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

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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.

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

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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