'pragmatic' when general environmental degradation proceeds insidiously.

Before real progress can be made in Danish environmental research, it is necessary to create an appropriate research environment. The Strategic Environmental Research Programme offers such a possibility. It remains to be seen how fruitful the initiative will be.

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# Influence of the Faecal Pollution of Marine Sediments on the Microbial Content of Shellfish

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**To determine the influence of faecally-contaminated sediments on the microbial content of shellfish, several indicator and pathogenic microorganisms were monitored over a period of 19 months in the Guadalhorce estuary (Malaga, South of Spain). All the microorganisms studied were found to be present in greater numbers in sediments and shellfish than in the overlying water.**

**The occurrence of the pathogens studied in shellfish does not appear to be correlated with the levels of any particular indicator group in sediments, except for sulphite-reducing clostridia and faecal streptococci, which showed a significant relationship with the presence of *Salmonella* and *Aeromonas hydrophila* in shellfish, respectively.**

**The results obtained in the present study suggest that an evaluation of the presence of indicator and pathogenic microorganisms in sediment may provide additional insight into long-term water quality conditions, but they do not improve the indication of pathogen presence in shellfish compared with the study of the seawater or the direct analysis of shellfish.**

Marine sediments of recent origin act as reservoirs of pollutant bacteria and viruses entering the marine ecosystem. These microorganisms are discharged to the marine environment by different ways: *1.* directly from land or rivers; *2.* through the offshore disposal of submarine sewage outfall; and *3.* from aquatic animal and bird faeces. The fate of these microorganisms in the aquatic environment depends on several factors, such as sedimentation processes linked to the adsorption on particulate matter; survival capability of the microorganisms; and influence of tides or currents on the release of microorganisms from sediment, to name a few.

Generally, a large number of the microorganisms discharged into marine environments settle in the sediment bottom layer, where a higher concentration of indicators of faecal pollution and pathogens, such as *Salmonella* and viruses, have been demonstrated in comparison with the numbers of those microorganisms in surrounding seawater (Chen *et al.*, 1979; Gerba *et al.*, 1979; LaBelle *et al.*, 1980). These microorganisms (bacteria and viruses) survive longer in sediments than in the water column (De Flora *et al.*, 1975; Babinchak *et al.*, 1977; Smith *et al.*, 1978; Rao *et al.*, 1984; Lear, 1985). The importance of the study of contamination of marine sediments in swimming and shellfish-harvesting areas is based on the fact that the microorganisms associated with sediments may be resuspended both by several natural processes (currents, rainwater runoff, storms, and changes in salinity and organic matter) (Gerba & Schaiberger, 1975), or by man activities (dredging or boat traffic) (Grimes, 1975, 1982), affecting the microbial quality of seawater and shellfish consequently.

The objective of this study is to determine the influence of faecally-contaminated sediments around the Guadalhorce river mouth on the microbial content of shellfish grown in the area of influence.

## Materials and Methods

*Samples collection and processing*

Water samples were collected from five sampling stations (sites 1–5) in the Guadalhorce estuary (Malaga, Spain) (Fig. 1) from October 1988 to May 1990. The mean water temperatures were 15.8°C and 16.2°C for fall 1988 and 1989, respectively; 14.2°C and 13°C for winter 1988–89 and 1989–90, respectively; 15°C and 16.1°C for spring 1989 and 1990, respectively; and 20°C for summer 1989. A total of 60 samples of seawater were taken at 2–3 cm depth using 500 ml sterile glass flasks and stored at 4°C until analysis in the laboratory.

A total of 52 samples of sediments samples were collected from the same stations as seawater (Fig. 1) using an anchor dredge. The top 2–3 cm of sediment was transferred into sterile glass flasks and transported to the laboratory at 4°C, where they were processed within 4 h of collection. The samples were suspended in 0.1% saline peptone water to obtain 1:100 dilutions, they were shaken vigorously, and decimal dilutions were performed before inoculation in appropriate media.

Fifty-five samples of shellfish composed of cockles (*Cardium edule*) and striped venus (*Chamelea gallina*) were harvested from the same sampling stations (Fig. 1) by trawling with rakes provided with thin grid nets, that were towed along the sea bottom about 250 m in parallel to the coast. Then, shellfish were placed in sterile plastic bags, and stored on ice for transport to the laboratory. The elapsed time between sampling and analysis never exceeded 4 h.

Shellfish specimens were washed and scrubbed under running tap water and their surface was sterilized by dipping in 70% ethanol; then, they were opened aseptically and the flesh was weighed and transferred to a sterile plastic bag with equal volume of sterile 0.1% peptone water or saline peptone water (ICMSF, 1978). The samples were then blended for 1 min using a Stomacher (Seward & Co. Ltd, London) to obtain a 1:10 dilutions.

*Microbiological analysis*

Total aerobic (TA) and total anaerobic (TAN) bacteria of serially diluted samples were determined according to standard procedures (Lake & Lynt, 1984; APHA, 1985) using Plate Count agar (Difco Laboratories, Detroit, Mich.) and the pour-plate technique. Incubation was carried out at 35 ± 0.5°C for 48 ± 3 h in aerobic conditions for TA, and at 35°C for 72 h using GasPak jars (BBL Microbiology Systems, Cockeysville, Md.) for TAN.

Total coliforms (TC), faecal coliforms (FC), *Escherichia coli* (Ec), and faecal streptococci (FS) were enumerated by means of the five-tube Most Probable Number (MPN) technique, using the following media (presumptive and confirmatory tests) and incubation conditions: MacConkey-purple broth (Difco) (36 ± 1°C, 48 h) and EC broth (Difco) (44.5 ± 0.5°C, 24 h) for total and faecal coliforms, respectively (Avila *et al.*, 1989). Presence of *E. coli* in gas-producing EC cultures was confirmed by streaking cultures on Levine's eosin-methylene blue (EMB) agar (Difco) plates and final confirmation of lactose-positive colonies was performed on API 20-E (Analytab Products Inc., Plainview, NY). Analyses of faecal streptococci were carried out using azide-dextrose (Rothe) broth (Bio-Merieux, Madrid, Spain) (36 ± 1°C, 48 h) and con-

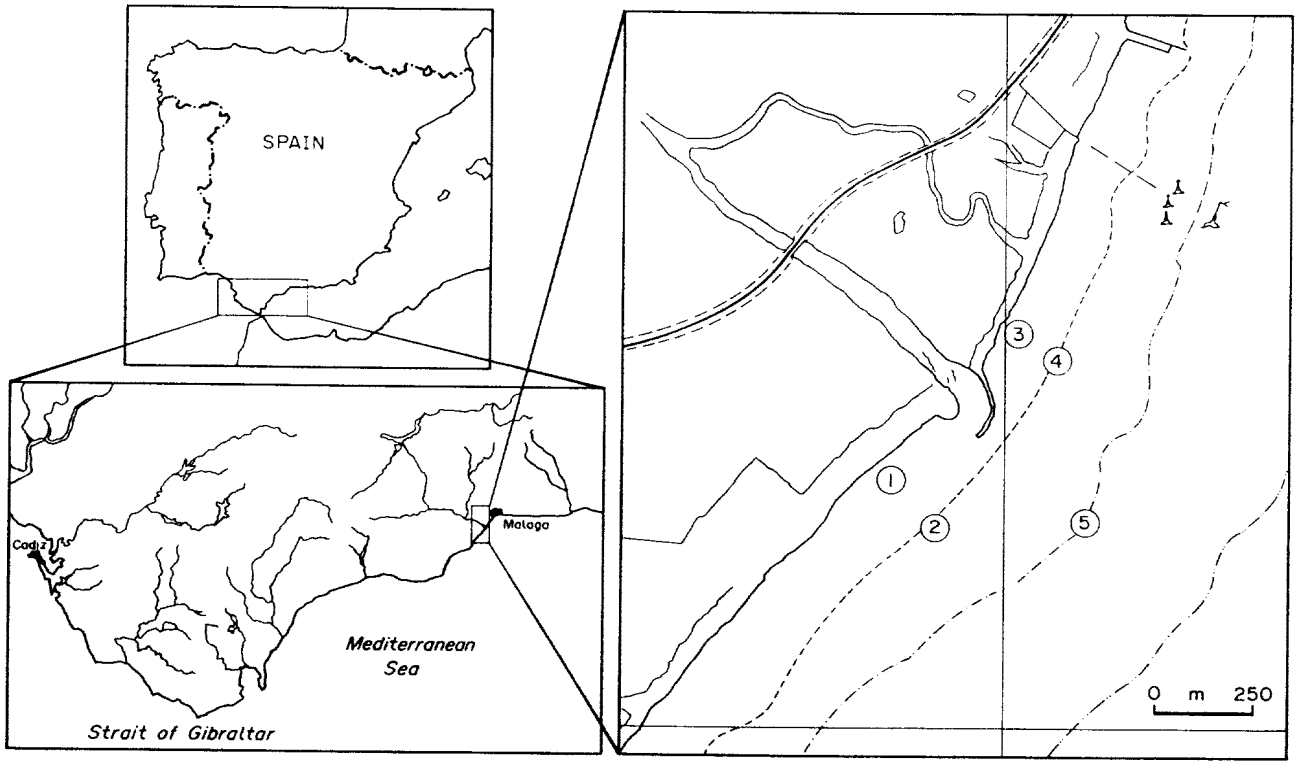


**Fig. 1** Geographical location of the sampling stations in the Guadalhorce estuary (Malaga, Spain): no. 1: S.SW 0–2 m depth; no. 2: S.SW 2–5 m depth; no. 3: N.NE 0–2 m depth; no. 4: N.NE 2–5 m depth; no. 5: SE 10 m depth.

firmation was performed on azide violet ethyl (Litsky) broth (BioMerieux) (36 ± 1°C, 24 h) (Reuter, 1985). From Litsky tubes that showed growth, the presence of faecal streptococci was finally confirmed by streaking cultures on KF agar (BioMerieux) plates supplemented with 1% of triphenyl tetrazolium chloride (Sigma Chemical Co., Sto. Louis, MO) and incubation at 36 ± 1°C for 24 h.

To enumerate sulphite-reducing clostridium spores (Csr) the pour-tube technique described by Bonde (1977) was used. Vegetative forms were eliminated by heating the samples at 80°C in a thermostatic bath for 10 min. Tryptone-sulphite-cycloserine (TSC) agar (Harmon *et al.*, 1971) was used as a selective medium incubated at 36 ± 1°C for 18–20 h in Gas-Pak jars (BBL) with nitrogen and hydrogen atmosphere.

Coliphages (Ph) were enumerated by using the Most Probable Number (MPN) technique described by Kott (1966), employing *Escherichia coli* K12 (PC 0008 from University of Utrecht Phagenkollection) as bacterial host and Phage Assay broth (PAB) (Kott, 1966) as enrichment medium. The final confirmation of the coliphage presence was performed by the drop test using Luria agar (Adams, 1959).

The MPN technique was used to detect and enumerate all the pathogenic microorganisms listed below.

*Salmonella* spp. (S): Pre-enrichment in Buffered Peptone Water (BPW) (Edel & Kampelmacher, 1969) incubated at 36 ± 1°C for 18–20 h, and culture of 0.1 ml amounts in tubes of RV/43 broth (Vassiliadis *et al.*, 1976) incubated at 43 ± 1°C for 72 h. The enriched cultures were streaked on Brilliant Green (BG) agar (Difco), Bismuth Sulphite (BS) agar (Difco), Xylose-Lysine-Deoxycholate (XLD) agar (Difco), and Hektoen Enteric (HE) agar (Difco) media. Typical *Salmonella* colonies grown on the media were screened biochemically using API 20-E system (API) and confirmed serologically with somatic (O) and flagellar (H) antisera (Difco).

*Vibrio parahaemolyticus* (Vp): Alkaline Saline Peptone Water (ASPW) (Dupray & Cormier, 1983) incubated at 36 ± 1°C for 8 h and streaked on TCBS agar (Difco) plates. Presumptive *V. parahaemolyticus* colonies were confirmed by applying the following tests (FDA, 1978): nitrate reduction, indole production, methyl red and Voges Proskauer tests, reactions of Kligler agar, arginine dihydrolase, growth at 6% and 8% of NaCl, growth at 42°C, and fermentation of the following carbohydrates: mannose, galactose, sucrose, and cellobiose.

*Aeromonas hydrophila* (Ah): Alkaline Peptone Water (APW) (Shread *et al.*, 1981) and incubation at 28 ± 0.5°C for 24 h. Colonies on Starch-Biliar salts (SBS) agar (Hansen & Bonde, 1973) were isolated from the tubes with growth. The identification scheme followed was that specified by Schubert (1974) modified later by Popoff (1984): methyl red and Voges Proskauer tests, arginine and esculine hydrolysis, growth at 0% of NaCl, and fermentation of galactose, mannitol, and inositol.

*Staphylococcus aureus* (Sa): m Staphylococcus broth (Difco) supplemented with sodium azide (Sigma) and incubation at 36 ± 1°C for 48 h (APHA, 1985). The confirmation was carried out by streaking the positive tubes on KRANEP agar (Sinell & Baumbart, 1967), and identification by use of the following tests (Schleifer & Kloos, 1975): Gram-stain, catalase, cytochrome-oxidase, O/F test, methyl red and Voges Proskauer tests, production of acid from glycerol in presence of erythromycin, coagulase, DN-ase, and phosphatase production.

*Statistical analyses*

To establish whether the mean concentrations of the microorganisms studied were significantly different, factorial analysis of the variance (F-ANOVA) was applied (Sokal & Rohlf, 1980) using a Stat-View 512 Plus program. To the significant results, a confirmation test was applied, which consists in a paired t-student test among two variables according to the following formula:

$$Ac = T_{0.05(d)}(V \times r)^{1/2}(1/n_1 + 1/n_2)^{1/2},$$

where $T_{0.05(d)}$ is t-student at 95% confidence level for 'd' freedom degree; $(V \times r)^{1/2}$ is the square root of the residual variance; and $n_1$ and $n_2$ are the samples. If the difference between the means of the two samples compared is higher than Ac, then, this value is considered significant. To study the relationship between indicator and pathogen concentrations in the samples, a correlation matrix was applied using a SPSS program. The microbiological quality of the shellfish was also studied by calculating the probability of detection of pathogens as a function of the median of the log-normal distribution ($XX_{50}$) of the indicators (El-Shaarawi & Pipes, 1982).

## Results

*Microbiological characteristics of the three types of samples studied*

Concentrations of indicators and pathogenic microorganisms in all the sample types were determined over a 19-month period. The mean concentrations, standard deviation and percentages of detection of indicators and pathogens per 100 ml of seawater or 100 g of shellfish and sediments are drawn in Figs 2 and 3, respectively.

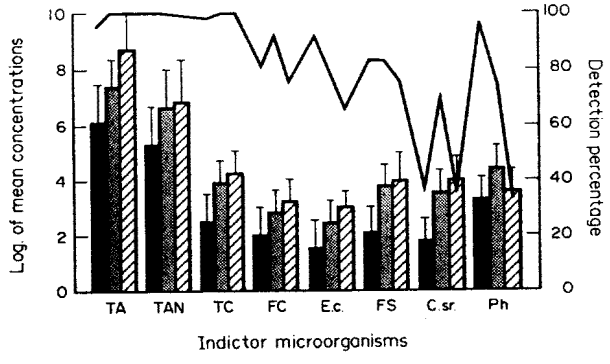


**Fig. 2** Mean concentrations and detection percentages of indicator microorganism in seawater, shellfish and sediments.
■ Seawater; ▩ Shellfish; □ Sediments.

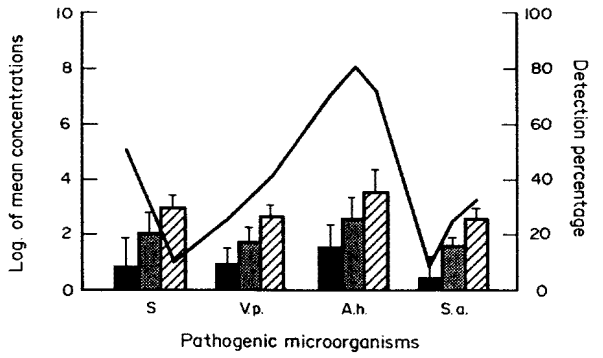


**Fig. 3** Mean concentrations and detection percentages of pathogen microorganism in seawater, shellfish and sediments.
■ Seawater; ▩ Shellfish; □ Sediments.

In seawater, detection percentages of TA and TAN are 95 and 100%, respectively, with log mean concentrations of 6.07 100 $ml^{-1}$ and 5.26 100 $ml^{-1}$ for both parameters. With regard to the indicators, their means range from 3.28 100 $ml^{-1}$ for Ph to 1.54 100 $ml^{-1}$ for Ec, and detection percentages from 35% for Csr to 95% for TC and Ph, most of them over 70%.

In shellfish, TA and TAN also have the higher titres 7.38 100 $g^{-1}$ and 6.59 100 $g^{-1}$, respectively, and 100% detection. The lowest mean is for Ec (2.45 100 $g^{-1}$) and the lowest detection percentage for Ph (67%).

In sediments the log of mean concentrations range from 8.67 100 $g^{-1}$ for TA to 3.07 100 $g^{-1}$ for Ec. The detection percentages range from 100% for TA and TAN to 34% for Ph.

With regard to pathogenic microorganisms, in seawater Sa shows the lowest percentage of detection (4%) and the lowest mean (0.45 100 $ml^{-1}$), whereas Ah is the most frequently detected (72%) with the highest concentration (1.65 100 $ml^{-1}$). Similar results were obtained in shellfish and sediments, but in the latter environment, *Salmonella* is the less frequent (only 10% of detection).

There were significant differences depending on the season of the year for the levels of the indicators TC, FC, and FS and for the pathogens *Salmonella* and Vp in seawater, and only for coliphages and *A. hydrophila* in shellfish. For sediments significant differences were only found for Vp concentrations (Table 1).

When applying the confirmatory statistical test, it is observed that the means for indicator bacteria and *Salmonella* in water in spring and summer are significantly lower than in fall and winter. On the contrary, Vp concentrations were higher at low seawater temperatures (fall and winter). For Ph and *A. hydrophila*, in shellfish, mean levels obtained in spring are significantly higher than in the other seasons. The main characteristic of sediments is their high stability over the different seasons of the year. The levels of TAN increase only in fall, whereas the levels of *V. parahaemolyticus* are higher in spring. No significant differences for the other microbiological parameters were observed.

*Relationships between microbiological parameters detected in seawater and sediments*

The relationships between microbiological parameters in seawater and sediments are given as a correlation matrix in Table 2. In general, there is a significant correlation between the concentration of each microorganism studied in seawater and the same parameter in sediments; only for TA, TC, Ph and *Salmonella* there is no correlation. Studying correlations between the concentration of indicators in seawater compared to the concentration of pathogens in sediments, only *E. coli* presents significant correlation with *V. parahaemolyticus* and *A. hydrophila*; although *A. hydrophila* in seawater is strongly and significantly correlated with all the pathogenic microorganisms tested in sediments (*Salmonella*, *V. parahaemolyticus*, *A. hydrophila* and *S. aureus*).

*Relationships between microbiological parameters in sediment and shellfish samples*

Table 3 shows the correlation matrix between microbiological concentrations detected in sediments and those detected in shellfish. Total aerobes (TA), TAN, FS, Csr, *Salmonella*, *A. hydrophila* and *V. parahaemolyticus* detected in sediments are significantly correlated with the same parameters detected in shellfish; *E. coli* and *S. aureus* have a good but not significant correlation: and TC, FC and Ph are not correlated.

Studying the correlation between indicators in sediments and concentrations of the pathogens in shellfish, only Csr possesses a strong correlation with

**TABLE 1**

F-ANOVA of the microbial concentrations in the three environments studied depending on the season of the year and the depth of sampling.

| Microorganisms | Season | | | Deep | | |
|---|---|---|---|---|---|---|
| | Seawater | Shellfish | Sediment | Seawater | Shellfish | Sediment |
| Total aerobes (TA) | 0.062 | 0.178 | 0.063 | 0.017* | 0.920 | 0.505 |
| Total anaerobes (TAN) | 0.469 | 0.021* | 0.008* | 0.483 | 0.670 | 0.097 |
| Total coliforms (TC) | 0.032† | 0.073 | 0.348 | 0.981 | 0.479 | 0.587 |
| Faecal coliforms (FC) | 0.026† | 0.076 | 0.385 | 0.980 | 0.396 | 0.418 |
| Faecal streptococci (FS) | 0.048† | 0.367 | 0.405 | 0.793 | 0.466 | 0.221 |
| Clostridium spores (Csr) | 0.125 | 0.199 | 0.402 | 0.836 | 0.696 | 0.330 |
| Coliphages (Ph) | 0.616 | 0.011* | 0.515 | 0.675 | 0.627 | 0.430 |
| *Salmonella* spp. | 0.031† | 0.124 | 0.434 | 0.383 | 0.711 | 0.317 |
| *Vibrio parahaemolyticus* | 0.002* | 0.068 | 0.009* | 0.387 | 0.428 | 0.710 |
| *Aeromonas hydrophila* | 0.643 | 0.014* | 0.119 | 0.756 | 0.887 | 0.966 |
| *Staphylococcus aureus* | 0.560 | 0.348 | 0.098 | 0.046† | 0.268 | 0.119 |

*Significant at confidence level of 99.9% ($p < 0.001$).
†Significant at confidence level of 95% ($p < 0.05$).

**TABLE 2**

Matrix of correlation between the concentrations of the indicator microorganisms tested from seawater and the concentrations of indicators and pathogens in sediment samples.

| Microorganisms in sediments | Seawater | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TA | TAN | TC | FC | Ec | FS | Csr | Ph | S | Vp | Ah | Sa |
| Total aerobes (TA) | 0.11 | – | – | – | – | – | – | – | – | – | – | – |
| Total anaerobes (TAN) | – | 0.40† | – | – | – | – | – | – | – | – | – | – |
| Total coliforms (TC) | – | – | 0.21 | – | – | – | – | – | – | – | – | – |
| Faecal coliforms (FC) | – | – | – | 0.27§ | – | – | – | – | – | – | – | – |
| *Escherichia coli* (Ec) | 0.21 | 0.05 | 0.29§ | 0.19 | 0.44† | – | 0.17 | 0.23 | – | – | 0.15 | – |
| Faecal streptococci (FS) | – | – | – | – | – | 0.49* | – | – | – | – | – | – |
| Clostridium spores (Csr) | – | 0.43† | – | – | – | – | 0.62* | – | – | – | – | – |
| Coliphages (Ph) | – | – | – | – | – | – | – | 0.17 | – | – | – | – |
| *Salmonella* spp. (S) | – | 0.01 | 0.19 | 0.05 | 0.03 | 0.08 | −0.12 | −0.02 | 0.02 | – | 0.25§ | – |
| *Vibrio parahaemolyticus* (Vp) | 0.18 | 0.03 | 0.14 | 0.04 | 0.28† | −0.24 | −0.03 | −0.03 | – | 0.42* | 0.43* | – |
| *Aeromonas hydrophila* (Ah) | 0.27‡ | 0.12 | 0.17 | 0.21 | 0.42† | 0.18 | 0.04 | 0.17 | – | – | 0.58* | – |
| *Staphylococcus aureus* (Sa) | 0.10 | −0.10 | 0.14 | 0.03 | 0.06 | −0.21 | 0.07 | −0.02 | – | – | 0.42* | 0.24§ |

*Significant at confidence level of 99.9% ($p < 0.001$).
†Significant at confidence level of 99% ($p < 0.01$).
‡Significant at confidence level of 95% ($p < 0.05$).
§Significant at confidence level of 90% ($p < 0.1$).

**TABLE 3**

Matrix of correlation between the concentrations of the indicator microorganisms tested from sediments and the concentrations of indicators and pathogens in shellfish samples.

| Microorganisms in shellfish | Sediments | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TA | TAN | TC | FC | Ec | FS | Csr | Ph | S | Vp | Ah | Sa |
| Total aerobes (TA) | 0.35† | – | – | – | – | – | – | – | – | – | – | – |
| Total anaerobes (TAN) | – | 0.30‡ | – | – | – | – | – | – | – | – | – | – |
| Total coliforms (TC) | – | – | 0.12 | – | – | – | – | – | – | – | – | – |
| Faecal coliforms (FC) | – | – | – | 0.09 | – | – | – | – | – | – | – | – |
| *Escherichia coli* (Ec) | −0.31 | 0.30§ | 0.25 | 0.14 | 0.25 | 0.12 | 0.29§ | 0.36‡ | – | – | 0.61* | – |
| Faecal streptococci (FS) | – | – | – | – | – | 0.58* | – | – | – | – | – | – |
| Clostridium spores (Csr) | – | 0.43† | – | – | – | – | 0.63* | – | – | – | – | – |
| Coliphages (Ph) | – | – | – | – | – | – | – | 0.09 | – | – | – | – |
| *Salmonella* spp. (S) | 0.07 | 0.05 | 0.01 | −0.24 | −0.21 | 0.08 | 0.29§ | −0.23 | 0.30‡ | – | 0.25§ | – |
| *Vibrio parahaemolyticus* (Vp) | 0.17 | 0.16 | 0.18 | 0.02 | 0.17 | −0.09 | −0.03 | 0.11 | – | 0.33‡ | 0.43* | – |
| *Aeromonas hydrophila* (Ah) | 0.04 | 0.18 | 0.10 | −0.06 | 0.15 | −0.41* | 0.04 | 0.08 | – | – | 0.51* | – |
| *Staphylococcus aureus* (Sa) | 0.15 | 0.02 | 0.10 | −0.05 | 0.22 | −0.03 | 0.07 | 0.00 | – | – | 0.42* | 0.20 |

*Significant at confidence level of 99.9% ($p < 0.001$).
†Significant at confidence level of 99% ($p < 0.01$).
‡Significant at confidence level of 95% ($p < 0.05$).
§Significant at confidence level of 90% ($p < 0.1$).

*Salmonella* and *E. coli*, and FS with *A. hydrophila*. It is interesting to note the high correlation between *A. hydrophila* concentrations in sediments with all the pathogenic microorganisms studied in shellfish.

*Determination of the health-hazard associated with the content of pathogenic microorganisms in shellfish*

Detection percentages of pathogens in shellfish at different concentration levels of indicators in sediments are shown in Table 4. For TC, FC and *E. coli* the percentages of detection of *V. parahaemolyticus* and *S. aureus* is greater at high indicator levels, but for *Salmonella* and *A. hydrophila* the opposite is the case. At low levels of FS, detection percentages of pathogens are higher except for *Salmonella*. At different levels of Csr the percentage of detection is similar for *A. hydrophila* and *S. aureus*, but the percentage of detection increases with the indicator levels for *Salmonella* and *V. parahaemolyticus*. At low concentrations of Ph the percentage of detection of pathogens is higher except for *Salmonella*.

The microbiological quality of shellfish may also be defined by the relationship between the probability of detection of pathogens in shellfish and the characteristic values of the indicators used as sanitary quality criteria. Thus, the occurrence of each pathogenic microorganism in terms of the median of the indicator log-normal distributions ($XX_{50}$) was studied.

Table 5 shows the results obtained from linear correlations between detection percentages of pathogens and the medians of the log-normal distribution ($XX_{50}$) of the indicators. The highest correlations were obtained between the $XX_{50}$ of Csr and the presence of *Salmonella* and *A. hydrophila*; and between the concentrations$_{50}$ of FS and the presence of *V. parahaemolyticus*. High but no significant relationships were obtained between the $XX_{50}$ of TC, FC, and *E. coli* and the probability to detect *Salmonella*. No significant correlations were obtained between the

**TABLE 4**

Detection percentages of pathogenic microorganisms in shellfish depending on the indicator concentration intervals in marine sediments.

| Indicators | Concentration intervals* | No. of samples | Detection percentages (concentration ranges)* *Salmonella* | *V. parahaemolyticus* | *A. hydrophila* | *S. aureus* |
|---|---|---|---|---|---|---|
| Total coliforms | $< 10^2$ | 24 | 37.5 (< 0.3–21) | 16.7 (< 0.3–0.4) | 83.3 (< 0.3– ≥ 240) | 25.0 (< 0.3–0.9) |
| | $\geq 10^2$ | 28 | 25.0 (< 0.3–21) | 25.0 (< 0.3–7.5) | 64.3 (< 0.3– ≥ 240) | 32.1 (< 0.3–0.7) |
| Faecal coliforms | $< 10^2$ | 27 | 44.4 (< 0.3–21) | 22.2 (< 0.3–2.8) | 85.2 (< 0.3– ≥ 240) | 14.8 (< 0.3–0.9) |
| | $\geq 10^2$ | 19 | 15.8 (< 0.3–0.4) | 36.8 (< 0.3–7.5) | 68.5 (< 0.3– ≥ 240) | 31.6 (< 0.3–0.4) |
| *Escherichia coli* | < 10 | 25 | 32.0 (< 0.3–21) | 16.0 (< 0.3–2.8) | 72.0 (< 0.3– ≥ 240) | 20.0 (< 0.3–0.9) |
| | ≥ 10 | 10 | 30.0 (< 0.3–2) | 40.0 (< 0.3–7.5) | 60.0 (< 0.3– ≥ 240) | 30.0 (< 0.3–0.7) |
| Faecal streptococci | $< 10^2$ | 18 | 33.3 (< 0.3–21) | 33.3 (< 0.3–7.5) | 83.3 (< 0.3– ≥ 240) | 44.4 (< 0.3–0.7) |
| | $\geq 10^2$ | 29 | 34.5 (< 0.3–0.4) | 20.7 (< 0.3–0.4) | 69.0 (< 0.3– ≥ 110) | 13.8 (< 0.3–0.4) |
| Clostridium spores | < 10 | 30 | 23.3 (< 0.3–21) | 16.7 (< 0.3–7.5) | 80.0 (< 0.3– ≥ 240) | 26.7 (< 0.3–0.9) |
| | ≥ 10 | 13 | 30.8 (< 0.3–0.4) | 23.1 (< 0.3–0.7) | 76.9 (< 0.3– ≥ 110) | 23.1 (< 0.3–0.4) |
| Coliphages | < 50 | 35 | 40.0 (< 0.3–21) | 43.2 (< 0.3–7.5) | 91.4 (< 0.3– ≥ 240) | 24.3 (< 0.3–0.9) |
| | ≥ 50 | 9 | 44.4 (< 0.3–4.3) | 22.2 (< 0.3–0.4) | 66.7 (< 0.3–15) | 11.1 (< 0.3–0.4) |

*Expressed per g of shellfish or sediment sample.

**TABLE 5**

Correlation between concentrations$_{50}$ of indicators ($XX_{50}$) in sediment and detection percentages of pathogenic microorganisms from shellfish samples.

| Indicators | Pathogenic microorganisms *Salmonella* a | r | *V. parahaemolyticus* a | r | *A. hydrophila* a | r | *S. aureus* a | r |
|---|---|---|---|---|---|---|---|---|
| Total coliforms | 8.03 | 0.44 | −25.85 | 0.39 | −29.97 | 0.70* | −1.09 | 0.05 |
| Faecal coliforms | −8.16 | 0.45 | 1.72 | 0.02 | −14.78 | 0.50 | −7.95 | 0.28 |
| *Escherichia coli* | 22.87 | 0.56 | −31.24 | 0.28 | −56.73 | 0.52 | −7.54 | 0.21 |
| Faecal streptococci | −12.45 | 0.53 | 63.01 | 0.74† | −24.67 | 0.43 | 0.52 | 0.02 |
| Clostridium spores | 20.46 | 0.74* | −3.11 | 0.11 | 28.71 | 0.70* | 17.64 | 0.39 |
| Coliphages | −8.51 | 0.65 | −3.90 | 0.29 | −13.87 | 0.79* | −11.65 | 0.54 |

Significant at *$p < 0.1$; †$p < 0.05$. a: slope; r: coefficient of correlation.

$XX_{50}$ concentrations for any of the indicators and the probability to detect *S. aureus*.

## Discussion

From the results given in Figs 2 and 3 it can be deduced that the estuary of the Guadalhorce river is affected by pollution not exclusively faecal in origin, since total coliform levels were always higher than other indicators more closely related to faecal discharges, such as faecal coliforms, *E. coli* or faecal streptococci (Goyal *et al.*, 1977; Abeyta, 1983; Al-Jeboury & Trollope, 1984). In general, the numbers of indicator and pathogenic microorganisms at all the sampling sites were lower for seawater than for sediment and shellfish samples. In addition, there was comparatively fewer fluctuations in the bacterial concentration in sediments than in water or shellfish samples. According to Greenberg (1956), adsorption and sedimentation tend to remove organisms from suspension and concentrate them in layers of sediment, where they continue metabolically and physiologically active, thus they may pose a hazard to human health (Goyal *et al.*, 1977). The concentrations of total aerobic bacteria, total anaerobic bacteria, total coliforms, faecal coliforms, *E. coli*, faecal streptococci and sulphite-reducing clostridia in seawater are similar to those obtained by other authors (Murcelano *et al.*, 1975; Kaper *et al.*, 1979; Ellender *et al.*, 1980; LaBelle *et al.*, 1980) in areas with a moderate level of pollution. In contrast, Sayler *et al.* (1975) found lower numbers of indicators in sediment than in water samples, although they collected the samples from deeper stations further from the coast than in our study. In relation to shellfish, the results obtained in the present study for indicators are similar to those reported by Goyal *et al.* (1979) and Abeyta (1983) in areas classified as 'prohibited' for shellfish harvesting. On the other hand, for sediment samples our results are in agreement with those reported by Goyal *et al.* (1979) and LaBelle *et al.* (1980). Detection frequencies of coliphages were high, reaching levels of 95% in seawater, 67% in shellfish, but only 34% in sediment samples. In contrast to these results, Vaughn & Metcalf (1975) found lower detection percentages, with mean values of 10.6% in seawater, 50.6% in shellfish, and 2.7% in sediments. The phage technique and the host bacteria used in the latter study would explain the wide differences between both results.

Pathogenic microorganisms showed detection percentages lower than those presented by indicators. *Salmonella* was detected more frequently in seawater and shellfish than in sediments, but for *V. parahaemolyticus* and *S. aureus* the contrary was the case. *A. hydrophila* was the most frequently pathogen isolated in the three environments, results that are in agreement with those reported by Kaper *et al.* (1981).

With regard to seasonal variation, sediments may be considered as a stable environment, since only significant increases in the levels of total anaerobic bacteria and *V. parahaemolyticus* in fall and spring, respectively, have been demonstrated. However, the number of the indicator microorganisms total coliforms, faecal coliforms and faecal streptococci was usually higher in winter months than in the summer. These data are in accordance with those of Sayler *et al.* (1975), Faust *et al.* (1975) and Goyal *et al.* (1977), who found higher coliform levels during the winter months in open bay and canal waters. A possible explanation for these observations is based on the fact that faecal bacteria survival in estuarine water may be strongly influenced by temperature, with die-off increasing rapidly with elevated temperatures (Borrego *et al.*, 1983). Sharp peaks were observed in the number of coliphages in water samples during the summer months, which corresponds to the increase of faecal wastes discharged during this season. This peak in the seawater did not correspond to a concentration increase in sediment bottom; this observation agrees with the results reported by Goyal *et al.* (1977).

From the results given in Table 2 it can be deduced that the microbial relation between seawater and sediments of a shallow estuary is established by a continuous process of precipitation and resuspension of microorganisms (Van Donsel & Geldreich, 1971). However, the lack of significant correlation between the microorganisms in both environments is explained by their different survival and accumulation rate capabilities. For this, *A. hydrophila* has been proposed as a reliable and universal indicator of pollution (Seidler *et al.*, 1980; Alvarez & Bitton, 1983; Biamon & Hazen, 1983).

The occurrence of pathogens in shellfish (Table 3) appears not to be correlated with the concentration of any particular indicator group in sediments. Only detection of *Salmonella* and *A. hydrophila* from shellfish showed a significant correlation with the levels of sulphite-reducing clostridium spores and faecal streptococci in sediments, respectively. Similar results have been reported by Moriñigo *et al.* (1990) in the case of natural contaminated-seawaters. For some indicators, the percentage of detection of some pathogens increase at the highest concentration intervals of indicators. This is the case of total coliforms, faecal coliforms, *E. coli* vs. *V. parahaemolyticus* and *S. aureus*; faecal streptococci and coliphages vs. *Salmonella*; sulphite-reducing clostridium spores vs. *Salmonella* and *V. parahaemolyticus* (Table 4), but this increase was only significant for *E. coli* vs. *V. parahaemolyticus* and faecal coliforms vs. *S. aureus*.

Lineal correlation between detection percentages of pathogens in shellfish and the median of log-normal distribution of indicators in sediments are shown in Table 5. The closest and highest correlations were obtained between $XX_{50}$ of sulphite-reducing clostridium spores and the presence of *Salmonella*, $XX_{50}$ of faecal streptococci and the presence of *V. parahaemolyticus*, and between $XX_{50}$ of coliphages and *A. hydrophila* occurrence. No significant correlation has been established between any of the indicators and *S. aureus* detection. However, the use of $XX_{50}$ of the indicators in sediments to predict the presence of pathogens in shellfish harvested in that area, is more reliable than the application of mean counts of indicators.

In summary, the discharges of polluted river waters affect the microbiological quality of sediments and shellfish of that area. Sediment is the most stable environment and allows the survival of microorganisms, being a reservoir of pathogens. No indicator system exhibits a significant correlation with the pathogens, but *A. hydrophila*, as suggested by other authors, may be considered as a reliable indicator system. Sulphite-reducing clostridium spores is the only microbial indicator parameter which may indicate the potential health-hazard associated with the presence of *Salmonella* in shellfish. The study of sediments of a shellfish-growing area does not improve the safeguard of the area compared to the study of the surrounding seawater or the direct analysis of shellfish.


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