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Microbiological Pollution of Ria Formosa (South of Portugal)

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A 24-month survey on the microbiological quality of recreational shore marine waters in three sampling stations located at Ria Formosa (South of Portugal) was conducted. The classical indicators of faecal pollution, such as total coliforms (TC), faecal (thermotolerant) coliforms (FC), faecal streptococci (FS) and somatic coliphages, as well as several physico-chemical parameters (temperature, salinity, dissolved oxygen (DO) and transparency) were determined in the sampling stations, which exhibited different degrees of faecal pollution. The relationships between faecal indicators and several pathogenic microorganisms (*Salmonella*, *Pseudomonas aeruginosa* and *Candida albicans*) were also established in these recreational marine zones. The results obtained indicate that none of the indicators tested may be considered as a universal index of the presence of pathogens in water; however, faecal streptococci showed a higher and significant relationship with sewage discharges. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: pollution; marine environment; faecal indicators; pathogenic microorganisms; water quality criteria; lagoon system.

Introduction

Telluric microorganisms are introduced into aquatic environments mainly by discharges of non-treated wastewater and sewage, that are the main sources of faecal pollution in natural aquatic environments (Wallis, 1977; Borrego and Figueras, 1997; Figueras *et al.*, 1997). The discharged microorganisms are not able to grow in natural waters, and they die off after a short time period, due to the influence of several biotic and abiotic factors which vary depending on the kind of water and the prevailing conditions (Borrego et al., 1983; Rheinheimer, 1992). Although several studies have been performed, the mechanisms affecting microbial die-off in aquatic environments are not well established yet (Fattal *et al.*, 1983; Borrego and Romero, 1985; de Vicente *et al.*, 1988; Moriñigo *et al.*, 1989; 1990; Cornax *et al.*, 1990; Pereira and Alcantara, 1993).

Faecal pollution of recreational waters may be a health hazard for bathers due to the presence of several microbial pathogens, including bacteria, viruses, fungi and protozoa (Moe, 1997). Routine analyses for these pathogenic microorganisms are very difficult to perform because of the diversity and complexity of their specific methodologies (Borrego, 1994). The presence of these microbial pathogens in natural waters are currently monitored using microbial indicators of faecal pollution, such as total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS) (Dufour, 1984; Geldreich, 1997). However, a direct and consistent relationship between indicators and microbial pathogens has not been established yet (Cabelli *et al.*, 1982; Ferley *et al.*, 1989; Kay *et al.*, 1994). Thus, the use of classical bacterial indicators of water quality has been questioned (Borrego *et al.*, 1983, Saliba and Helmer, 1990; Fewtrel and Jones, 1992; Borrego and Figueras, 1997).

The main objective of the present work was to monitor Ria Formosa waters microbiologically to determine the relation among different groups of microorganisms in waters with different levels of faecal pollution. In addition, the influence of several physico-chemical parameters on the evolution of the microbial numbers in these waters was also studied.

Materials and Methods

Description of the study area and sampling

The investigations were carried out in Ria Formosa, near Faro (South of Portugal) over a two-year period. Ria Formosa extends about 55 km from Ancao (37°1′30″N–8°2′30″W), and has a variable width that reaches 6 km from Faro to Santa Maria Cape. The submerged area is calculated to be about 11 800 Ha (Monteiro, 1989), and the area includes the towns of Faro, Olhao, Loule, Tavira and Vila Real de San Antonio.

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It is protected from the Atlantic Ocean by a large string of sand dunes, from alluvium origin, composed of five barrier islands and it is cut by sixbars connecting the sea and the land. The lagoon is protected from sea storms by barrier islands and the bars regulate the flow of tides. This system is characterized as an intertidal lagoon (Dronkers and Zimmerman, 1982). However, it is also been categorized as an estuary ecosystem because of its low salinity, values of nutrients, and the high rate of water renewal (Pera, 1986).

Sampling was conducted at three stations, Ilha (I), Portas do Mar (PM) and Ponte Cais (PC) with different degrees of pollution from the discharge of urban effluents. In Ilha, the sampling place is further away from the domestic discharges. PM is the sampling station most exposed to pollution by domestic sewage, because it is located very close to the main sewage discharge of Faro. PC is located between the two above-mentioned sampling stations. In this station, commercial shellfish-growing beds and a pleasure harbour are located.

Water samples were collected, using autoclaved glass bottles in conjunction with modified ZoBell samplers, from the subsurface layer (30 cm) and transported to the laboratory in cold boxes at 4°C, where they were processed within 4 h of collection.

Physico-chemical parameters

The temperature of the water was determined using a thermometer combined with a Nansen sampler. Transparency was measured with a Secchi disc and expressed in metres. To determine in water samples, the Winkler method modified by Grasshof (1976) was used to measure dissolved oxygen (DO) which was expressed as mg of oxygen per litre. Salinity was measured by use of a multisounding CTD 100 plus (SIS).

Microbiological parameters

TC, FC, FS, *Pseudomonas aeruginosa* and *Candida albicans* were enumerated by the membrane filtration technique, according to Greenberg *et al.* (1992), and the results expressed as colony forming units (CFU) per 100 ml. The selective and recovery media used and incubation conditions were the following: mEndo Les agar (Difco Lab., Detroit, Mich.) (36 ± 1°C, 24 h) for TC; mFC agar (Difco) (44.5 ± 0.2°C, 24 h) for FC; mEnterococcus (Difco) (36 ± 1°C, 48 h) for FS; Selective agar (Diagnostics Pasteur, Paris, France) (36 ± 1°C, 48 h) for *P. aeruginosa*; and both BiGGY agar (Oxoid, Basingstoke, England, UK) and Sabouraud agar (Difco) supplemented with 1 mg/ml of chloramphenicol (Sigma Chemical, St. Louis, MO) (36 ± 1°C, 72 h) were used for the enumeration of *C. albicans*. All presumptive *P. aeruginosa* colonies were confirmed by culture on King'A, King's B and Columbia media (de Vicente *et al.*, 1988).

Salmonella was detected and quantified as described by Moriñigo *et al.* (1986). The enumeration of somatic coliphages was performed by direct counting as described by Borrego and Romero (1985), using *Escherichia coli* C as host bacteria, and modified Sholten agar (1.2% and 0.7% agar) (Havelaar and Hogeboom, 1983) as bottom and top agar layers, respectively.

Statistical analysis

The quantitative analyses by membrane filtration technique were carried out with five replicates. All the data were transformed in decimal logarithms and processed by the Microsoft Excel (Microsoft version 5) computer application.

Results

Temporal and seasonal evolution of the physico-chemical and microbiological parameters

Table 1 shows the figures obtained for the temperature of the surface water samples collected between 8:00 and 10:00 a.m. The highest value was recorded in June (25°C), and the lowest in January (11.8°C). Comparing the values for the three sampling stations (I, PM and PC), in PC station, with the greatest depth, the water temperatures were similar to or a little lower than those obtained at the other two sampling stations (I and PM), except for the coldest months (November to February), when higher temperature values were observed. No significant spatial variations among the three sampling stations were obtained. Temporal variations were related to the seasonal evolution of the atmospheric temperature.

The salinity mean values were between 33.9 and 37.1‰ for Ilha (I), 31.6 and 36.8‰ for PM, and 35.3 and 36.9‰ for PC (Table 1). Minimum figures were always recorded when rain had occurred on the sampling day or on the eve (November and December). Maximum values were obtained in spring and summer (Table 1).

In PC samples, the DO values (Table 1) were always higher compared to those obtained for PM, and in 64% of the samples, the values were higher than the values of Ilha. Maximum values for all the stations were recorded in May. Minimum values were obtained in summer months for I and PM, and for PC in winter months.

Table 1 also shows the extreme values of transparency. The maximum value advisable by Portuguese law is 2 m for recreational waters with direct contact, and the imperative value is 1 m. The values obtained for all the sampling stations were lower than 2 m, although in 70.8% of the samples values were over 1 m.

Numbers of total coliforms are given in Table 2. The highest values were recorded in PM samples, while in Ilha samples the lowest ones were obtained. Large fluctuations in the total coliform titres were present in PM (2 orders of magnitude); on the contrary, only one order of magnitude between the maximum and minimum counts was recorded for Ilha samples. For faecal coliforms the same trend was observed as for total coliforms (Table 2), with fluctuations of up to three orders of magnitude for PC samples, and only one for Ilha. The

TABLE 1
Seasonal evolution of the physico-chemical parameters in the three sampling stations at Ria Formosa (South of Portugal).[a]

Season	Temperature (°C)			Salinity (‰)			Dissolved oxygen mg l^{-1}			Transparency (m)		
	I	PM	PC	I	PM	PC	I	PM	PC	I	PM	PC
Spring	17.7 ± 2.1	17.7 ± 2.1	16.8 ± 1.4	36.4 ± 0.7	36.2 ± 0.3	36.9 ± 0.2	5.4 ± 1.4	5.7 ± 1.1	6.8 ± 1.6	0.8 ± 0.1	0.7 ± 0.2	1.0 ± 0.2
Summer	24.2 ± 0.8	24.2 ± 1.8	23.0 ± 2.3	37.1 ± 0.2	36.6 ± 0.1	36.6 ± 0.2	5.5 ± 0.7	3.7 ± 0.9	5.8 ± 1.0	1.1 ± 0.4	1.1 ± 0.4	1.3 ± 0.1
Autumn	18.8 ± 3.8	19.0 ± 3.6	18.5 ± 3.5	36.2 ± 0.5	35.5 ± 0.6	35.3 ± 1.1	5.8 ± 0.3	3.7 ± 0.8	6.1 ± 0.7	1.6 ± 0.1	1.4 ± 0.1	1.6 ± 0.1
Winter	11.8 ± 0.8	12.3 ± 0.6	12.9 ± 0.1	33.9 ± 1.8	31.6 ± 5.9	35.8 ± 0.9	8.1 ± 1.4	6.3 ± 1.6	7.7 ± 2.1	0.8 ± 0.3	0.9 ± 0.6	1.2 ± 0.4
Spring	16.0 ± 1.9	15.9 ± 1.8	15.1 ± 1.0	36.2 ± 0.6	35.6 ± 0.5	36.1 ± 0.4	9.3 ± 5.1	7.2 ± 3.6	10.7 ± 7.0	1.3 ± 0.2	1.4 ± 0.3	1.6 ± 0.1
Summer	25.0 ± 1.0	24.3 ± 0.6	22.7 ± 1.1	37.1 ± 0.8	36.8 ± 0.1	36.6 ± 0.2	4.8 ± 1.6	2.5 ± 0.4	5.9 ± 1.24	1.3 ± 0.1	0.8 ± 0.3	1.2 ± 0.3
Autumn	17.4 ± 2.7	17.3 ± 2.8	17.4 ± 2.4	36.2 ± 0.7	36.3 ± 0.5	36.0 ± 0.4	5.5 ± 0.8	3.7 ± 0.5	6.0 ± 0.7	1.6 ± 0.3	1.2 ± 0.2	1.9 ± 0.4
Winter	12.6 ± 1.1	12.9 ± 1.0	14.1 ± 0.5	35.5 ± 0.4	35.5 ± 0.1	35.8 ± 0.1	5.7 ± 0.1	4.5 ± 0.5	5.3 ± 0.6	1.4 ± 0.5	1.0 ± 0.1	1.7 ± 0.5

[a] Mean ± SD; I – Ilha; PM – Portas do Mar; PC – Ponte Cais.

TABLE 2
Seasonal evolution of the microbiological parameters in the three sampling stations at Ria Formosa (South of Portugal).

Microbial parametres	Season	Ilha			Portas do Mar ($\times 10^3$)			Ponte Cais ($\times 10^2$)		
		Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
TC	Spring	460	200	800	5300	140	30 000	44	14	140
	Summer	1500	450	3000	350	10	500	550	16	2800
	Autumn	750	170	1600	750	230	1900	120	43	300
	Winter	1200	120	2300	390	80	980	92	18	250
FC	Spring	130	20	260	86	14	160	11	2.1	28
	Summer	71	30	130	70	1.2	160	54	0.6	270
	Autumn	100	37	190	110	35	280	16	3.6	32
	Winter	240	60	520	67	14	130	18	6.1	50
FS	Spring	51	23	97	3.3	0.9	8.2	1.2	0.3	2.4
	Summer	45	12	82	2.9	0.7	5.7	1.9	0.4	6.7
	Autumn	30	17	48	3.9	0.8	7.6	1.2	0.5	2.2
	Winter	72	23	210	4.2	0.8	15	6.2	0.8	260
Coliphages	Spring	37	17	62	3.7	1.6	6	1.7	0.6	3.8
	Summer	30	8	110	4.8	1	9.2	1.8	0.4	5.5
	Autumn	38	20	64	3	0.2	7.7	1.8	0.2	3.0
	Winter	81	40	140	2.9	1.2	7.1	3.2	1.1	7.6
Salmonella	Spring	< 41	< 2	240	1.6	0.02	4.6	< 1	< 0.02	4.6
	Summer	< 1.5	< 2	9	< 0.05	< 0.002	0. 1	< 0.9	< 0.02	1.7
	Autumn	< 7.2	< 2	43	< 0.1	< 0.002	0.5	< 0.02	< 0.02	< 0.02
	Winter	< 2	< 2	< 2	< 0.3	< 0.002	1.1	< 1.5	< 0.02	4.6
P. aeruginosa	Spring	< 2.3	< 1	8	< 0.004	< 0.001	0.1	< 0.1	< 0.01	0.5
	Summer	< 2.8	< 1	12	< 0.005	< 0.001	0.07	0.4	0.08	1.7
	Autumn	< 17	< 1	87	0.1	0.002	0.3	1	0.02	5.4
	Winter	< 6.8	< 1	24	0.2	0.002	0.6	0.4	0.05	1.7

data obtained from the three sampling stations for faecal streptococci are given in Table 2. The highest values for this parameter were obtained for PM and the lowest for Ilha. In the case of phages of *E. coli* C (somatic coliphages), the results ranged between 8 plaque forming units (PFU) per 100 ml for Ilha and 9.2×10^3 pfu 100 ml^{-1} for PM.

The concentration intervals and maximum and minimum values of the three pathogenic microorganisms tested are also given in Table 2. In all the sampling stations *Salmonella* spp. were detected, with a maximum level of 2.4×10^2 100 ml^{-1} for Ilha, 4.6×10^3 100 ml^{-1} for PM and 4.6×10^2 100 ml^{-1} for PC. *P. aeruginosa* levels in all the stations were lower, with a maximum of 8.7×10^1 cfu/100 ml for Ilha, 5.7×10^2 cfu 100 ml^{-1} for PM and 5.4×10^2 cfu 100 ml^{-1} for PC. Only three samples were positive for *C. albicans*, all of them collected from PM sampling station, with a maximum of 1.2×10^1 cfu 100 ml^{-1}.

Relationships between physico-chemical and microbiological parameters

To establish the relationship between the parameters tested in the three sampling stations, the Pearson coefficient test was applied (Table 3). None of the indicator microorganisms used showed significant correlation with the number of the pathogenic microorganisms tested (*Salmonella, P. aeruginosa* and *C. albicans*), although in Ilha coliphages showed the highest values of Pearson's coefficient for *Salmonella* and *P. aeruginosa*). In PM station, faecal streptococci showed the highest relationship with the pathogenic microorganisms. In the case of PC samples, faecal streptococci and total

TABLE 3

Relationship between all the parameters tested in the three sampling stations at Ria Formosa (South of Portugal).[a]

Parametres	Ilha						Portas do Mar						Ponte Cais					
	TC	FC	FS	Ph	Sal	Pa	TC	FC	FS	Ph	Sal	Pa	TC	FC	FS	Ph	Sal	Pa
FC	0.375						0.759*						0.841*					
FS	0.284	0.257					0.236	0.241					0.450**	0.653*				
Coliphages	−0.127	0.385**	0.164				0.151	0.388**	0.450**				0.309	0.535*	0.473**			
Salmonella	0.059	0.004	0.014	0.105			−0.201	−0.238	0.296	0.111			−0.196	0.018	0.274	−0.066		
P. aeruginosa	−0.009	0.148	0.007	0.305	−0.171		0.090	0.310	0.332	0.193	−0.212		0.218	0.169	−0.045	0.066	−0.167	
Temperature	0.247	−0.527*	−0.239	−0.727*	0.008	−0.107	−0.084	−0.200	−0.154	0.097	0.473**	−0.173	0.311	0.066	−0.250	−0.286	−0.054	0.279
Salinity	−0.126	−0.423**	−0.275	−0.650*	0.020	−0.187	−0.075	−0.217	−0.289	−0.127	−0.037	−0.248	−0.069	−0.141	−0.130	−0.378	0.451**	0.016
Diss. oxygen	−0.073	0.193	0.162	0.151	−0.090	−0.243	0.419**	0.141	0.093	0.003	−0.259	−0.196	−0.156	−0.215	−0.185	−0.153	0.013	−0.481**
Transparency	−0.455**	−0.256	−0.599*	−0.039	−0.176	0.205	0.226	0.086	−0.021	−0.301	0.223	0.208	0.021	−0.057	−0.292	0.124	0.464**	0.209

[a] TC – total coliforms; FC – faecal coliforms; FS – faecal streptococci; Ph – coliphages; Sal – *Salmonella*; Pa – *P. aeruginosa*.
* Statistically significant ($p < 0.01$).
** Statistically significant ($p < 0.05$).

coliforms showed the highest correlation with *Salmonella* and *P. aeruginosa*, respectively (Table 3).

For Ilha samples, significant relationships were obtained between faecal coliforms vs coliphages ($p < 0.05$); faecal coliforms vs temperature and salinity ($p < 0.01$, and $p < 0.05$, respectively); coliphages vs temperature and salinity ($p < 0.01$); and transparency vs total coliforms and faecal streptococci ($p < 0.05$, and $p < 0.01$, respectively) (Table 3).

For PM samples, the following significant relationships were obtained: total coliforms vs faecal coliforms and DO ($p < 0.01$, and $p < 0.05$, respectively); coliphages vs faecal coliforms and faecal streptococci ($p < 0.05$); and *Salmonella* vs temperature ($p < 0.05$) (Table 3).

In the case of the PC samples, significant relationships were established between: total coliforms vs faecal coliforms and faecal streptococci ($p < 0.01$, and $p < 0.05$, respectively); coliphages vs faecal coliforms and faecal streptococci ($p < 0.01$, and $p < 0.05$, respectively); faecal coliforms vs faecal streptococci ($p < 0.01$); *Salmonella* vs salinity and transparency ($p < 0.05$); and *P. aeruginosa* vs DO ($p < 0.05$).

Discussion

Direct discharges of sewage into the marine environment cause an ecological alteration due mainly to the amount of organic matter and toxic materials which change the balance of the autochthonous marine bacterial population and can alter the food web. The marine environment possesses a self-depurating capability that can restore the balance. However, in closed systems such as bays, estuaries or lagoons, the self-depurating power of seawater is not strong enough to counteract the alterations produced by continuous wastewater discharges.

In normal conditions, the natural marine environment has a specific microbiota adapted to the particular environmental conditions. The external contributions of sewage increase the microbiological load but its persistance is local and transitory depending on several factors, of physico-chemical, biological and hydrological origin. These factors contribute to the decrease of telluric microorganisms on the basis of their specific resistance to the environmental conditions (Baleux *et al.*, 1988; Rheinheimer, 1992).

In the present study, the low number of significant relationships obtained between the microbial and physico-chemical parameters studied may be a consequence of the complexity and diversity of the Ria Formosa ecosystem. Due to the interference of many variables, simple linear correlations could not be expected. In these ecosystems, it is important to study the influence of the environmental factors on the microbiological population, their relationships, and the mechanisms by which the biological systems were affected (Pratt and Reynolds, 1974).

In waters with strong microbiological contamination by direct discharges of domestic sewage, an increase in temperature results in a rise of the activity and a reduction in the generation time of the microorganisms (Borrego, 1994). However, the negative and harmful effects are more frequent and the autolysis of the microorganisms is greater at high temperatures (Rheinheimer, 1992). Microorganisms die rapidly in a hostile environment, as in the case of the enteric bacteria discharged into the marine environment (Barbosa, 1989; Pereira and Alcantara, 1993). Alkan *et al.* (1995) verified under laboratory conditions that temperatures between 10°C to 30°C do not have a significant effect on the survival of bacteria in aquatic environments when light was present. These authors concluded that light causes the same effect as temperature.

The degree of salinity in the aquatic environment is a factor that determines the distribution of the organisms. The extreme values of salinity recorded from the samples over a two-year period were between 25‰ and 37‰. The minimum value for this parameter was recorded in winter at the station PM, which is located near the main sewage discharges of Faro (South of Portugal). In that sampling station, the water depth is around 1 m at low tide. Similar results were reported in Ria Formosa by Benoliel (1984), with values ranging between 26‰ and 38‰.

A direct relationship between temperature and salinity ($r = 0.726, p < 0.01$ for Ilha; $r = 0.465, p < 0.05$ for PM; and $r = 0.144, p < 0.05$ for PC) was observed in the three stations. However, salinity showed only significant correlation with faecal streptococci and coliphages (Table 3). Comparing the salinity values obtained in the three sampling stations, the highest values for this parameter were recorded in Ilha, although no significant differences were obtained. In this sampling station, there is a smaller telluric contribution, and as a consequence, a higher influence of the ocean, demonstrated by the higher abundance of halophilic bacteria in these samples (data not shown). The higher influence of the marine environment provokes bacterial inactivation of the allochthonous microbiota from sewage discharges, except for the halotolerant microorganisms *Vibrio* and staphylococci (Borrego *et al.*, 1988; Borrego and Figueras, 1997). However, salinity is not the main factor responsible for the inactivating capability of the marine environment (Anderson *et al.*, 1979; Rhodes and Kator, 1988; Hoff, 1989; Barcina *et al.*, 1997), but the bactericidal action of seawater is a result of a synergic effect of other biotic and abiotic factors (Fujioka and Narikawa, 1982; Mason *et al.*, 1986; Sorensen, 1991; Ahl *et al.*, 1995).

Turbidity is another factor which significantly affects the fate of the allochthonous microorganisms in the marine environment. The organic and/or inorganic material in suspension provokes the adsorption of microorganisms, which increases microbial survival in waters (Rheinheimer, 1992). In addition, this material in suspension adsorbs toxic substances and protects the microorganisms against the effects of sunlight.

In the present study, significant correlations were obtained between transparency and number of microorganisms in only two sampling stations, Ilha and PC (Table 3). In Ilha, transparency was related to total coliforms and faecal streptococci, and in PC, transparency only was significantly correlated with the number of *Salmonella*.

Alkan *et al.* (1995) studying in the laboratory the survival of enteric bacteria observed that turbidity, vertical mixture and effluents were the factors that affected significantly and directly the bacterial inactivation rate in the presence of light. These results are consistent with those obtained in the present study, since the highest bacterial titres corresponded with the smallest values of transparency.

The concentration of DO in seawater exerts an important influence on the physiological processes of the microorganisms. In waters with a low oxygen content, slight variations in this parameter can produce important modifications in the bacterial population (Rheinheimer, 1992). In PM samples, a significant negative correlation was obtained between temperature and DO concentrations. The low depth of this sampling station may be responsible for the strong effect of temperature on the DO. Cachola and Sampayo (1984) reported values of DO ranging between 7.34 mg l^{-1} (high tide) and 12.78 mg l^{-1} (low tide) in a study carried out in Ria Formosa. In our study, lower values were recorded which may be attributed to the sampling time, before 12.00 a.m., when photosynthesis activity has not compensated yet for the use of oxygen during the night.

The results obtained during a two-year sampling period allowed us to evaluate the microbiological quality of recreational waters of Ria Formosa, establishing the impact of the main output of sewage. According to the abundance of indicator and pathogenic microorganisms obtained in all the sampling stations, a correlation matrix was performed in order to obtain information about their relationships and, in addition, to determine the influence of several factors on their numbers. In all the cases, the highest microbial values were recorded in PM and the lowest in Ilha.

Nunes (1984) and Barbosa (1989) studying the sampling area (Ria Formosa) demonstrated a decrease in the number of mesophilic microorganisms and coliforms in zones nearest the open ocean. This decrease may be due to different biotic and abiotic factors which exert a negative effect either individually or by synergism on the allochthonous microorganisms in the marine environment (Gauthier, 1980; Borrego *et al.*, 1983; Martin and Bonnefort, 1986).

Mean concentrations of faecal indicators in the different sampling stations are given in Table 2. The continuous sewage discharges from Faro and other places explain the higher values of indicator and pathogenic microorganisms at the PC station. Cachola and Lima (1984), in a study on the water quality and live resources of the coast of Algarve (South of Portugal), recorded the highest values of faecal coliforms in water and shellfish samples in areas near outfalls, which suggests that this microbial indicator is only useful in areas with a high degree of faecal pollution with continuous and/or recent discharges. On the other hand, as it was expected, in Ilha there was no significant variation in the number of microbial parameters because this station is located far from domestic sewage discharges.

Although a direct relationship seems to exist between the concentrations of faecal indicators and the amounts of sewage discharge, in the present study significant seasonal variations in the indicator titres have not been established. Several authors have reported that visible light provokes a strong inactivating effect on microorganisms discharged by sewage effluents (Evison and Tosti, 1980; Fattal *et al*., 1983; Moriñigo *et al*., 1989; Cornax *et al*., 1990). This effect is proportional to the intensity and period of radiation (Gameson and Gould, 1975), and to the presence of dissolved organic matter in water (Fujioka *et al*., 1981). Ria Formosa, due to its geographic location, has a very long insolation period in summer, and this fact could explain the decrease of microbial parameters observed in this season.

The pathogenic microorganisms tested showed low detection rates at the three sampling stations, *P. aeruginosa* being the pathogenic microorganism most frequently detected in water samples. *P. aeruginosa* in recreational waters is important because it caused ear infections among bathers (Hoadley and Knight, 1975; Cabelli *et al*., 1982; Borrego and Mariño, 1995). On the other hand, this microorganism is also considered as an indicator of water quality by several authors (Kenner and Clark, 1974; Bonde, 1977; de Vicente *et al*., 1986). The enumeration of *Salmonella* in waters presented the shortcomings of its low number in these samples, and the low detection and enumeration efficiency of its specific methodology (Petrili, 1979; Hoadley, 1981; Moriñigo *et al*., 1992). *C. albicans* is part of the normal microbiota of the intestine of vertebrates and birds, and its occurrence in natural waters is associated with faecal pollution (Buck and Bubucis, 1978). In the present study, only a low frequency isolation was obtained, because of the low incidence of this yeast in polluted natural waters (Mariño *et al*., 1995).

The relationships established between indicator and pathogenic microorganisms in the samples tested are shown in Table 3. As it can be seen, none of the indicators used presented a significant correlation with the pathogen levels, although *P. aeruginosa* showed a high relationship with coliphages ($r = 0.305, p > 0.05$) in Ilha, and with faecal coliforms and faecal streptococci in PM ($r = 0.310$ and $r = 0.332$, respectively, $p > 0.05$). Similar results have been reported by several authors (Borrego *et al*., 1983; Goyal, 1983; de Vicente *et al*., 1986; Moriñigo *et al*., 1989), who suggested that the different survival capabilities, the methods used, and the intermittent presence of pathogens in sewage discharges are the most probable causes of the lack of a significant relationship.

In the case of the relationships established between indicator microorganisms, only faecal coliforms and coliphages showed a significant relationship in all the sampling stations (Table 3). In the PM station faecal streptococci and coliphages, and total coliforms and faecal coliforms were significantly correlated. Finally, in the PC sampling station, faecal streptococci were significantly correlated with total coliforms, faecal coliforms and coliphages, and in addition, total coliforms were correlated with faecal coliforms. Other authors have obtained similar relationships between total coliforms and faecal coliforms in the same sampling zone (Nunes, 1984; Cachola and Sampayo, 1984; Baptista, 1993).

In short, from the results obtained in the present study, we can conclude that none of the microbial indicators tested may be considered adequate to determine the presence of pathogenic microorganisms in marine recreational waters. However, faecal streptococci, on the basis of its greater survival capability in seawater and its relationship with gastro-intestinal disturbances (Cabelli *et al*., 1982), may be considered as the best indicator system for marine waters.

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