3B2v8.06a/w (Dec 5 2003).51c XML:ver:5.0.1 WR : 5205 Prod.Type:COM pp.1–7(col.fig.:NIL) ED:B.P.Ramya PAGN:Mamatha SCAN:Global

ARTICLE IN PRESS

ELSEVIER

Water Research ▮ (▮▮▮▮) ▮▮▮–▮▮▮

WATER RESEARCH

www.elsevier.com/locate/watres

Relative survival of *Escherichia coli* and *Salmonella typhimurium* in a tropical estuary

Abirosh Chandran, A.A. Mohamed Hatha*

School of Environmental Sciences, Mahatma Gandhi University, Gandhi Nagar, P.O., Kottayam 686 008, Kerala, India

Received 21 October 2003; received in revised form 28 May 2004; accepted 11 January 2005

Abstract

Microcosm studies have been carried out to find out the relative survival of *Escherichia coli* and *Salmonella typhimurium* in a tropical estuary. Survival has been assessed in relation to the important self-purifying parameters such as biotic factors contained in the estuarine water, toxicity due to the dissolved organic and antibiotic substances in the water and the sunlight. The results revealed that sunlight is the most important inactivating factor on the survival of *E. coli* and *S. typhimurium* in the estuarine water. While the biological factors contained in the estuarine water such as protozoans and bacteriophages also exerted considerable inactivation of these organisms, the composition of the water with all its dissolved organic and inorganic substances was not damaging to the test organisms. Results also indicated better survival capacity of *E. coli* cells under all test conditions when compared to *S. typhimurium*.

Keywords: *Escherichia coli*; *Salmonella typhimurium*; Survival; Estuary; Sunlight inactivation; Competition

1. Introduction

One of the characteristic features of the estuarine system is the constant pollution from various human and non-human sources. Population explosion and rapid industrialisation have resulted in an ever-increasing load of waste input into this ecosystem. Large number of pathogenic bacteria enters this system mainly through sewage input. Rivers are the main contributors to the estuary, which transport a large volume of teluric land materials and dump them in the estuary. However, all the natural systems have got considerable self-purifying capacities owing to various physicochemical and biological parameters.

Most sanitary indicator organisms as well as the enteric water borne pathogens are bacteria whose natural environment is the intestine of man and warm-blooded animals. When discharged in the faeces, these microorganisms frequently gain entry into a body of water. Once these bacteria are deposited into the water, they are in an environment that is not favourable to the maintenance of viability of most bacteria. The survival of enteric bacteria in natural aquatic ecosystems has been of interest to public health and microbial ecology (Barcina et al., 1986; Borrego and Figueras, 1997; Dionisio et al., 2000).

Several factors are involved in the disappearance of the pollutant microorganism in the aquatic environment, the two most important being physical dilution and microbial inactivation (Morinigo et al., 1989). Both processes depend on various physicochemical and

*Corresponding author. Present address: Department of Biology, School of Pure and Applied Sciences, The University of the South Pacific, Private Mail Bag, Suva, Fiji. Tel.: +679 321 2550; fax: +679 331 5601.

E-mail addresses: abdulla_m@usp.ac.fj, mohamedhatha@-hotmail.com (A.A. Mohamed Hatha).

0043-1354/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2005.01.010

biological factors such as water temperature (Vasconcelos and Swartz, 1976; Anderson et al., 1983), adsorption and sedimentation processes (Mitchell and Chamberlain, 1975; Geldreich, 1978), sunlight action (Davies and Evison, 1991; Sinton et al., 1999, 2002), predation by bacteria or protozoa (Rhodes and Kator, 1990), bacteriophage lysis (Ricca and Cooney, 1999), lack of nutrients (Sinclair and Alexander, 1984), competition with autochthonous microbiota (Enzinger and Cooper, 1976; McCambridge and McKeekin, 1981) and antibiosis (Colwell, 1978). However, there is considerable disagreement among the observations made by various researchers. Also, the applicability of seawater and freshwater studies to estuarine waters is doubtful because of the likely effects on microbial inactivation of differences in optical characteristics (Davies-Colley et al., 1993), salinity (Evison, 1988; Solic and Krstulovic, 1992), and autochthonous microbiota (Klein and Alexander, 1986; Gonzalez et al., 1990; Rhodes and Kator, 1990).

Escherichia coli is considered as typical faecal indicator bacteria and its presence in natural waters is considered as indicator for the presence of possible pathogens. However, its absence does not necessarily guarantee the quality of water (Dutka, 1973). Therefore, it is interesting to know the inactivation kinetics that environmental factors exert on this faecal indicator bacterium and pathogen *Salmonella typhimurium*, since their relative survival rates in the aquatic environment may determine the validity of *E. coli* as suitable indicator for *Salmonella*. In our studies on the prevalence of indicator bacteria and *Salmonella* from Cochin estuary (Hatha et al., in press), we could consistently isolate number of *E. coli* strains including many diarrhegenic serotypes, though the isolation of *Salmonella* was very low.

In the present investigation, microcosm studies have been carried out to determine the effects of various self-purifying factors such as biotic, physical and chemical factors on the survival of *E. coli* and *S. typhimurium* in estuarine water.

2. Materials and methods

Test organisms: *E. coli* and *Salmonella* isolated from the estuary were used. *S. typhimurium* was used as it is considered as a typical species of *Salmonella*.

Preparation of inocula: *E. coli* and *S. typhimurium* cells were inoculated in to Tryptone Soya Broth (TSB) and incubated overnight (16–18 h) at 37 °C. After incubation, the cells were concentrated by centrifugation at 3000 rpm for 15 min and washed twice with sterile saline solution. After the final wash, the cells were suspended in the saline solution at a concentration of 10^8 colony-forming units per millilitre. From this final suspension, 1 ml was inoculated into 250 ml Erlenmeyer flask with 100 ml of the test solution so as to give an initial inoculum density of 10^6 cells per millilitre of test solution.

Test solution to study the effect of biological factor: Raw estuarine water with all its self-contained biotic factors was used. Estuarine water from different stations were collected, pooled and then a subsample of 100 ml was taken to suspend the test organisms. The test organisms were suspended at a final concentration and survival and injury were estimated at regular intervals.

The total heterotrophic bacterial (THB) load in the sample was determined by standard plate count method using nutrient agar prepared in filtered estuarine water. Protozoans were analysed qualitatively with the help of a microscope. Bacteriophages were enumerated by plaque assay using double-layer agar method (Kennedy et al., 1986), which is described below.

Forty-five millilitre of the sample and 5 ml of *E. coli*/*Salmonella* culture was inoculated into 45 ml of deca strength phage broth and incubated at 37 °C for 24 h. After incubation, the cells were centrifuged at 2500 rpm for 10 min and the supernatant was filtered through 0.45 μm filter. Then 0.1 ml of the filtrate was mixed with 1 ml of *E. coli*/*Salmonella* culture and 5 ml of 0.6% nutrient agar (used as top agar) and poured over nutrient agar plates with 1.2% agar concentration (basal agar). The plates were then incubated at 37 °C for 24 h and the plaques counted and expressed as plaque forming units (pfu) per millilitre.

Test solution to study the toxic effect of dissolved organic matter and antibiotics in the estuarine water: The effect of antibiotics and other dissolved organic substances was studied by suspending the test organisms in filter sterilised (0.22 μm) estuarine water, which excluded all the biotic factors including microbacteria and bacteriophages, while preserving the dissolved organic components.

Test solution to study the effect of sunlight: Filter sterilised (0.22 μm) estuarine water was used. Test solutions were taken in sterile glass bottles, which were suspended at about half a feet below the water surface in a glass tank (200 l capacity) maintained at the roof top. The experiment started at 10 a.m. and continued up to 6 p.m. with sampling at 2-h intervals.

All the test solutions except the one to determine the effect of sunlight were incubated at 30 °C and also at 20 °C, in order to find out the survival at low temperature as the temperature goes down to 20 °C in winter as well as at a certain depth. The test solutions were incubated in the dark, except the test solution to study the effect of luminous factors, which was kept under natural sunlight.

3. Enumeration techniques

Enumeration was accomplished using two plating media in parallel, one selective and the other non-selective, with spread plating technique and incubation at 37 °C for 24 h. Selective and non-selective media were used in order to find out the injury exerted by the test solutions as the characteristic feature of the injured cells is that they fail to develop on the selective medium while maintaining the ability to grow on a non-selective medium. Dilution of the samples whenever necessary was done using sterile saline solution.

Quantification of *E. coli* cells was done with tryptone soya agar (TSA) as the non-selective medium and eosine methylene blue (EMB) agar as the selective media. Quantification of *S. typhimurium* cells was done with TSA as non-selective medium. The selective medium used to enumerate *S. typhimurium* was xylose lysine deoxycholate (XLD) agar.

The samples from the test solution were taken and assayed after 1, 2, 3 and 4 days with the spread plating technique. All the samples were replicated two-fold. The percentage of survivors and injured cells at time '*t*' was calculated according to the following formulae:

$$\text{Percentage of survival of } E.\ coli \text{ cells at time '}t\text{'} = \frac{\text{Count on TSA plates at time '}t\text{'}}{\text{Count on TSA plates at time '0'}} \times 100.$$

$$\text{Percentage of injury of } E.\ coli \text{ cells at time '}t\text{'} = 1 - \frac{\text{Count on EMB plates at time '}t\text{'}}{\text{Count on TSA plates at time '}t\text{'}} \times 100.$$

$$\text{Percentage of survival of } S.\ typhimurium \text{ cells at time '}t\text{'} = \frac{\text{Count on TSA plates at time '}t\text{'}}{\text{Count on TSA plates at time '0'}} \times 100.$$

$$\text{Percentage of injury of } S.\ typhimurium \text{ cells at time '}t\text{'} = 1 - \frac{\text{Count on XLD plates at time '}t\text{'}}{\text{Count on TSA plates at time '}t\text{'}} \times 100.$$

4. Results and discussion

The results are presented in Figs. 1–5 and Table 1. The results (Figs. 1 and 2) indicated a rapid inactivation of the suspended test organisms in raw estuarine water. The experiment started with around 10^6 cells of test organisms, which reduced by almost 3 logs by the end of the 2nd day of the experiment. T_{90} (time required for the reduction of 90% of cells) for *E. coli* is reached in 1 day and that of *S. typhimurium* took less than 24 h, suggesting an enhanced removal of *Salmonella* when compared to *E. coli*. Towards the later stages of the experiment, *E. coli* showed some level of acclimatisation

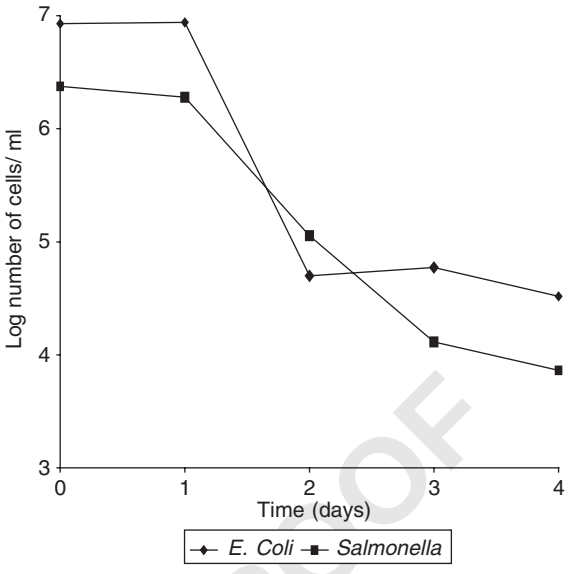

Fig. 1. Relative survival curves of *E. coli* and *S. typhimurium* in estuarine water as a function of the biotic factors at 20 °C.

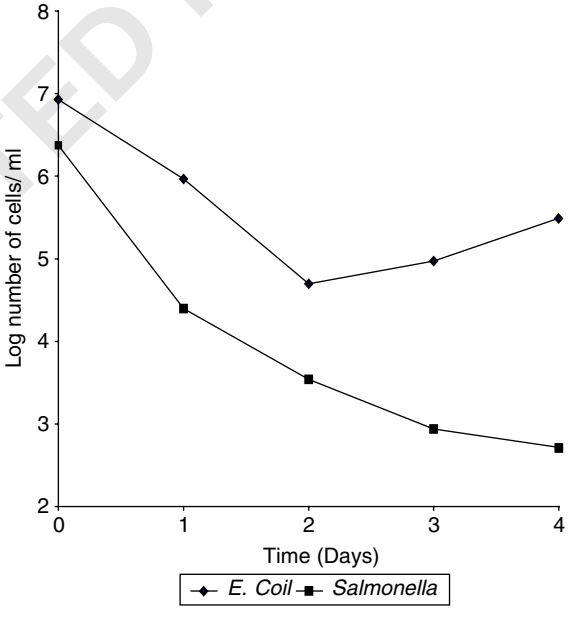

Fig. 2. Relative survival curves of *E. coli* and *S. typhimurium* in estuarine water at 30 ° as a function of biotic factors.

to the test solutions and did not show any further reduction. However, the reduction of the *S. typhimurium* cells in the test solution was linear with time. Test solutions were incubated at room temperature and also at 20 °C (Fig. 1) in order to see the survival capacity at a reduced temperature, which is a common feature of the study environment (Cochin estuary) during monsoon as

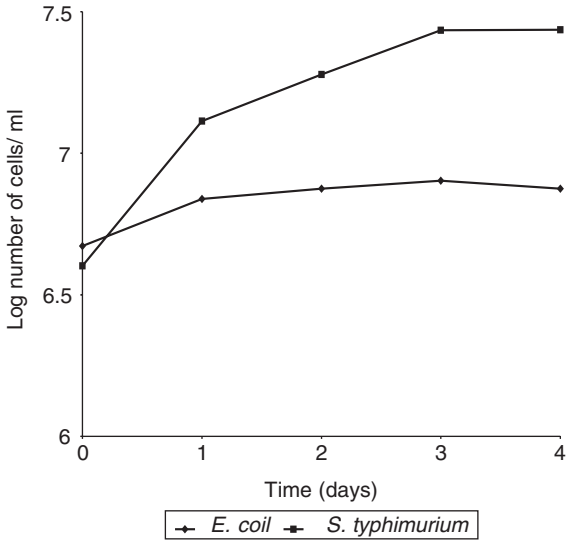

Fig. 3. Relative survival curves of *E. coli* and *S. typhimurium* in estuarine water at 20 °C as a function of the dissolved organic and inorganic substances.

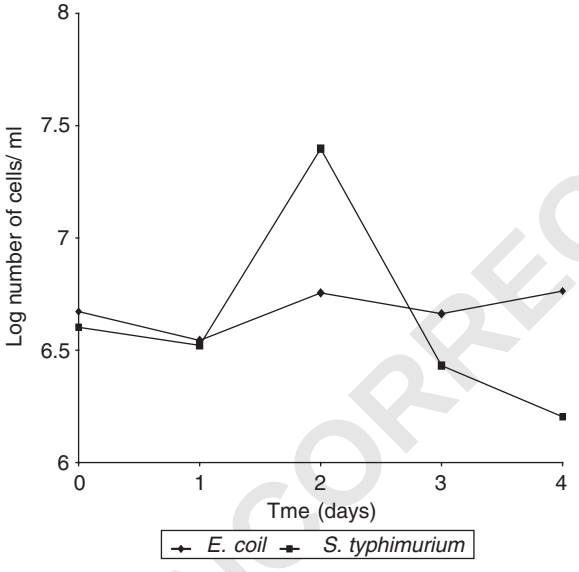

Fig. 4. Relative survival curves of *E. coli* and *S. typhimurium* in estuarine water at 30 °C as a function of dissolved organic and inorganic substances.

well as in winter. While *E. coli* cells showed a slight growth in the test solutions at room temperature after 2 days (Fig. 2), the *S. typhimurium* cells showed a better adjustment at lower temperature.

The findings are in agreement with the observations of Morinigo et al. (1989) and Cornax et al. (1990), who studied the survival of indicator and pathogenic bacteria along the coast of Spain. The role of biological factors

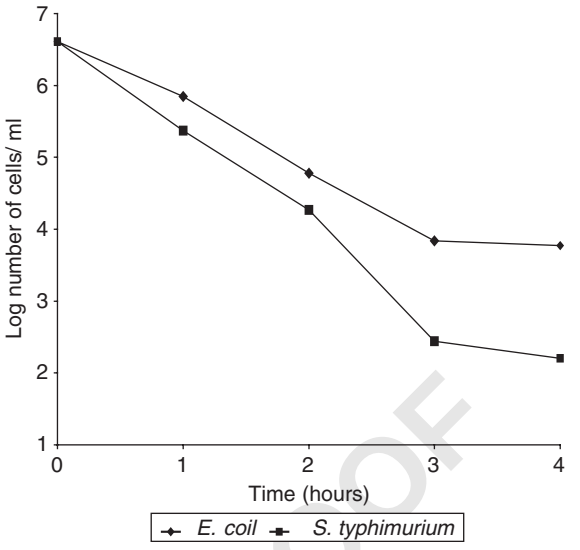

Fig. 5. Relative survival curves of *E. coli* and *S. typhimurium* in estuarine water as a function of sunlight.

were further strengthened by our observations that when the biological factors contained in the estuarine water were removed by filtration (0.22 μm) the test organisms showed enhanced survival (Figs. 3 and 4). Morinigo et al. (1990) also observed an extended survival of *Salmonella* and other indicator microorganisms in their studies using a membrane diffusion chamber, which prevents the entry of bacterial predators inside. We have also estimated the THB population contained in the estuarine water (Table 1), which showed around 10^5–10^6 cells per millilitre of the raw estuarine water indicating severe competition from these autochthonous microorganisms. Rhodes and Kator (1990) reported a higher mortality of *E. coli* cells in the estuarine environment due to autochthonous microbiota. Also, the possible predators such as protozoans and coliphages have been assessed (Table 1). Majority of the protozoans were found to be ciliates, which are reported to do active grazing on bacteria. Mitchell and Morris (1969) demonstrated the existence of microbial predators by adding untreated seawater to agar containing dense suspensions of *E. coli*, and observed discrete clear areas (plaques). Inspection of different plaques revealed a variety of protozoa and bacteria having lytic activity towards *E. coli*. Enzinger and Cooper (1976) reported that the survival of *E. coli* in natural waters is a function of protozoan predators and observed a higher number of protozoan predators resulted in a rapid decline of *E. coli* cells. Bacteriophages have also been considered as a factor in the removal of coliforms from natural environments. We were also able to detect the bacteriophages specific to *E. coli* and *S. typhimurium* in the

Table 1
Range and mean value of total heterotrophic bacteria, bacteriophages and protozoans in the estuarine water

Sample	Mean THB load (cfu/ml)	Bacteriophage (pfu/ml)	Protozoans
Estuarine water	6.36×10^4	$1.27 \times 10^{3*}$ $3.71 \times 10^{2**}$	Detected[a] (mostly ciliates)

* Coliphages, ** Bacteriophages predating *S. typhimurium*.
[a]Protozoans were estimated only qualitatively.

sample by plaque assay and the population varied as 10^2–10^3 plaque forming units (Table 1).

The relative ability of *E. coli* and *S. typhimurium* to resist the biotic factors indicated a better survival capacity of *E. coli* in raw estuarine water (Figs. 1 and 2). The capacity of *E. coli* cells to resist phagocytosis has been reported earlier. Also, selective grazing by protozoans on *S. typhimurium* cells can also lead to a relative reduction of these cells in the test solution. Selective grazing of protozoans has been reported earlier (Barcina et al., 1992). The results of the significance testing by paired student '*t*' test revealed that these two organisms do not differ significantly ($p \leqslant 0.3$) in their capacity to survive in the raw estuarine water.

The toxicity of the dissolved organic substances and antibiotics in the estuarine water for the survival of the test organisms has been assessed by filtering out the biological factors from it as well as incubating in the dark in order to avoid interference from the light factor. The results (Figs. 3 and 4) indicated that the dissolved organic and inorganic substances in the estuarine water are well suited for the survival of both these organisms. Especially, *E. coli* cells showed a gradual and steady increase in the number of cells throughout the experimental period. The most significant feature of the growth curve of *S. typhimurium* was the sudden spurt in growth in the initial days of the experiment, especially in the test solution maintained at room temperature. The cells showed a reduced growth at 20 °C in case of *E. coli* and *S. typhimurium* suggesting their mesophilic nature. The growth in the test solution may be due to the high level of nutrients that are available in the estuarine water. The growth pattern also shows a utilisation of the available nutrients in the initial days and then stagnation, possibly due to nutrient limitation. The relative survival curves of *E. coli* and *S. typhimurium* (Figs. 3 and 4) suggest that *E. coli* is better acclimatised to the composition of the estuarine water, both at 30 and 20 °C. The observed negative effect of the filter sterilised estuarine water on the test organism, though negligible, is based on the presence of antibiotic substances and heavy metal ions within the system. The statistical tests revealed that the survival of both the organisms in the filtered estuarine water was highly significant at two different temperatures ($p \leqslant 0.01$). The difference in the survival capacity of the *E. coli* and *S. typhimurium* in this test solution was also significant ($p \leqslant 0.05$).

Effect of sunlight on the test organisms has been studied by suspending the test organisms in filter-sterilised water and exposing them to natural sunlight. The experiment has been conducted during the daytime for an 8-h duration from 10 a.m. to 6 p.m. The results (Fig. 5) indicated remarkable inactivation of both *E. coli* and *S. typhimurium*. The reduction of cells was linear in relation to time and the T_{90} values reached within 120 min. While the *E. coli* cells showed slight stabilisation in the last 2 h of the experiment, the *S. typhimurium* cells continued to decline throughout the experimental period. The observations agree with the findings of Fujioka et al. (1981) and Fujioka and Narikawa (1982) who reported sunlight as the major inactivation factor affecting the survival of indicator bacteria in the natural environment. Our findings are also in agreement with the observations of Sieracki and Sieburth (1986) and Rhodes and Kator (1990) who observed a higher mortality and sublethal stress during the first 4 h of the experiment in their studies with *E. coli* in estuarine environment. Sinton et al. (1999, 2002) also showed considerable sunlight inactivation of *E. coli* in waste stabilisation pond effluent as well as in sewage polluted seawater.

We had observed that the injury caused by the sunlight was almost 100% as the cells failed to develop on the selective medium. The injury level was much higher when compared to the injury of cells in the other test solutions such as raw estuarine water and filter sterilised estuarine water , which were incubated in the dark. The relative ability of *E. coli* and *S. typhimurium* to survive under sunlight revealed a better survival capacity of *E. coli* (Fig. 5). The *E. coli* cells were found to acclimatise after 6 h of exposure, while the *S. typhimurium* continued to decline. However, the survival capabilities of the two organisms in test solutions supplemented with sunlight were not found to differ significantly ($p \leqslant 0.6$). The stabilisation might be resulting from the recovery of the damaged cells or selection of more resistant organisms. The effect of the visible light may be the result of the accumulation of exogenous and endogenous peroxidases produced by the respira-

tory chain or catalase system (Leclerc et al., 1977; Kapuscinski and Mitchell, 1983).

5. Conclusions

The results of the present investigation revealed that sunlight is the most important inactivating factor on the survival of faecal indicator bacteria *E. coli* and pathogen such as *S. typhimurium* in the estuarine water. While biological factors contained in the estuarine water such as protozoans and bacteriophages in general also exert considerable inactivation of these organisms, the dissolved organic and inorganic substances in the estuarine water did not exert any considerable damage to the test organisms. The results also indicated better survival capacity of *E. coli* cells under all test conditions when compared to *S. typhimurium*, reiterating its role as the ideal indicator organism. However, the detection of *Salmonella* may be less even when there are high numbers of *E. coli* due to two possible reasons, such as the reduced numbers of *Salmonella* entering into the system as well as lower survival capacity of this organism when compared to *E. coli*.

Acknowledgements

The study has been carried out as a part of the DST FAST Track project funded by Department of Science and Technology (SR/OY/LS-18/2001), Govt. of India. The financial assistance to A.A.M. Hatha to carry out the project is thankfully acknowledged.

References

Anderson, J.C., Rhodes, M.W., Kator, H.I., 1983. Seasonal variation in the survival of *E. coli* exposed in situ in membrane diffusion chambers containing filtered and nonfiltered estuarine water. Appl. Environ. Microbiol. 45, 1877–1883.

Barcina, I., Arana, I., Iriberri, J., Egea, L., 1986. Influence of light and natural microbiota of the Butron River on *E. coli* survival. Antonie van Leeuwenhoek 52, 555–566.

Barcina, I., Arana, I., Astorga, A.F., Iriberri, J., Egea, L., 1992. Survival strategies of plasmid-carrier and plasmidless *Escherichia coli* strains under illuminated and non-illuminated conditions, in a fresh water ecosystem. J. Appl. Bacteriol. 73, 229–236.

Borrego, J.J., Figueras, M.J., 1997. Microbiological quality of natural waters. Microbiol. SEM 13, 413–426.

Colwell, R.R., 1978. Bacterial and viruses-indicator of environmental changes in estuaries. Environ. Int. 1, 223–231.

Cornax, R., Morinigo, M.A., Romero, P., Borrego, J.J., 1990. Survival of pathogenic microorganisms in seawater. Curr. Microbiol. 20, 293–298.

Davies, C.M., Evison, L.M., 1991. Sunlight and the survival of enteric bacteria in natural waters. J. Appl. Bacteriol. 70, 265–274.

Davies-Colley, R.J., Vant, W.N., Smith, D.G., 1993. Colour and Clarity of Natural Waters. Science and Management of Optical Water Quality. Ellis-Horwood, New York, NY.

Dionisio, L.P.C., Joao, M., Ferreiro, V.S., Fidalgo, M.L., Garcia Rosado, M.E., Borrego, J.J., 2000. Occurrence of *Salmonella* spp in estuarine and coastal waters of Portugal. Antonie van Leewenhoek 78, 99–106.

Dutka, B.J., 1973. Coliforms are an inadequate index of water quality. J. Environ. Health 36, 39–46.

Enzinger, R.M., Cooper, R.C., 1976. Role of bacteria and protozoa in the removal of *E. coli* from estuarine waters. Appl. Environ. Microbiol. 31, 758–763.

Evison, L.M., 1988. Comparative studies on the survival of indicator organisms and pathogens in fresh and seawater. Water Sci. Technol. 20, 309–315.

Fujioka, R.S., Narikawa, O.T., 1982. Effect of sunlight on survival of indicator bacteria under field conditions. Appl. Environ. Microbiol. 44, 395–401.

Fujioka, R.S., Hashimoto, H.H., Siwak, E.B., Young, R.H.T., 1981. Effect of sunlight on survival of indicator bacteria in seawater. Appl. Environ. Microbiol. 41, 690–696.

Geldreich, E.E., 1978. Bacterial populations and indicator concepts in feces, sewage, storm water and solid wastes. In: Berg, G. (Ed.), Indicators of Viruses in Water and Food Ann Arbor Science, Ann. Arbor, Michigan, pp. 51–97.

Gonzalez, J.M., Irriberri, J., Egea, L., Barcina, I., 1990. Differential rates of digestion of bacteria by freshwater and marine phagotrophic protozoa. Appl. Environ. Microbiol. 56, 1851–1857.

Hatha, A.A.M., Abirosh, C., Mujeeb Rahiman, K.M. Prevalence of diarrhegenic serotypes of *Escherichia coli* in the Cochin estuary, along west coast of India. Indian J. Mar. Sciences, in press.

Kapuscinski, R.B., Mitchell, R., 1983. Solar radiation induces sublethal injury in *Escherichia coli* in seawater. Appl. Environ. Microbiol. 41, 670–674.

Kennedy Jr., J.E., Wei, C.I., Oblinger, J.L., 1986. Methodology for enumeration of coliphages in foods. Appl. Environ. Microbiol. 51, 956–962.

Klein, T.M., Alexander, M., 1986. Bacterial inhibitors in lake water. Appl. Environ. Microbiol. 52, 114–118.

Leclerc, H., Mossel, D.A., Trinel, A.A., Gabini, F., 1977. Microbiological monitoring: a new test for faecal contamination. In: Hoadley, A., Dutka, B.J. (Eds.), Bacterial Indicators/Health Hazard Associated Water. American Society for Testing and Materials, Philadelphia, pp. 21–31.

McCambridge, J., McKeekin, T.A., 1981. Effect of solar radiation and predacious microorganisms on survival of faecal and other bacteria. Appl. Environ. Microbiol. 40, 907–911.

Mitchell, R., Chamberlain, 1975. Factors influencing the survival of enteric microorganism in the sea, an overview. In: Gameson, A. (Ed.), Discharge of Sewage from Sea Outfalls. Pergamon press, London, pp. 237–251.

Mitchell, R., Morris, B.C., 1969. The fate of intestinal bacteria in the sea. In: Jenkins, S.H. (Ed.), Advances in Water Pollution Research. Pergamon Press, New York, pp. 811–817.

Morinigo, M.A., Cornax, R., Munoz, M.A., Romero, P., Borrego, J.J., 1989. Viability of *Salmonella* spp. in natural waters. Curr. Microbiol. 18, 267–273.

Morinigo, M.A., Cornax, R., Castro, D., Martinez-Manzares, E., Borrego, J.J., 1990. Viability of *Salmonella* spp and indicator microorganisms in seawater using membrane diffusion chambers. Antonie van Leewenhoek 57, 109–117.

Rhodes, M.W., Kator, H.I., 1990. Effect of sunlight and autochthonous microbiota on *Escherichia coli* survival in an estuarine environment. Curr. Microbiol. 21, 67–73.

Ricca, D.M., Cooney, J.J., 1999. Coliphages and indicator bacteria in Boston Harbour, Massachusetts. Environ. Toxicol. 14 (4), 404–408.

Sieracki, M.E., Sieburth, J.M., 1986. Sunlight induced growth delay of planktonic marine bacteria in filtered seawater. Mar. Ecol. Prog. Sci. 33, 19–27.

Sinclair, J.L., Alexander, M., 1984. Role of resistance to starvation in bacterial survival in sewage and lake water. Appl. Environ. Microbiol. 48, 410–415.

Sinton, L.W., Finlay, R.K., Lynch, P.A., 1999. Sunlight inactivation of faecal bacteriophages and bacteria in sewage-polluted seawater. Appl. Environ. Microbiol. 65 (8), 3605–3613.

Sinton, L.W., Hall, C.H., Lynch, P.A., Davies-Colley, R.J., 2002. Sunlight inactivation of faecal indicator bacteria and bacteriophages from waste stabilisation pond effluent in fresh and saline waters. Appl. Environ. Microbiol. 68 (3), 1122–1131.

Solic, M., Krstulovic, N., 1992. Separate and combined effects of solar radiation, temperature, salinity, and pH on the survival of faecal coliforms in seawater. Mar. Pollut. Bull. 24, 411–416.

Vasconcelos, G.I., Swartz, R.G., 1976. Survival of bacteria in seawater using diffusion chamber in situ. Appl. Environ. Microbiol. 31, 913–920.