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Evaluation of microbiological quality of coastal waters in Greece

A. C. Vantarakis, A. Tsibouxi, D. Venieri, G. Komninou, A. Athanassiadou and M. Papapetropoulou

ABSTRACT

To evaluate the microbiological water quality of bathing sites along the Achaia coastline (south western Greece), a survey was conducted to determine the concentration of faecal bacterial and phage indicators as well as the presence of human viruses. Seawater samples (234) were collected from nine bathing sites on the Achaia coastline and were analysed for the presence of: total coliforms, faecal coliforms, faecal streptococci, *Escherichia coli*, somatic coliphages, F-RNA bacteriophages, bacteriophages infecting *Bacteroides fragilis*, enteroviruses, adenoviruses and hepatitis A viruses. Most of the bacteriological analysis results were in accordance with the European Union standards. In all sites, bacteriophages were detected occasionally. Enteroviruses and adenoviruses were detected in 24 samples (10.26%) and 37 samples (15.81%) respectively. No samples were positive for the presence of hepatitis A virus. The overall data indicates that bathing sites are impacted by human faecal material. Both bacterial indicators and phages have low predictive capability for the presence of human viruses in coastal waters. None of the environmental parameters analysed was strongly related to the presence of the indicator organisms and viruses. Appropriate and effective administrative measures that should be taken into account may be considered in order to improve water quality and reduce public health risk.

Key words | bacterial indicators, bacteriophages, Greece, seawater, viruses

A. C. Vantarakis (corresponding author)
Laboratory of Hygiene and Environmental Protection, Medical School,
Democritus University of Thrace,
Greece
Tel: 0030-25510-30505
Fax: 0030-25510-30505
E-mail: *avanta@otenet.gr*

A. Tsibouxi
D. Venieri
G. Komninou
M. Papapetropoulou
Laboratory of Public Health, Medical School,
University of Patras,
Patras Greece

A. Athanassiadou
Laboratory of Biology, Medical School,
University of Patras,
Greece

INTRODUCTION

The south west of Greece is a popular area with tourists who benefit the local economy. Despite this popularity, there has only been one survey of the microbiological quality of recreational coastal waters (Vantarakis & Papapetropoulou 1998).

One of the public concerns over recreational water use is the risk of illness resulting from exposure to waters contaminated by sewage. The risk to human health associated with contaminated coastal water has been well documented. Some studies have shown that swimmers were at a greater risk of becoming ill than non-swimmers; moreover swimmers were at a greater risk when swimming at beaches affected by pollution compared with beaches that were considered to be unpolluted (Petrilli *et al.* 1980; Fleisher *et al.* 1996). Children swimming in contaminated seawater are more likely to develop illnesses than those who do not (Alexander *et al.* 1992; Kay *et al.* 1994).

The sanitary quality of seawater along bathing beaches currently relies on the detection of total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FST) as bacterial indicators of faecal pollution (Anon 1976). Apart from the three main aforementioned bacterial organisms used to evaluate the possibility of faecal contamination of recreational waters, the European Union (EU) Bathing Water Directive (Anon 1976) lists a number of microbial standards including the absence of enteroviruses in 10 l of seawater in 95% of the samples examined, specifying that they should be monitored when they are suspected to be present.

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Studies have shown that bacterial indicators do not provide adequate information about viruses, particularly in terms of their survival in environmental conditions and their resistance to treatment (Merrett-Jones *et al.* 1991). Human viral pathogens are more persistent in these waters than coliform bacteria and are not efficiently removed by treatment processes such as chlorination (Hejkal *et al.* 1981). Total and faecal coliform bacterial indicators often do not indicate the persistence of pathogens, especially viruses. Total and faecal coliforms can be readily isolated in warm seawater from areas far removed from human activity and thus are not adequate indicators of faecal contamination and human health risks. In addition, Wyer *et al.* (1995) have shown poor statistical relationship between bacterial indicators of faecal pollution and enteroviruses in coastal waters resulting in a low predictive capability. The comparative survival of viruses and indicator bacteria in the marine environment has shown that bacteria released in the marine environment may be undetectable within a few days while enteric viruses may be present in an infectious state at detectable levels for several months (Wheeler 1990). This situation leads to the conclusion that current bacterial indicators are inadequate as a monitoring tool for pollution by viruses.

Thus, studies have been directed towards identifying more specific indicators of viral contamination. Two approaches are possible. One involves the detection of enteroviruses by cell culture or molecular techniques. The other involves looking for particular types of bacteriophage commonly found in human faeces.

Enteric viruses, in the environment, pose a public health risk because they can be transmitted via the faecal-oral route through contaminated water, and even low numbers are able to initiate an infection in humans. The US Environmental Protection Agency (USEPA) describes the enteric viruses group itself as the most meaningful, reliable and effective index for environmental monitoring (Karaganis *et al.* 1983). Numerous studies have documented the presence of enteroviruses in coastal and recreational waters (Abbaszadegan *et al.* 1993; Kopecka *et al.* 1993; Tsai *et al.* 1993; Puig *et al.* 1994; Vantarakis & Papapetropoulou 1998; Lipp *et al.* 2001). Enteroviruses have been recorded in seawater in many parts of the Mediterranean (Puig *et al.* 1994; Vantarakis & Papapetropoulou 1998). On the other hand, adenoviruses are the only human enteric viruses containing DNA and are therefore human pathogens. However, their presence in coastal waters and their role as originators of gastroenteritis and respiratory illness has probably been underestimated (Irving & Smith 1981; Hurst *et al.* 1988; Griffin *et al.* 2001). Numerous studies have documented the presence of adenoviruses in coastal and recreational waters (Girones *et al.* 1993; Puig *et al.* 1994; Enriquez & Gerba 1995; Enriquez *et al.* 1995; Vantarakis & Papapetropoulou 1998).

Three types of bacteriophage have been proposed as specific indicators of viral contamination: somatic coliphages (Morinigo *et al.* 1992), the F-RNA phages (Havelaar *et al.* 1986) and the phages infecting *Bacteroides fragilis* (Jofre *et al.* 1986). The *Bacteroides fragilis (B. fragilis)* HSP40 phages seem to be specific indicators of human faecal contamination whereas the other two types of phage may be indicators of both human and non-human faecal contamination (Havelaar *et al.* 1986; Jofre *et al.* 1986).

Member states of the European Union are required to implement the Bathing Water Directive 76/160/EEC. The European Commission proposed to revise this 25-year-old directive in October 2002 and the revised directive is now under international consultation (Council of the European Communities 2002). This revised draft directive will replace enumeration of total and faecal coliforms with *Escherichia coli* (*E. coli*) and intestinal enterococci enumeration. In addition to enumeration activities, a sanitary inspection of the recreational site to identify pollution sources and their significance will need to be carried out. Ideally this will result in prediction of water quality linked to management systems and improved protection of public health (WHO 2003).

In order to evaluate the water quality of seawater in our region, a pilot study was conducted at nine bathing sites, representative of bathing water of the Achaia coastline. The locations of these bathing sites were selected by the Ministry of Environment. All sites were identified as bathing areas and their microbiological quality reflects the water quality of the coastal line of our Prefecture. In addition to the monitoring of seawater for bacterial indicators (total coliforms, faecal coliforms, *E. coli*, faecal streptococci), the study included the enumeration of somatic coliphages, F-RNA phages, and phages infecting *Bacteroides fragilis*, as well as the detection of viral pathogens such as entero-

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viruses, adenoviruses and hepatitis A virus. Additional objectives of this study were: a) broad assessment of the level of faecal input throughout the Achaia coastline; b) evaluation and ranking of the nine most popular tourist destinations in our region according to their microbiological quality; and c) evaluation of the possibility of using bacteriophages as possible indirect indicators of viral contamination in the seawater of our region.

MATERIALS AND METHODS

Sampling

Nine bathing sites along the Achaia coastline, selected by the Greek Ministry of Environment, were sampled. These sites were sampled a total of 20 times each, about once every three or four days, between 1 May 2000 and 1 August 2000 (main bathing period). All nine sites were sampled on the same day. Table 1 lists each sampling site, its location and some of its characteristics. Salinity, pH and seawater temperature in the water and water temperature from each water sample were also monitored (Table 2). Also weather data (wind direction, wind speed, temperature) were obtained from the meteorological survey of Patras.

Table 1 | Sampling sites and their characteristics

Site location	Characteristics
Alissos	Between Patras and Pyrgos, tourist and bathing site, 50 m from a hotel
Agyia	Patras, 1,500 m from a sewage pump in Patras, not a popular bathing site
Proastio	6 km from Patras, next to a hotel, bathing site
Rio	10 km from Patras, very popular tourist site, bathing site
Araxovitika	20 km from Patras, tourist and bathing site
Logos	Between Patras and Egio, tourist and bathing site
Selianitika	Between Patras and Egio, tourist and bathing site
Enallax	Egio, 50 m from a restaurant, not a popular bathing site
Inoa	Egio (a city of 40,000 people), close to sailing club, not a popular bathing site

Table 2 | Range of physicochemical parameters at study sites

Site	Water temperature (°C)	pH	Salinity (‰)
Alissos	16–27	7.7–8.4	36.5–39.9
Agyia	17–27	7.8–8.2	36.5–38.5
Proastio	17–27	7.8–8.2	36.4–38.7
Rio	20–27	7.9–8.3	36.7–39.1
Araxovitika	18–26	7.5–8.2	36.2–39.7
Logos	19–27	7.8–8.2	35.4–38.9
Selianitika	17–27	7.8–8.2	35.6–39.7
Enallax	18–26	7.8–8.2	36.2–39.6
Inoa	18–27	7.8–8.2	35.9–37.9

Bacterial analysis

Four bacterial indicators (total coliforms, faecal coliforms, *E. coli*, faecal streptococci) were monitored in all water samples. The membrane filtration method was used and 100 ml of seawater was assayed for each parameter. The bacteria were detected by the conventional culture method (*Standard Methods* 1995). Results were expressed as colony forming units (cfu) per unit volume.

Bacteriophage analysis

For bacteriophage analysis, 10 ml of water sample were analysed. The analysis was performed by means of plaque assay method using the double-agar layer technique (Dore & Lees 1995; Puig *et al.* 1999). The results were expressed as number of plaque forming particles per unit of volume (pfu ml^{-1}).

The number of somatic coliphages was determined using the ISO (1999a) 10705-2 method. The host strain used was *E. coli* WG5 and the reference phage was ϕX174.

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For the detection of F-RNA bacteriophages (Havelaar *et al.* 1986), the host strain used was *Salmonella typhimurium* WG49 and the reference phage MS2 was used. The culture media used were Tryptone Yeast Glucose Broth (TYGB) TYGAgar, ssTYGA (Oxoid, Unipath, Basingstoke, UK) (Debartolomeis & Cabelli 1991). The method is validated by ISO 10705-1 (1996).

For the detection of *B. fragilis* phages, the double-agar layer technique was also used, as previously described (Tartera *et al.* 1992). The host strain used was *B. fragilis* RYC2056 and the reference phage was B56-3. The method is validated by ISO 10705-4 (1999b).

Human virus analysis

For human virus analysis, 400 litres of seawater were collected at each site. The sampling for virus analysis was performed according to the modified methodology previously described (Vantarakis & Papapetropoulou 1998).

The seawater samples were concentrated by filtration through Virosorb filters (AMF Cuno Meriden, Connecticut). Filter elution and concentration of the viruses were performed using organic flocculation and ultracentrifugation (Puig *et al.* 1994). Nucleic acid extraction, cDNA synthesis for enteroviruses and HAV, PCR and nested PCR for all viruses were performed according to Puig *et al.* (1994). From the final concentrate (0.2 ml), 50 μl were assayed twice by PCR representing 100 litres of the original sample. The primer sequences used were previously suggested (Puig *et al.* 1994). All the preventive measurements to avoid cross-contamination in nested PCR were taken.

Statistical analysis

Statistical analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, Illinois). The Kruskal-Wallis test was used to determine if the sampling date or sampling site had an effect on the median values of the indicator organisms. The Chi square test of independence was used to assess which environmental factors were related with the detection of bacterial and phage indicator organisms and the viruses in the water samples.

Correlation analysis between water temperature, salinity, pH, time of sampling and presence of microbiological parameters (bacteria and phages) was performed. Also, correlation analysis between the presence of all indicator bacteria and phages was also performed. Finally, a t-test was performed to correlate the mean amounts of bacterial and phage indicators in samples with the presence or absence of viruses. Any probability less than or equal to 0.05 was considered significant.

The presence of viral indicators and the existence or absence of faecal pollution according to the EU Directive was compared among the samples. Every sample in which the bacterial burden exceeded the EU Directive was considered as a 'faecally polluted sample' (Anon 1976).

RESULTS

Seven indicators (bacteria and phages) of faecal pollution were assessed at each bathing site. Among all water samples analysed, 13 (5.56%), 11 (4.7%) and 11 (4.7%) were positive for the presence of total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FST), respectively, according to EU regulations (TC $> 10^4$ cfu 100 ml^{-1}, FC $>$ 2,000 cfu 100 ml^{-1}, FS $>$ 100 cfu 100 ml^{-1}). *E. coli* was detected in levels above 400 cfu 100 ml^{-1} (proposed level by EU) in 8.12% of the samples analysed. Somatic coliphages, F-specific RNA phages and phages infecting *B. fragilis* were found to be present in 64 (28.63%), 36 (15.81%) and 30 (12.82%) samples, respectively. The RT-PCR virus data was reported as presence or absence. All viral data was confirmed by nested PCR. Of the samples analysed, 24 (10.26%) were enterovirus positive by nested PCR while the corresponding number for adenoviruses was 37 samples (15.81%) (Figure 1). No site was positive for hepatitis A virus.

Total coliform level (in all sites) averaged 599.03 cfu 100 ml^{-1} (the geometric mean value was 5.56 cfu 100 ml^{-1}). One of the sites (Agyia) was in violation of the EU Directive for bathing water quality. The average faecal coliform level was 39.6 cfu 100 ml^{-1} (geometric mean 2.22 cfu 100 ml^{-1}) and six samples in one site (Agyia) were in violation of the EU Bathing Water Directive. Average *E. coli* concentration was 690.33 cfu 100 ml^{-1} and the geometric mean was 9.01 cfu 100 ml^{-1}. Seventeen samples from three sites (Agyia, Proastio, Inoa) were in violation of the standard

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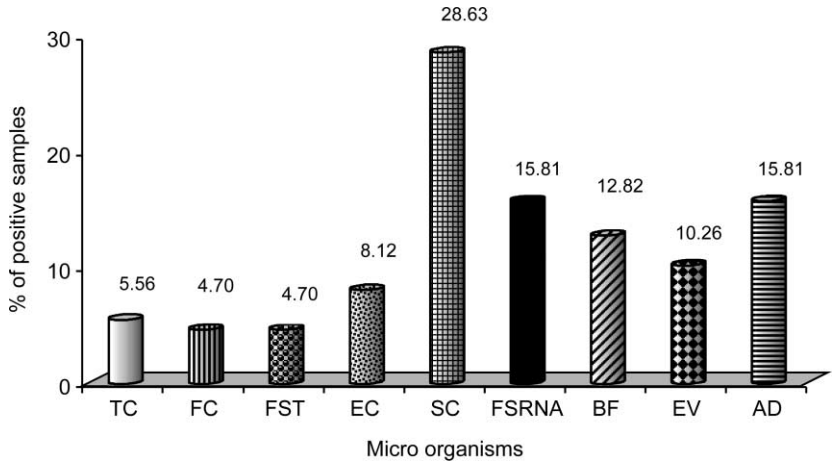

Figure 1 | Percentages of water samples with presence of microbial parameters in all sites. TC: total coliforms, FC: faecal coliforms, FST: faecal streptococci, EC: *E. coli*, SC: somatic coliphages, FSRNA: F-specific RNA phages, BF: phages against *Bacteroides fragilis*, EV: enteroviruses, AD: adenoviruses.

proposed in the revised EU Bathing Water Directive (*E. coli* < 400 cfu $100\,ml^{-1}$) (Council of the European Communities 2002). Faecal streptococci averaged 21.04 cfu $100\,ml^{-1}$ and the geometric mean was 1.81 cfu $100\,ml^{-1}$. Seven samples from two sites (Agyia, Proastio) were in violation of the EU Bathing Water Directive. Somatic coliphages averaged 65.81 pfu $10\,ml^{-1}$ (geometric mean was 6.49 pfu $10\,ml^{-1}$). Average F-RNA phage levels were 247.54 pfu $10\,ml^{-1}$ (geometric mean was 9.01 pfu $10\,ml^{-1}$). Phages against *B. fragilis* averaged 57.79 pfu $10\,ml^{-1}$ (geometric mean was 4.90 pfu $10\,ml^{-1}$). Table 3 summarizes these results.

Table 4 lists the sites in order from the west end (Alissos) of the coastline to the east end of Achaia (Inoa). The indicator (bacteria and phage) concentrations were given rankings of 1 (the lowest levels of bacteria and coliphages within each group) to 9 (the highest levels of bacteria and coliphages within each group) in order to compare the sites with each other. After each category was ranked, a total score was calculated and an overall ranking was assigned per site. Logos and Selianitika, which are considered bathing areas, had the best microbial seawater quality. Agyia, which was located inside the port of Patras had the worst microbial seawater quality. Table 5 lists the sites from west to east and includes the bacteria and phage site rankings in addition to the RT-PCR human virus data.

The median values of all bacterial indicators and bacteriophages differed with sampling site according to the Kruskal-Wallis test ($p < 0.05$). Also the median values of all bacterial indicators or phages differed with sampling date according to the Kruskal-Wallis test presenting significantly higher numbers of all microorganisms in June ($p < 0.05$).

The presence of adenoviruses and enteroviruses differed with sampling site according to the Chi-square test of independence ($p < 0.05$). Also the positive values of the independent samples t-test indicates that the mean amounts of bacterial and phage indicators are significantly greater in samples with the presence of enteroviruses or adenoviruses ($p < 0.05$).

No significant correlation between all the microbiological parameters was found. The presence of viruses cannot easily be predicted by the presence of bacterial indicators although it shows a positive relation to high numbers of indicator bacteria ($p < 0.05$). Also the virus presence cannot be predicted by the presence of bacteriophages. Finally, the presence of phages does not show statistical correlation with the presence of indicator bacteria ($p > 0.05$).

The physicochemical parameters (salinity, water temperature, pH) do not affect the presence of bacteria, phages or human viruses in the water sample, probably because the sampling period was too short and the environmental conditions were rather stable for our area ($p > 0.05$).

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Table 3 | Average and geomean values of indicators of seawater quality at study sites

Site		TC (cfu 100 ml^{-1})	FC (cfu 100 ml^{-1})	FST (cfu 100 ml^{-1})	EC (cfu 100 ml^{-1})	SC (cfp 10 ml^{-1})	F-RNA (cfp 10 ml^{-1})	BF (cfp 10 ml^{-1})
Alissos	Average	231.54	64.27	31.58	125.12	21.27	37.31	13.42
	Geomean	55.21	41.91	12.72	22.06	4.38	2.90	2.62
Agyia	Average	1,352.92	97.88	63.12	4,705.73	486.77	1,950.27	449.50
	Geomean	1,400.67	692.14	40.93	345.30	48.52	9.45	5.84
Proastio	Average	729.85	34.88	69.69	980.54	24.85	104.77	17.73
	Geomean	100.59	62.73	17.03	29.36	16.78	4.61	4.94
Rio	Average	894.27	16.31	2.88	36.81	9.92	13.04	7.69
	Geomean	18.85	12.31	4.09	3.95	5.69	1.46	1.16
Araxovitika	Average	1,754.04	24.00	2.88	48.12	4.54	25.88	3.62
	Geomean	23.19	17.43	3.11	6.52	2.47	1.55	1.16
Logos	Average	40.46	87.50	0.00	10.77	2.27	5.31	1.96
	Geomean	4.53	3.04	1.48	1.98	2.28	1.35	0.00
Selianitika	Average	16.35	0.00	0.00	21.54	5.62	4.42	3.81
	Geomean	13.33	6.57	3.29	2.33	1.26	0.00	0.00
Enallax	Average	63.42	4.81	0.00	45.81	5.50	4.81	3.77
	Geomean	15.88	5.99	3.03	1.60	2.05	1.19	0.00
Inoa	Average	308.46	25.77	16.23	238.54	31.54	82.08	18.58
	Geomean	43.33	26.94	12.27	9.17	17.12	2.86	2.01
Total	Average	599.03	39.60	21.04	690.33	65.81	247.54	57.79
	Geomean	5.56	2.22	1.81	9.01	6.49	9.01	4.90

TC: total coliforms, FC: faecal coliforms, FST: faecal streptococci, EC: *E. coli*, SC: somatic coliphages, F-RNA: F-specific RNA phages, BF: phages against *Bacteroides fragilis*

DISCUSSION

The cleanest sites were located close to Logos and Selianitika, which are both bathing areas and very popular; their location is 3 km distant from any sewage outfalls. Even when a site on the shore has a tide, enteroviruses and adenoviruses were detected on a few occasions. Enallax (rank 3) is an area previously reported with a high incidence of enteroviruses and adenoviruses (Vantarakis & Papapetropoulou 1998). In this study, the area was ranked high (very clean) probably because of the shutdown of several enterprises (restaurants, hotels) located in the area, resulting in the elimination of the local and occasional sewage outfalls. Therefore, the quality of seawater of this site was significantly improved compared with our previous study. Araxovitika (rank 4) has a very old sanitation system due to

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Table 4 | Site rankings based on the prokaryote data (sites are listed in order from west to east)

Site	TC	FC	FST	EC	SC	F-RNA	BF	Overall rank
Alissos	4	7	7	6	6	6	6	6
Agyia	9*	9*	9*	9*	9*	9*	9*	9
Proastio	6	6	8	8	7	8	7	8
Rio	7	3	5	3	5	4	5	5
Araxovitika	9	4	5	4	2	5	2	4
Logos	2	8	2	1	1	3	1	1.5
Selianitika	1	1	2	2	4	1	4	1.5
Enallax	3	2	2	5	3	2	3	3
Inoa	5	5	6	7	8	7	8	7

TC: total coliforms, FC: faecal coliforms, FST: faecal streptococci, EC: *E. coli*, SC: somatic coliphages, F-RNA: F-specific RNA phages, BF: phages against *Bacteroides fragilis*
*Indicates a value that exceeds the EU standards for any parameter

Table 5 | RT-PCR virus results (sites are listed in order from west to east)

Site	Overall rank	EV	ADV			HAV
			% positive		% positive	
Alissos	6	+(1)	3.8	+(2)	7.6	–
Agyia	9	+(7)	26.9	+(10)	38.5	–
Proastio	8	+(4)	15.3	+(5)	19.2	–
Rio	5	+(2)	7.7	+(2)	7.7	–
Araxovitika	4	+(2)	7.7	+(2)	7.7	–
Logos	1.5	+(1)	3.8	+(1)	3.8	–
Selianitika	1.5	+(1)	3.8	+(3)	11.5	–
Enallax	3	+(3)	11.5	+(5)	19.2	–
Inoa	7	+(2)	7.7	+(6)	23	–

EV: enteroviruses, ADV: adenoviruses, HAV: hepatitis A virus
The number of samples positive for the presence of enteroviruses and adenoviruses is shown in parentheses. All samples were negative for the presence of hepatitis A virus.

which contamination problems arise occasionally. In Logos (rank 5), several contamination problems appear due to the seasonal operation of two large hotels, which during the summer, host more than 5,000 people.

A small hotel is located on the western site of the monitored area (Alissos, rank 6), which utilizes its own septic tank for sewage treatment. Inoa (rank 7) is close to a small, protected boat basin. Its poor sanitary quality is due to the occasional disposal from the boats. Therefore, the microbiological burden is variable in this area. Proastio (rank 8) is a bathing site for local people with a small hotel in the area, which utilizes septic tanks for sewage disposal. Several problems appear occasionally when overflow occurs. Agyia (rank 9) is not a popular bathing site. The sampling site was 1500 m from a sewage outfall. Three sites (Agyia, Proastio and Inoa) were among the highest-ranked sites for faecal pollution because they were close to sewage outfalls. The results of the study were given to the Ministry of Environment and proved valuable for the evaluation of the origin of human pollution as well as the administrative measures that should be taken.

Previous research has demonstrated that total coliforms are not good indicators of faecal pollution in seawater while other investigators have suggested that alternative indicators are more reflective of microbiological pollution; for example USEPA has promoted the use of faecal streptococci levels in marine waters as a better indicator of health risks (Dufour *et al.* 1986). Also, Cabelli (1983) showed that levels of faecal streptococci greater than 35 cfu 100 ml^{-1} are associated with an increased risk of illness. The average level of faecal streptococci (21.04 cfu 100 ml^{-1}) found in our study, was below that level.

The low numbers of bacteriophages (coliphages, F-RNA phages, phages infecting *B. fragilis*) isolated in this study may indicate rapid die-off of phages, as a result of high salinity (characteristic of east Mediterranean area) and high water temperatures monitored during the sampling period. Our results are in accordance with the results of Griffin *et al.* (1999). Salinity values ranged from 35.4 to 39.9 ppt (average 37.6 ppt) during the sampling period and water temperature varied from 17 to 27°C (average temperature 22.4°C). The pH of seawater did not vary significantly (average pH 8.08). Salinity and water temperature may significantly affect viral viability as Patti *et al.* (1987) suggested.

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The number of samples positive for enteroviruses and adenoviruses suggest an impact of the wastewater (human pollution) along the Achaia coastline. In addition, at several sites, even where they have met microbiological standards, viruses have been detected. It is useful to estimate periodically the occurrence of enteroviruses and adenoviruses in seawater, in order to assess the public health risk associated with exposure to these pathogens (Griffin *et al.* 2003). The number of samples positive for the presence of viruses analysed during this period was similar to previous studies (Vantarakis & Papapetropoulou 1998). Our study demonstrated that adenoviruses are more often detected in coastal waters than enteroviruses. Apart from their better stability and survival in seawater than that of enteroviruses (Enriquez *et al.* 1995), adenoviruses constitute the most frequently isolated virus group from the population of Greece (Kouloumbis *et al.* 1981; Krikelis *et al.* 1986). Our results are also in agreement with those of Puig *et al.* (1994) who reported a greater presence of adenoviruses in waters than enteroviruses. The presence of human pathogenic viruses in seawater is responsible for outbreaks of human illnesses. In the majority of the studies that monitored for both bacterial indicators and pathogenic viruses, viruses were detected when indicators levels were below public health water quality threshold levels.

The comparison among the bacterial faecal pollution (exceeding EU standards), and the presence of bacteriophages and the presence of viral indicators gave no statistically significant correlation. It seems that in our geographical region, the presence of enteric viruses is not necessarily linked to the presence of *E. coli* or other microorganisms used as indicators. Our results are, also, in agreement with those of Wyer *et al.* (1995) who reported poor statistical relation between concentrations of bacterial indicators of sewage pollution and enteroviruses in marine waters.

The presence of somatic coliphages in a water sample usually indicates pollution from human or animal faeces or by wastewater containing these extracts. Somatic coliphages provide a relatively simple and inexpensive means of detecting faecal pollution. Compared with the faecal indicator bacteria routinely used to evaluate bathing water quality, their survival more closely mirrors those of human enteric viruses. Exceptionally, however, in pristine waters the presence of coliforms other than *E. coli* may permit multiplication of somatic coliphages, thereby reducing their significance as indicators of faecal contamination.

CONCLUSIONS

The rationale of this pilot study was to broadly evaluate the level of faecal input and the possible presence of human pathogens throughout the Achaia coastline as well as to rank bathing sites according to microbiological quality. The survey of nine locations indicated that compliance with EU bacterial regulations is not sometimes sufficient in order to reduce the risk to public health of the presence of human pathogenic viruses. Also bacteriophages, in our high temperature seawaters, could not be used as virus indicators for seawater faecal pollution.

The constant surveillance of the coastline with all bacterial indicators and occasionally with viruses, gives us the opportunity of 'true' microbiological monitoring of the area as well as the control of any effects of the improper disposal of sewage in this area. As a result of this study, proper management actions could be applied in order to improve significantly the sanitary quality of the area and reduce the public health risk.

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