beaches situated over a length of 200 km of the littoral of the province of Cadiz, in the southwest of the Iberian Peninsula. This characterization has been carried out with two related objectives: (I) to evaluate the possibility of utilizing the bacterial group "*Clostridium perfringens*" (CP) in the sanitary control of beaches, as a "long-term" microbial indicator of faecal pollution; and (II) to compare the microbial results obtained in samples of water, sediments and elutriates of sediments in bathing zones.

Material and methods

Description of the sampling zone

The zone of study is situated in the extreme southwest of Europe, between 5° and 6° of longitude west, and between 36° and 37° of latitude north, close to the Strait of Gibraltar, at the confluence of the Atlantic Ocean and the Mediterranean Sea. Fig. 1 shows the location of the beaches selected for this study.

The littoral strip sampled has a semidiurnal tidal range, and mean tidal heights between 1.20 and 3.30 m. Most of the beaches are composed of fine-medium grain size sand ($D_{50} = 250\,\mu m$) consisting of quartz at 85–95% and calcium carbonate at 5–15% (Muñoz et al., 2001). The station situated in the inner waters of the Bay of Cadiz (station no. 7) present a variable composition of mud and sand ($D_{50} = 100$–$330\,\mu m$) (Forja et al., 2004).

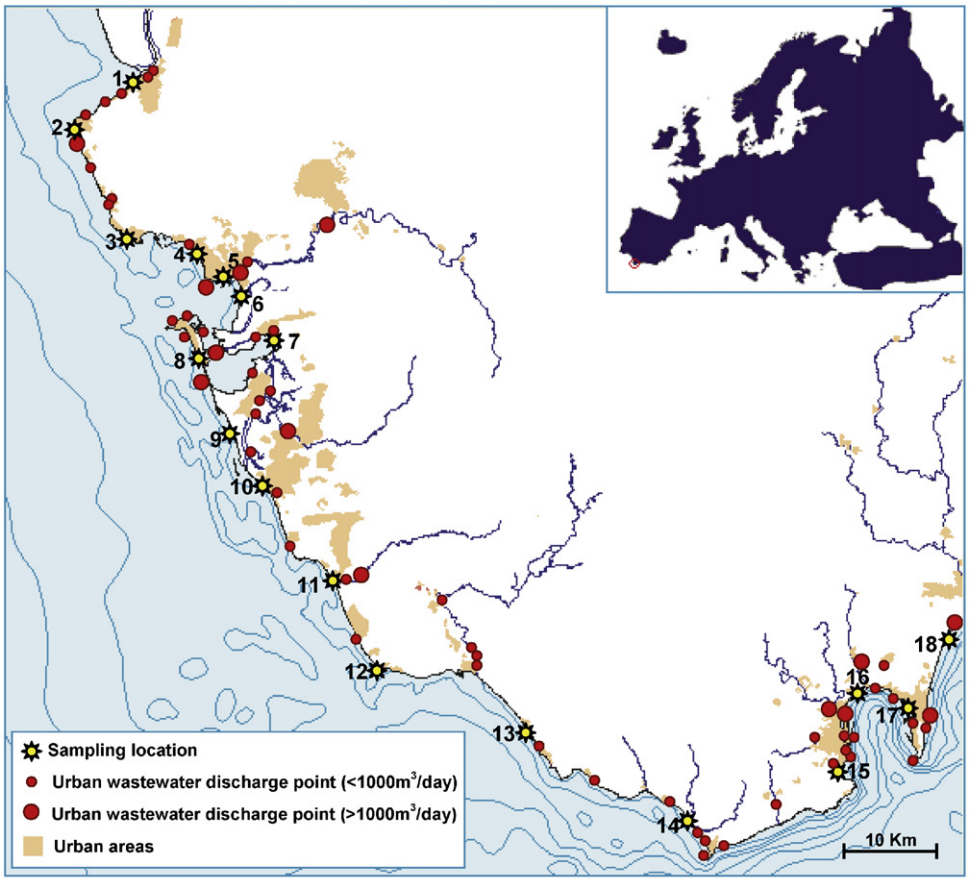
During the period of sampling (5 consecutive days of April–May just before the bathing season), the ambient temperature ranged 20–22 °C and the sky was clear with practically no cloud.

Sampling strategy

Sample locations were selected as a single point located in the area of highest bather density of each beach. In each location, water and sediments samples were taken in at low tide, just on the change from ebb to flood flow, and according to the sampling methodology approved in Directive 2006/7/CE (ECC, 2006). First, a sample of the water column was taken at 30 cm below the surface, to avoid the layer of water directly affected by the ultraviolet radiation of the sun. Water samples were collected in sterile borosilicate bottles. Next, a single-surface (top 4 cm) sediment samples were collected using a small Van Been grab sampler (1 l) and stored in a sterile bag. Both samples were stored in a cool box (temperature around 4 °C and darkness), and immediately they were transported to the laboratory for analysis. The time between sampling and analysis was less than 4 h.

Microbial analysis of the samples

The determination of the microbial indicators in liquid samples was done using the technique of



Station ID	City Name	Beach Name
1	Sanlucar de Barrameda	Bajo de Guia
2	Chipiona	(La) Regla
3	Rota	(La) Costilla
4	El Puerto de Santa Maria	Fuentebravia
5	El Puerto de Santa Maria	La Puntilla
6	El Puerto de Santa Maria	Valdelagrana
7	Puerto Real	(La) Cachucha
8	Cadiz	Victoria
9	San Fernando	Camposoto
10	Chiclana de la Frontera	(La) Barrosa
11	Conil de la Frontera	(Los) Bateles
12	Barbate	Caños de Meca
13	Barbate	Zahara
14	Tarifa	(Los) Lances
15	Algeciras	Getares
16	Los Barrios	Palmones
17	La Linea de la Concepción	Poniente
18	La Linea de la Concepción	(La) Alcaidesa

Fig. 1. Locations and codes of sampling bathing zones.

membrane filtration and incubation in a culture medium at an adequate temperature (APHA, 1992). Each sample was analysed in triplicate. The nutritive medium m-FC Broth Base (Difco Laboratories) was utilized for the analysis of FC. The incubation temperature was $45 \pm 0.5\,^{\circ}C$, and the incubation time was 24 h. Dark blue colonies were counted as FC. CP were enumerated on m-CP Agar Base (Scharlab, S.L.) and incubated anaerobically for 48 h at $45 \pm 0.5\,^{\circ}C$. The anaerobic incubation atmosphere was obtained utilizing a Gas-Pack (Mitsubishi Gas Chemical Company, Inc.) jar and an AnaeroGen™ (Oxoid Limited) system for the

generation of anaerobic medium, controlled by a BR-55 (Oxoid Limited) indicator for anaerobiosis. Yellow colonies that turned dark pink to magenta after exposure to ammonium hydroxide fumes were counted as CP.

The most probable number (MPN) was the technique employed for the analysis of the sediment samples (USEPA, 1986). Prior to the seeding, sediment was mixed with buffered water (pH = 7.2) in the proportion 1:1 (weight/volume). Four series of dilutions of the mixture on logarithmic scale with the nutritive medium were prepared: 1/1, 1/10, 1/100 and 1/1000 (volume/volume). The 1/1 dilution is obtained making a double-concentrated culture medium. Each dilution was analysed in quintuplicate. For the analysis of FC, the A-1 (Difco Laboratories) medium was utilized. For the analysis of CP, the medium utilized was Lactose Sulfite broth (Scharlab, S.L.). The temperatures, time and conditions of incubation were the same as those described in the microbial analysis of waters. For the identification of positive tubes, a Durhan-type bell was introduced into each tube before proceeding to sterilize the culture medium. Once the period of incubation had elapsed, those tubes that presented gas in the bell, produced by microbial decomposition, were identified as positive. Each sample was analysed in triplicate.

Microbial analysis of elutriates from the sediment

It is important to know the concentration of microorganisms in the pore water because these could migrate most easily to the water column under turbulent conditions than those attached to the sediment. For this, it is customary to perform procedures of elutriation of the sediments and the subsequent analysis of the aqueous fraction of elutriates (USEPA, 1991). The procedure of elutriation consisted of mixing the sediment with sterilized synthetic seawater (formula from APHA, 1992) in 1:4 (weight/volume) proportion (USEPA/USACE, 1998). The mixture is stirred vigorously for 30 min with a magnetic stirrer. At 10 min intervals, the mixture is also stirred manually to ensure complete mixing. After the 30 min mixing period, the mixture is allowed to settle for 1 h (USEPA, 1991). The supernatant is then extracted using sterile pipette. The aqueous phase obtained was analysed using the technique of the MPN as described previously.

Expression of the results

The microbial results obtained have been referred to a volume of 100 mL in the analyses utilizing the membrane filtration technique, and to a dry weight of 100 g when the MPN technique was utilized in the analysis of sediment and elutriate. With the objective of being able to compare the number of bacteria obtained in each phase, the specific density of water has been approximated to 1 g/mL.

Chemical analysis of waters and sediments

Temperature of water was measured in each location before the sampling strategy using an electronic portable thermometer (Hanna instruments checktemp). Mean water temperature was $17.9 \pm 0.2\,^{\circ}C$.

Some complementary analyses were performed on waters and sediments in the laboratory. In waters, the pH (Crison GLP-21 pHmeter) and the salinity (Crison GLP-32 conductivimeter) of the samples were measured according to the procedures proposed by Grasshoff et al. (1983). In sediments, the dry-weight/wet-weight ratio, necessary for the expression of the microbial results, was determined gravimetrically, drying about 10 g of sediment in an oven (Selecta S.A. Digitronic) at $105 \pm 1\,^{\circ}C$ to constant weight (ASTM, 1996). Also the organic matter content in the sediments was determined by means of calcination of the sample in a Mufla oven (Selecta S.A. Select-Horn) at 550 °C and subsequent determination of the dry residue and the volatile fraction (APHA, 1992). Water was analysed within 6 h after sampling time whereas the sediment analysis requires more time, i.e., 24 h for the wet–dry ratio plus 24 h for the organic matter determination.

Results and discussion

Table 1 and Fig. 2 shows the results obtained from the analysis of the microbial indicator FC in samples of water (FC(w)), sediment (FC(s)) and elutriate (FC(e)) in the bathing zones sampled.

All the concentrations obtained for FC(w) in this study (single samples) are under the guideline concentration of 200 CFU/100 mL (annual geometrical mean) (Directive 76/160/CEE). They also are under the demanding requirements for coastal bathing waters of excellent quality that are included in the new Directive (Directive 2006/7/EC) of 250 CFU/100 mL (95th percentile evaluation for *E. coli*). With the exception of stations 4 and 5, which present the highest concentrations of FC(w), the levels of FC(w) at the rest of the stations ranged 0–8 CFU/100 mL.

The concentrations of FC(s) and FC(e) are much higher than in the samples of water, at all stations except station 4. The concentrations of FC(s) ranged 4–12 MPN/100 g dry weight, with the exception of stations 7, 16 and 28, where the concentrations are higher (117, 34 and 28 MPN/100 g dry weight, respectively).

Table 1. Mean concentration (and standard deviation, $n = 3$) of *faecal coliforms* in water, sediment and elutriate of the bathing zones

Station no.	Beach name	FC(w) (CFU/100 mL)	FC(s) (MPN/100 g dry weight)	FC(e) (MPN/100 g dry weight)
1	Bajo de Guia	0 (0)	28 (23)	13 (2)
2	La Regla	0 (0)	10 (1)	6 (1)
3	La Costilla	3 (3)	10 (6)	12 (2)
4	Fuentebravia	17 (8)	10 (1)	7 (1)
5	La Puntilla	22 (3)	4 (1)	6 (1)
6	Valdelagrana	8 (8)	10 (1)	25 (2)
7	La Cachucha	2 (3)	117 (2)	60 (2)
8	Victoria	2 (3)	12 (1)	6 (1)
9	Camposoto	0 (0)	10 (1)	5 (1)
10	La Barrosa	0 (0)	10 (1)	5 (1)
11	Los Bateles	0 (0)	10 (1)	5 (1)
12	Caños de Meca	0 (0)	10 (1)	6 (1)
13	Zahara	3 (6)	4 (6)	7 (1)
14	Los Lances	3 (6)	10 (1)	6 (1)
15	Getares	0 (0)	10 (6)	6 (1)
16	Palmones	5 (5)	34 (10)	23 (4)
17	Poniente	0 (0)	4 (1)	6 (1)
18	La Alcaidesa	2 (3)	14 (95)	75 (10)

Table 2 presents the results obtained from the analysis of CP in samples of water (CP(w)), sediment (CP(s)) and elutriate (CP(e)).

The concentrations of CP in the three media are found to be clearly higher than those obtained for the parameter FC (Table 1). This difference is especially striking in the case of the samples of sediment, where the concentrations of CP are between one and two orders of magnitude greater than those of FC. These differences may be due to the greater resistance of the CP spores in marine environments, compared with the FC (Skanavis and Yanko, 2001).

In a work done in Hawaiian streams, Fujioka and Shizumura (1985) suggested that concentrations of CP higher than 50 CFU/100 mL are indicative of human faecal contamination in water. Other authors (Lipp et al., 2001) used this criterion to determine the faecal origin of CP in coastal zones. In accordance with this criterion, of the 18 bathing zones sampled, four would be considered to be affected by faecal contamination in the water column, while 16 would be so considered with respect to the sediment.

In Table 3, the complementary chemical results analysed in the bathing waters and sediments sampled are shown. Table 4 shows the Pearson Correlation matrix among all the chemical and microbial variables determined in this study.

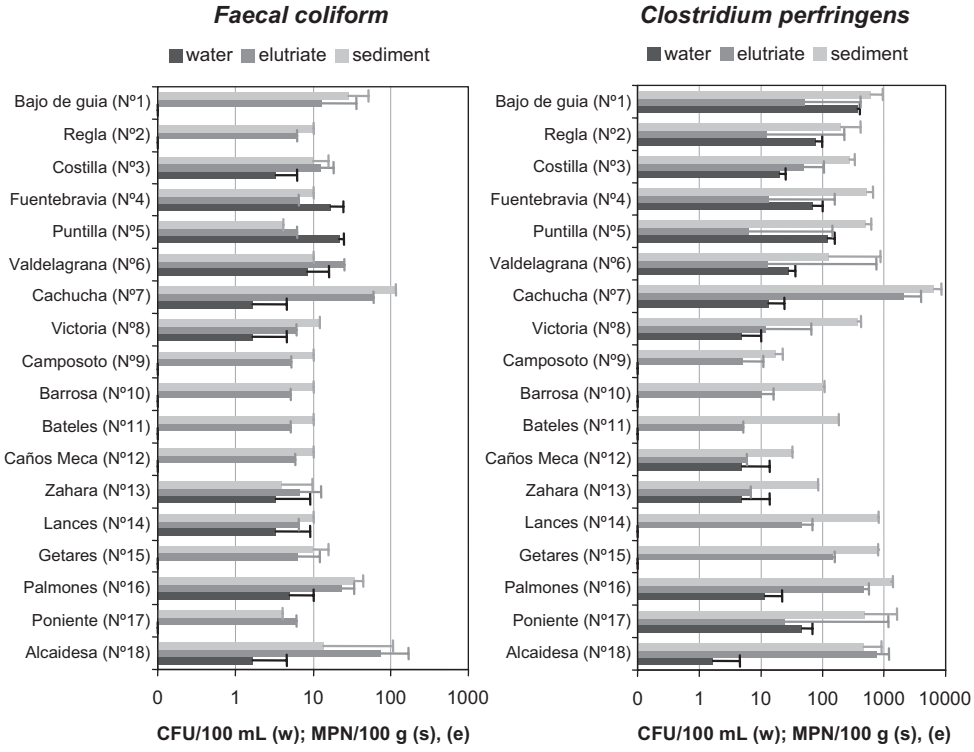


Fig. 2. Mean density (⊥ Standard Deviation) of *Faecal coliform* and *Clostridium perfringens* in water (w), sediment (s) and elutriate (e) of 18 bathing zones.

Table 2. Mean concentration (and standard deviation, $n = 3$) of *Clostridium perfringens* in water, sediment and elutriate of the bathing zones

Station no.	Beach name	CP (w) (CFU/100 mL)	CP (s) (MPN/100 g dry weight)	CP (e) (MPN/100 g dry weight)
1	Bajo de Guia	363 (35)	614 (354)	51 (3)
2	(La) Regla	77 (20)	197 (214)	12 (6)
3	(La) Costilla	20 (5)	274 (55)	50 (23)
4	Fuentebravia	70 (30)	522 (143)	13 (7)
5	(La) Puntilla	122 (33)	495 (137)	6 (2)
6	Valdelagrana	28 (8)	126 (750)	13 (2)
7	(La) Cachucha	13 (10)	6596 (1939)	2061 (512)
8	Victoria	5 (5)	367 (53)	12 (3)
9	Camposoto	0 (0)	17 (6)	5 (2)
10	(La) Barrosa	0 (0)	102 (6)	10 (1)
11	(Los) Bateles	0 (0)	180 (5)	5 (2)
12	Caños de Meca	5 (9)	32 (3)	6 (4)
13	Zahara	5 (9)	84 (4)	7 (1)
14	(Los) Lances	0 (0)	807 (23)	45 (11)
15	Getares	0 (0)	805 (10)	148 (22)
16	Palmones	12 (10)	1315 (95)	467 (31)
17	Poniente	47 (21)	485 (1161)	24 (22)
18	(La) Alcaidesa	2 (3)	464 (445)	754 (117)

Table 3. Complementary chemical analysis of the samples

Station no.	Beach name	pH (w)	Salinity (w)	% Organic matter (s)
1	Bajo de Guia	8.01	29.2	0.75
2	(La) Regla	8.19	36.0	0.92
3	(La) Costilla	8.30	37.8	1.20
4	Fuentebravia	8.21	38.1	1.34
5	(La) Puntilla	8.26	38.0	1.26
6	Valdelagrana	8.31	38.6	0.83
7	(La) Cachucha	8.68	39.4	2.96
8	Victoria	8.14	39.0	0.97
9	Camposoto	8.05	38.0	1.01
10	(La) Barrosa	8.05	38.1	1.13
11	(Los) Bateles	8.04	38.0	0.89
12	Caños de Meca	8.15	37.7	1.10
13	Zahara	8.12	36.8	1.11
14	(Los) Lances	8.25	41.0	1.06
15	Getares	8.26	40.5	2.34
16	Palmones	8.16	37.6	0.72
17	Poniente	8.13	38.4	0.64
18	(La) Alcaidesa	8.26	37.9	0.54

The lack of correlation between the FC(w) and FC(s) or FC(e) indicates that the absence of FC in the water column does not necessarily imply its absence in the sediment. The sediments are usually reservoirs of the contamination that reaches the coastal waters. The difference in the concentration of microorganisms measured between water and sediment as shown by the results corroborates with the results obtained by other authors for bathing zones (Crabill et al., 1999; An et al., 2002; Wheeler et al., 2003; Shibata et al., 2004). In the water column, sunlight is one of the factors that has most influence on the disappearance of microorganisms of enteric origin. Deposition in the sediments provides these microorganisms protection from the ultraviol et radiation of the sun (Wheeler et al., 2003).

Table 4 shows a significant correlation between the concentrations of FC(s) and FC(e). In a bathing zone, where the depth of the water column does not exceed 1.5–2 m on average, the sediment is in continuous movement caused by the littoral currents, the entry and breaking of the waves on the beach, and the recreational activities of the bathers. Given that the procedure of elutriation simulates the natural agitation of the surface layers of the sediment with the water, this correlation indicates that a part of the FC present in the sediment migrates to the water column.

The average percentage of FC present in the sediment that passed to the aqueous phase following the process of elutriation was 56% (SD = 8, $n = 12$). Those stations where the samples of elutriate presented FC concentrations higher than those of the sediment have not been considered.

No correlation has been obtained between the concentrations of CP in water and those in sediment or elutriate. There is a clear correlation between CP in sediment and elutriate. This latter result has the same implications as those presented in the case of the FC: that it is possible for CP to be transferred from the sediment to the water column in turbulent conditions. Despite the mobility of the CP observed, this is found to be much lower than that of the FC. The average percentage of CP in the elutriate compared with that originally present in the sediment at the various sampling stations was 12.63% (SD = 10.82, $n = 17$).

Considering the sampling period was realized in a dry period (no rainfall occurred), the strong correlation existing between FC(s) and CP(s) could indicate that the main CP(s) source is human faecal pollution from domestic wastewater in this coastal area. Other possible sources such as runoff or re-growth in sediments could be present but not in important percentages with respect to the pollution charge of bacteria in this coastal area where the disinfection processes of domestic wastewater are deficient.

Only in the water column it has been observed a significant negative correlation between chemical and microbial indicators; this is between CP(w) and salinity. It indicates that the bathing waters influenced by continental waters are those that are at greater risk of presenting microbial contamination in the water column by CP. Table 4 confirms this interaction, since the stations situated at the mouths of two important rivers

Table 4. Matrix of Pearson correlations of the variables analysed in bathing zones

	pH (w)	Salinity (w)	Organic matter (s)	FC(w)	FC(s)	FC(e)	CP(w)	CP(s)	CP(e)
pH (w)	1.00								
Salinity (w)	0.43	1.00							
Organic matter (s)	*0.72*	0.38	1.00						
FC(w)	0.22	0.11	0.05	1.00					
FC(s)	*0.72*	0.02	*0.67*	−0.12	1.00				
FC(e)	*0.61*	0.06	0.19	−0.05	*0.60*	1.00			
CP(w)	−0.24	*−0.87*	−0.19	0.15	0.03	−0.10	1.00		
CP(s)	*0.80*	0.17	*0.76*	−0.03	*0.97*	*0.57*	−0.05	1.00	
CP(e)	*0.79*	0.17	*0.63*	−0.09	*0.93*	*0.80*	−0.13	*0.94*	1.00

Correlations in italics are significant at $p < 0.05$.

in the study area (stations 1 and 5) are the ones that present higher concentrations of CP(w), presumably due to the role that these rivers play as sources of contamination to the sea.

A large number of significant interactions between chemical and microbial indicators of the sediment have been determined. The positive correlations obtained between FC(s) and CP(s) and the content in organic matter are especially notable. The stations influenced by the mouths of rivers and the stations where the sediments have a greater content in organic matter, normally sediments of fine granulometry, are those that present the highest concentrations of microorganisms (Tables 1 and 2). The most serious microbial contamination by FC(s) and CP(s) and by organic matter corresponds to station 7 situated on a beach of the interior of the Bay of Cadiz, a beach with very fine sediment, subject to low wave action, and influenced by several wastewater discharge points and contaminated runoff. These characteristics could promote processes of accumulation, re-growth and/or survival of microorganisms in the sediment as several authors had shown in coastal areas (Howell et al., 1996; Crabill et al., 1999; Wheeler et al., 2003; Sanders et al., 2005; Evanson and Ambrose, 2006; Lee et al., 2006).

Conclusions

The principal results drawn from this study are the following:

(I) The absence of FC in bathing waters does not indicate that faecal contamination events have not taken place. The joint analysis of other types of more resistant microbial indicators such as CP provides a useful complementary information when classifying bathing zones as not influenced, in either the short or long term, by domestic wastewater that may represent a risk for the health of sea bathers and of others pursuing recreational activities in these waters.

(II) At present, the sanitary control of bathing waters only involves the microbial characterization of the waters themselves. The microbial results obtained in this study indicate that the absence of indicators of faecal contamination in the waters is not conclusive for stating that the sanitary quality of the bathing waters is guaranteed. Elutriation procedure simulates the turbulence that could occur in the sediments of bathing zones and recreational activities. Migration of FC and CP could takes place from the sediment to the water column. For these reasons it is recommended that microbial analysis of the sediment, both its solid phase and the pore water, should be included in the diagnosis of the microbial quality of bathing waters.

References

American Public Health Association (APHA), 1992. Standard Methods for the Examination of Water and Wastewater, 18th ed. APHA, Washington, DC.

American Society for Testing and Materials (ASTM), 1996. Standard guide for collection, storage, characterization, and manipulation of sediments for toxicological testing. ASTM E 1391–94. Annual Book of ASTM Standards, vol. 11.05, pp. 805–825.

An, Y.J., Kampbell, D.H., Breidenbach, G.P., 2002. *Escherichia coli* and total coliforms in water and sediments at lake marinas. Environ. Pollut. 120, 771–778.

Characklis, G.W., Dilts, M.J., Simmons III, O., Likirdopulus, C.A., Krometis, L.A.H., Sobsey, M.D., 2005. Microbial partitioning to settleable particles in stormwater. Water Res. 39, 1773–1782.

Crabill, C., Donald, R., Snelling, J., Foust, R., Southam, G., 1999. The impact of sediment faecal coliform reservoirs on seasonal water quality in Oak Creek, Arizona. Water Res. 33 (9), 2163–2171.

Craig, D., Fallowfield, H., Cromar, N., 2004. Use of macrocosms to determine persistence of *Escherichia coli* in

recreational coastal water and sediment and validation with in situ measurements. J. Appl. Microbiol. 96, 922–930.

Dahlen, D., Hunt, C., Emsbo-Mattingly, S., Keay, K., 2006. Are toxic contaminants accumulating in Massachusetts coastal sediments following startup of the Massachussetts Bay outfall: a comprehensive comparison of baseline and post-diversion periods. Mar. Pollut. Bull. 52, 1372–1388.

Davies, C.M., Bavor, I.I.J., 2000. The fate of storm water associated bacteria in constructed wetland and water pollution control pond system. J. Appl. Microbiol. 89 (2), 349–360.

Davies, C.M., Long, J.A.H., Donald, M., Ashbolt, N.J., 1995. Survival of faecal microorganisms in marine and freshwater sediments. Appl. Environ. Microbiol. 61 (5), 1888–1896.

Davies-Colley, R.J., Donnison, A.M., Speed, D.J., Ross, C.M., Nagels, J.W., 1999. Inactivation of faecal indicator microorganisms in waste stabilisation ponds: interaction of environmental factors with sunlight. Water Res. 33 (5), 1220–1230.

Edwards, D.D., Mc Feters, G.A., Venkatesan, M.I., 1998. Distribution of *Clostridium perfringens* and faecal sterols in a benthic coastal marine environmental influenced by the sewage outfall from McMurdo Station, Antarctica. Appl. Environ. Microbiol. 64, 2596–2600.

European Community Comission (ECC), 1976. Council Directive 76/160/CEC, of 8 of December of 1975, concerning the quality of bathing waters. Offic. J. L 31, 1–7.

European Community Comission (ECC), 2002. Proposal of Council Directive concerning the quality of bathing waters: COM 2002/0254, 581 final.

European Community Comission (ECC), 2006. Council Directive 2006/7/EC, of 15 February 2006, concerning the management of bathing water quality and repealing Directive 76/160/EEC. Offic. J. L 64, 37–51.

Evanson, M., Ambrose, F., 2006. Sources and growth dynamics of fecal indicator bacteria in a coastal wetland system and potential impacts to adjacent waters. Water Res. 40, 475–486.

Forja, J., Ortega, T., Del Valls, T.A., Gomez-Parra, A., 2004. Benthic fluxes of inorganic carbon in shallow coastal ecosystems of the Iberian Peninsula. Mar. Chem. 85, 141–156.

Fujioka, R.S., Shizumura, L.K., 1985. *Clostridium perfringens*, a reliable indicator of stream water quality. J. Water Pollut. Control Fed. 57, 982–986.

Grasshoff, K., Ehrhard, M., Kremlings, K., 1983. Methods of Seawater Analysis, second ed. Verlag Chemie, Weinheim.

Hill, R.T., Straube, W.L., Palmisano, A.C., Gibson, S.L., Colwell, R.R., 1996. Distribution of sewage indicated by *Clostridium perfringens* at a deep-water disposal site after cessation of sewage disposal. Appl. Environ. Microbiol. 62, 1741–1746.

Howell, J.M., Coyne, M.S., Cornelius, P.L., 1996. Effect of sediment particle size and temperature on fecal bacteria mortality rates and the fecal coliform/fecal streptococci ratio. J. Environ. Qual. 25, 1216–1220.

Hughes, K.A., Thompson, A., 2004. Distribution of sewage pollution around a maritime Antarctic research station indicated by faecal coliforms, *Clostridium prefringens* and faecal sterol markers. Environ. Pollut. 127, 315–321.

Lee, C.M., Lin, T.Y., Lin, Ch., Kohbodi, G.A., Bhatt, A., Lee, R., Jay, J.A., 2006. Persistence of fecal indicator bacteria in Santa Monica Bay beach sediments. Water Res. 40, 2593–2602.

Lipp, E.K., Farrah, S.A., Rose, J.B., 2001. Assessment and impact of microbial faecal pollution and human enteric pathogens in a coastal community. Mar. Pollut. Bull. 42 (4), 286–293.

Muñoz, J.J., Lopez de San Roman, B., Gutierrez-Mas, J.M., Moreno, L., Cuena, G.J., 2001. Cost of beach maintenance in the Gulf of Cadiz SW Spain. Coastal Eng. 42, 143–153.

Sanders, B.F., Arega, F., Sutula, M., 2005. Modeling the dry-weather tidal cycling of fecal indicator bacteria in surface waters of an intertidal wetland. Water Res. 39, 3394–3408.

Shibata, T., Solo-Gabriele, H.M., Fleming, L.E., Elmir, S., 2004. Monitoring marine recreational water quality using multiple microbial indicators in an urban tropical environment. Water Res. 38, 3119–3131.

Sinton, L.W., Davies-Colley, R.J., Bell, R.G., 1994. Inactivation of enterococci and faecal coliforms from sewage and meatwork effluents in seawater chambers. Appl. Environ. Microbiol. 60 (6), 2040–2048.

Skanavis, C., Yanko, W.A., 2001. *Clostridium prefringens* as a potential indicator for the presence of sewage solids in marine sediments. Mar. Pollut. Bull. 42 (1), 31–35.

Steets, B.M., Holden, P.A., 2003. A mechanistic model of runoff-associated fecal coliform fate and transport through a coastal lagoon. Water Res. 37, 589–608.

Stenstrom, T.A., Carlander, A., 2001. Occurrence and die off of indicator organism in the sediment in two constructed wetlands. Water Sci. Technol. 44 (11–12), 223–230.

Stephenson, G.R., Rychert, R.C., 1982. Bottom sediment a reservoir of *Escherichia coli* in rangeland streams. J. Range Manage. 35 (1), 119–123.

United States Environmental Protection Agency (USEPA), 1986. Recommended protocols for microbiological studies in Puget Sound. Puget Sound Estuary Program, Puget Sound Monitoring Program. USEPA, Region 10. Office of Puget Sound, Settle, WA.

United States Environmental Protection Agency (USEPA), 1991. Evaluation of Dredged Material Proposed for Ocean Disposal, Testing Manual. Office of Water (4504F), EPA 503/8-91/001.

United States Environmental Protection Agency (USEPA), United States Army Corps of Engineers (USACE), 1998. Evaluation of Dredged Material Proposed for Discharge in Waters of the US Washington, DC – testing manual, EPA-823-B-98-004.

Villar, C., De Cabo, L., Vaithiyanathan, P., Bonetto, C., 1999. Pore water N and P concentration in a floodplain marsh of the Lower Parana River. Hydrobiology 392 (1), 65–71.

Wells, P.G., 2003. State of the marine environment reports – a need to evaluate their role in marine environmental protection and conservation. Mar. Pollut. Bull. 46, 1219–1223.

Wheeler, E.A., Burke, J., Spain, A., 2003. Faecal indicator bacteria are abundant in wet sand at freshwater beaches. Water Res. 37, 3878–3983.