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MEDITERRANEAN ACTION PLAN

Meeting of MED POL National Coordinators

Athens, 18-22 March 1996

UNEP(OCA)/MED WG.111/Inf.8

**ASSESSMENT OF THE STATE OF MICROBIOLOGICAL
POLLUTION OF THE MEDITERRANEAN SEA**

In collaboration with:



WHO



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main parameters being microbiological. Thirty institutions from fourteen Mediterranean countries participated in the pilot project, which was coordinated by the World Health Organization. During the course of the pilot project, desirable environmental quality criteria for both recreational and shellfish-growing waters were elaborated by participants, with a view to their eventual proposal for adoption by Mediterranean Governments.

1-8. The Intergovernmental Review Meeting of Mediterranean Coastal States and the First Meeting of the Contracting Parties to the 1976 Convention (Geneva, 5-10 February 1979) recommended (UNEP, 1979) that:

"Work should be continued on the development of the scientific rationale for the criteria applicable to the quality of recreational waters, shellfish-growing areas, waters used for aquaculture and seafood. Based on this rationale, and taking into account existing national provisions and international arrangements and agreements, the criteria should be formulated on a scientific basis and submitted to the Governments and the EEC for their consideration".

1-9. The first or pilot phase of the MED POL programme was concluded in 1981. The Second Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its related Protocols, held in Cannes from 2 to 7 March 1981 (UNEP, 1981), approved the second phase of the programme, now termed the Long-term Programme of Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase II). Originally designed to cover the period 1981-1990, the programme was later extended until the end of 1995. The monitoring component of the programme (UNEP, 1983) included microbiological monitoring of recreational and shellfish areas within the framework of national marine pollution monitoring programmes to be upgraded or established. The research component included three activities wholly or partially devoted to microbiological pollution. The first of these activities, entitled "Development and testing of sampling and analytical techniques for monitoring of marine pollution", provided for the upgrading of microbiological methodology in current use, and the developing of standard methods for determination of microbiological parameters (particularly pathogens) for which no methods appropriate for Mediterranean conditions were then available. A considerable number of such methods were, in fact, developed during the course of MED POL Phase II. The second activity, entitled "Biogeochemical cycles of pollutants", included a sub-activity on survival of pathogens, on which several studies were performed during the same period. The third activity was entitled "Epidemiological studies related to the confirmation or possible revision of the proposed environmental quality criteria (standards of use) of bathing waters, shellfish-growing waters and edible marine organisms". A number of studies performed within the framework of this activity aimed at finding a correlation between the microbiological quality of coastal recreational waters and observed health effects on exposed population groups. The first such study was initiated in 1982 and completed in 1986, and was followed by others throughout the course of the programme. However, shortage of funds resulted in all of them being necessarily small-scale.

Previous assessments of microbiological pollution of the Mediterranean Sea

1-10. In view of the fact that interim microbiological criteria for recreational and shellfish waters had already been prepared during the first phase of the MED POL programme, these were proposed to the Governments of the region within the framework of an assessment on the current state of microbial pollution of the Mediterranean Sea (UNEP/WHO, 1985) which was prepared by WHO mainly on the basis of monitoring data from the MED POL VII pilot

project. The Fourth Ordinary Meeting of the Contracting Parties to the Convention and Protocols, held in Genoa from 9 to 13 September 1985 (UNEP, 1985a) decided to postpone the issue of shellfish waters and shellfish to a later date. Insofar as the recommendations regarding recreational waters were concerned, these were approved only in part, Contracting Parties adopting joint interim criteria for bathing waters based on maximum acceptable concentrations of only one indicator organism (faecal coliforms), instead of the two (faecal coliforms and faecal streptococci) proposed. Details of the proposals and the interim criteria for recreational waters adopted are contained in Part 4 of this document.

1-11. At their Fifth Ordinary Meeting, held in Athens from 8 to 11 September 1987, (UNEP, 1987), Contracting Parties adopted environmental quality criteria for shellfish waters, proposed on the basis of a revised assessment (UNEP/WHO, 1987) prepared by WHO on the recommendations of a meeting of experts convened by that Organization earlier that year (WHO/UNEP, 1987), which had the task of preparing alternative proposals to those submitted in 1985. The criteria recommended, and eventually adopted, were limited to shellfish waters, and were identical with the relevant EEC Directive on the subject (EEC, 1979). In this context, it was understood that the scope of the resolution in question, the operative parts of which are given in Part 4 of this document, was only designed to cater for acceptability of marine areas for shellfish growing and harvesting, and did not in any way intrude on acceptability of the shellfish for human consumption, which aspect would continue to be handled by appropriate public health or related legislation in the various countries (WHO, 1989).

1-12. The first *ad hoc* assessment of the state of pollution of the Mediterranean Sea by pathogenic microorganisms was prepared by WHO in 1991 (UNEP/WHO, 1991), and submitted to the seventh ordinary meeting of the Contracting Parties to the Barcelona Convention and Protocols (UNEP, 1991). Except for a brief review of the situation regarding temporal trends in concentrations of bacterial indicator organisms in coastal (mainly recreational) waters on the basis of an interim review of MED POL Phase II monitoring data (UNEP, 1989) and data from Mediterranean States contained in annual EEC reports on bathing waters, the document concentrated on pathogenic microorganisms recorded in the Mediterranean, their source, dispersal and fate, and on microbiological/epidemiological studies conducted to date on the correlation between coastal water quality and health effects on exposed population groups. A number of recommendations on data acquisition through monitoring and research were made. It was not, however, recommended that, at that particular stage in time, any formal action be taken to amend the current interim microbiological criterion for acceptability of bathing waters, even on a further interim basis, as the global situation regarding the validity of the several bacterial indicators in current use was in a state of flux.

Scope of the present document

1-13. In approving and adopting the workplan and time-table for the progressive implementation of the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources, it was understood by the Contracting Parties that any particular item should be updated as appropriate. In view of the relative importance of microbiological pollution of coastal areas, and the facts that (a) the interim criteria for bathing waters and the environmental quality criteria for shellfish areas were now ten and eight years old respectively, (b) a considerable amount of MED POL monitoring and related data, mainly concerning recreational waters, were available for evaluation and interpretation, (c) there have been

recent advance in the field of epidemiological studies correlating recreational water quality and health effects, and (d) a number of bathing water quality standards, including international ones, are in the process of revision, it has been considered that a comprehensive and updated review of the situation is due, with a view to the improvement of current prevention and control measures.

1-14. The recent amendments made to the 1976 Barcelona Convention on the Protection of the Mediterranean Sea against Pollution (UNEP, 1995b, 1995c) and, as already stated, those currently under negotiation regarding the 1980 Athens Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources (UNEP, 1995a, 1995b) in no way affect the importance accorded to the prevention and control of microbiological pollution of the Mediterranean Sea, or the general procedures for the preparation, development and adoption of any individual or joint measures designed to achieve this purpose.

1-15. The present document was prepared for the World Health Organization and the United Nations Environment Programme by a consultant (Dr L.J. Saliba, Malta), and was reviewed by a WHO/UNEP consultation on microbiological monitoring of recreational and shellfish-growing waters, held in Athens, Greece, from 29 November to 2 December 1995 within the framework of the MED POL Phase II programme. The present version of the document incorporates, or takes account of, as appropriate, the comments of this expert meeting. It follows the general lines of content and format agreed on for MED POL assessments, and attempts to consolidate and update all previous information on the state of microbiological pollution of the Mediterranean Sea with particular reference to coastal recreational and shellfish areas through the inclusion of monitoring and research data, drawn from national MED POL monitoring programmes, MED POL research projects, EC annual reports on bathing waters, and other national and international sources. Wherever appropriate, relevant data from previous documents has been included, either in the original or in an abridged form, as appropriate, to make the document more self-contained, and provide a better view of temporal trends. An overview of the current situation, together with recommendations for possible action, is also included.

PART 2

THE ORIGIN OF MICROBIOLOGICAL POLLUTION OF THE MEDITERRANEAN SEA

2.1 SOURCES OF POLLUTION

2-1. Pathogenic and other microorganisms enter the marine environment mainly through municipal wastewater discharges. As is the case in other regions, microbiological pollution of the Mediterranean Sea is principally the direct result of the discharge of untreated or partially-treated sewage into the immediate coastal zone. In this regard, on the basis of the results of a pilot project on pollutants from land-based sources in the Mediterranean, carried out in 1976-77 within the framework of the MED POL Phase I Programme, it was estimated that at least up to the late 1970s, over 80% of liquid municipal waste used to be discharged into the sea in the raw state UNEP/ECE/UNIDO/FAO/UNESCO/WHO/IAEA, 1983). The same project provided an estimate of approximately $2 \times 10^9 \text{ m}^3$ /year for the total volume of wastewater discharges from coastal communities in the region. This figure has been considered as marginal compared to the estimated amount of $420 \times 10^9 \text{ m}^3$ /year of freshwater discharged by rivers. However, although rivers may add a considerable amount of microbiological pollution, mainly from upstream wastewater discharges, their actual relative contribution to pollution of the Mediterranean Sea by microorganisms (pathogenic and otherwise) has not been assessed, and it has been assumed that the high concentrations of microorganisms in wastewater discharges directly into coastal waters makes such discharges the major source of microbiological pollution reaching the Mediterranean Sea (UNEP/WHO, 1985).

2-2. During the fourth ordinary meeting of the Contracting Parties to the Barcelona Convention and Protocols in Genoa in September 1985, Mediterranean States adopted a formal Declaration (subsequently termed the Genoa Declaration) wherein they committed themselves to the achievement of a number of environmental targets during the second decade of operation (1986-1995) of the Mediterranean Action Plan. These targets included the establishment as a matter of priority of sewage treatment plants in all cities around the Mediterranean with more than 100,000 inhabitants, and appropriate outfalls and/or appropriate treatment plants for all towns with more than 10,000 inhabitants (UNEP, 1985a).

2-3. This target has only been partially achieved, but the general situation has improved considerably. An updated survey on pollutants from land-based sources in the Mediterranean was commenced in 1989. Owing to problems in the acquisition of the necessary data in a number of countries, this survey is not yet complete. Preliminary (unpublished) results available show that although there is an indication that the total volume of wastewater discharges has increased in global terms, due to new coastal development projects implemented during the past two decades, a significant amount of municipal wastewater, at least in some areas, now undergoes various stages of treatment prior to discharge. Data available from the survey show that, at the time of submission, in France, 87% of a total volume of municipal wastewater of 361×10^6 cubic metres per year was undergoing treatment prior to discharge. The figures for Slovenia are 82% of a total of $6.13 \times 10^6 \text{ m}^3$ per year, for Spain, 69% of a total of $589.29 \times 10^6 \text{ m}^3$ per year, for Croatia, 14% of a total of $71.44 \times 10^6 \text{ m}^3$ per year, and for Cyprus 12% of a total of $16.66 \times 10^6 \text{ m}^3$ per year. The figure for Cyprus will be considerably increased when a new treatment plant becomes

operational. Information from other countries is either unavailable, or shows that still very little, if any, treatment of municipal wastewater is being carried out.

2-4. This, together with the number of new submarine outfalls constructed during the last decade in various parts of the Mediterranean, means that a certain proportion of wastewater is no longer being discharged in the immediate coastal zone, *i.e.* practically at the land/sea interface, as a result of which better dilution and dispersion is achieved. Guidelines for submarine outfall structures for Mediterranean small and medium-sized communities have recently been prepared and distributed (WHO/UNEP, 1994a). However, untreated or inadequately-treated sewage, and disposal of this into the immediate coastal marine environment through relatively short outfalls, in many cases at the coastline itself, still remains the main factor of concern in a number of Mediterranean areas, and will remain so until the targets set in Genoa in 1985 are fully achieved.

2-5. The atmosphere may also serve as a pathway for the entry of pathogenic and other microorganisms into the coastal marine environment. It has been stated (Brisou, 1976) that winds blowing from the continents towards the sea carry, *inter alia*, bacteria, viruses and parasites, and that rain facilitates the descent of these pollutants into rivers and oceans. One other possible source, which affects mainly coastal recreational areas, is bathers themselves. Recreational waters not affected by sewage effluent discharges can be contaminated with enteroviruses, and that the serotype found in the water is likely to be the same one predominating in concomitant human infections (Shuval, 1986). Therefore, bathing waters contaminated by the bathers themselves may at times serve as an effective route of transmission of some viral diseases. This could also apply to other bacterial and fungal infections (Papadakis *et al*, 1992), and it has been reported that skin counts of certain bacteria and fungi can increase after bathing, even if the water is unpolluted (Papapetropoulou and Sotiracopoulou, 1995). There is currently an increasing amount of evidence linking adverse health effects with bathing in high-population-density beaches, and the contribution of bathers themselves as a source of pollution of recreational waters by pathogenic microorganisms is a subject which calls for serious consideration (WHO/UNEP, 1995).

2-6. Apart from pathogenic microorganisms (principally bacteria, viruses and fungi) discharged into the marine environment in municipal sewage effluents or from other terrestrial sources, another group of naturally-occurring marine microorganisms, which can be considered as pathogenic through their ability to produce various toxins, to which man is exposed mainly through the consumption of contaminated shellfish, can pose a similar problem to human health when present in large numbers. These microorganisms, mainly dinoflagellate algae, constitute a phenomenon known as an algal bloom or a red tide when their concentration in seawater reaches levels of 10^4 to 10^6 cells per litre. While the environmental conditions under which these microorganisms are able to reproduce asexually at high rates does not yet appear to have been fully elucidated, the fact that red tides are essentially a coastal phenomenon indicates that terrestrial factors are at least partially responsible. Land drainage has been considered as playing a rôle in their initiation (WHO, 1984). It has also been considered that the size of algal blooms is in proportion to the magnitude of incoming nutrient masses through rivers (WHO, 1991). In a review of the problem, Shumway (1990) states that a number of factors are thought to enhance algal blooms, including nutrient enrichment (eutrophication), decreased grazing pressure, large-scale hydrometeorological changes, upwelling of nutrient-rich bottom water, heavy precipitation and run-off, and even the presence of previous blooms of other phytoplankton species.

2-7. Eutrophication can be defined as the process of enrichment of waters with plant nutrients, primarily nitrogen and phosphorus, that stimulates aquatic primary production. Apart from algal blooms (or red tides), its most serious manifestations are algal scum, enhanced benthic algal growth leading at times to massive growth of submerged and floating macrophytes (Vollenweider, 1968, 1981). Sometimes these manifestations are accompanied by, or alternate with, cycles of visible bacterial blooms (Aubert, 1988) and fungal development. Eutrophication as a water quality problem differs from pollution-related ones mainly in the increased difficulty in distinguishing the process of eutrophication caused by man from processes and phenomena that may also occur naturally. Although not its main cause, sewage and other forms of water pollution may directly or indirectly enhance or counteract eutrophication (Vollenweider *et al*, 1993). In this context, it has been stated (Shumway, 1990) that it has been firmly established that there is a direct correlation between the number of red tides and the extent of coastal pollution, particularly from sewage and some forms of industrial waste.

2-8. Marine eutrophication is mainly an inshore problem that affects lagoons, harbours, estuaries and coastal areas adjacent to river mouths. Major publicity has been accorded to the Northern Adriatic as a problem area but, in the Mediterranean as a whole, coastal marine areas and lagoons prone to eutrophication problems exist in practically every country (UNEP/FAO/WHO, 1995). No comprehensive estimate of the total nutrient load to the Mediterranean Sea as a whole appears to have been made, a lacuna difficult to fill because of the lack of exhaustive source and reliable input data for all the countries bordering the Mediterranean Sea. A provisional estimate of 590,000 to 1,070,000 tons nitrogen and 104,000 to 120,000 tons of phosphorus annually has recently been made (UNEP/FAO/WHO, 1995). Although the main body of the Mediterranean Sea as a whole is not yet seriously threatened by eutrophication, there are localized problems, which can be quite serious in their actual or potential socio-economic and sanitary impact on tourism, aquaculture, fisheries and other water uses and which, in the light of projected population increases, will be aggravated in future years unless the necessary remedial and preventive measures are taken (UNEP/FAO/WHO, 1995).

2.2 DISPERSION AND FATE OF MICROORGANISMS IN THE MEDITERRANEAN MARINE ENVIRONMENT

Dispersion

2-9. Seawater is not the natural environment for most of the microorganisms discharged in wastewater effluents, particularly those originating in the intestinal tract of humans and other warm-blooded animals. In the first assessment of the state of microbial pollution of the Mediterranean Sea (UNEP/WHO, 1985), it was explained that concentrations of the three major groups of indicator bacteria normally utilized for determining the state of pollution of the sea by sewage (total coliforms, faecal coliforms and faecal streptococci) would not remain unaltered in the receiving waters, but would progressively disappear. However, it was also stated that whether or not all the microorganisms discharged in wastewater effluents were permanently inactivated during the hours following their mixing with the receiving seawater was the subject of considerable debate and of continuing research. Over the past century, many studies have been carried out to estimate the fate of pathogenic microorganisms (viruses, bacteria, fungi and protozoa) and bacterial indicator organisms in estuaries and seawater through both field and laboratory observations and experiments. (WHO/UNEP, 1991).

2-10. Microorganisms contained in sewage are dispersed by the turbulence of diffusion where they are discharged into the sea. On discharge into seawater, they are rapidly adsorbed on to particles of every kind that float in the water. (plankton, mineral particles, assorted organic debris) and when routine counts are made, this adsorption results in an apparent diminution in the number of microorganisms per unit of volume of seawater (Brisou, 1976). These adsorbents are diluted, dispersed, flocculated, sedimented or carried back to the coast. The coarse particulate matter contained in sewage has a tendency to settle rapidly in seawater, fixing microorganisms which are adsorbed onto it. Although this process of sedimentation plays an important role in the vicinity of waste discharge points (Mitchell and Chamberlin, 1975; Geldreich, 1978), it does not appear to be an essential factor in microbial disappearance, considering the high bacterial concentrations detected in these waters. On the other hand, the fine particles undergo a diffusion process whereby they transport a large quantity of microorganisms with them (Gauthier, 1980; Mujeriego *et al*, 1982; Borrego, 1982).

2.11. The physicochemical processes of flocculation of microbial cells, and their subsequent sedimentation to the sea bottom, have also been considered as the mechanism responsible for the microbial enrichment of sediments in the areas surrounding wastewater discharges (Mitchell and Chamberlin, 1975). Natural turbulence and marine currents can become a plausible mechanism by which the contaminated sediments can be re-suspended, with the consequent impairment of the microbiological quality of the overlying seawater (Volterra and Aulicino, 1981; Velescu, 1983).

Adaptation and survival

2-12. The survival of enteric bacteria in the marine environment was originally considered as essentially of short duration, these bacteria supposedly being destroyed comparatively rapidly by an assembly of antagonistic factors including physical factors such as temperature and solar radiation, chemical factors such as salinity, heavy metals, pH and xenobiotics, and biological factors such as macropredators, lytic substances or antibiotics produced by marine bacteria, algae or fungi. The effects of each of these factors will be briefly discussed later in this part of the document. During the last two decades, it has been progressively recognised that these bacteria, instead of being destroyed, can be subjected to considerable stress from hostile factors, particularly under the conditions prevailing in the marine environment (essentially low temperature, high salinity and nutritional insufficiency). It has been demonstrated that these bacteria can evolve towards a viable but not cultivable state which could be irreversible. During the course of such evolution towards dormancy, the cells become subject to highly significant structural and metabolic modifications which render them progressively inert, and which are essentially attributed to food scarcity. In effect, a striking analogy exists between such evolution and that of autochthonous marine bacteria in oligotrophic waters (Gauthier, 1992b).

2-13. The capability of a microbial cell to adapt may be overcome by its contact with seawater, which constitutes a hostile environment, such contact resulting in physiological damage which may be either sublethal or so intensive that it causes the death of the cell. The physiological damage or stress exerted by the marine environment on the allochthonous microorganism can be studied by observing either the degree of structural disorganization in the microorganism, or its inability to carry out a determined metabolic function when grown in a selective medium. However, these stressed cells can develop in culture media which do not contain inhibitory substances (Romero and Borrego, 1991). The importance of the study of physiological damage to pathogenic cells is based on the non-detection of these

cells in the performance of standard microbiological tests which, in turn, are based on the examination of selective culture media (Hoadley, 1981).

2-14. Large variations in T_{90} values (the time taken for a 90% reduction in bacterial counts) are observed in different marine areas. These differences are attributable to very different environmental conditions (WHO, 1991). Salinity, natural light, temperature, dissolved substances and natural predators are among the factors known to affect the survival of these microorganisms in seawater. The saline content of seawater varies from 3.3 to 3.8%, while the most favourable habitat for allochthonous microorganisms requires a salinity level of close to 0.9%. This significant difference in salt concentration anticipates the sea water's ability to inactivate these microorganisms. Such inactivation may be achieved through osmotic shock or through the specific toxicity of the ions (Carlucci and Pramer, 1960). It is also commonly known that increase in cellular metabolism is dependent on temperature. The accelerated activity of microorganisms caused by this increase can produce a greater inactivation of the toxic factors in the water (Aubert and Aubert, 1969; Vasconcelos and Swartz, 1976). The effects of temperature depend closely on the organism tested. Some bacteria or viruses (e.g. *Salmonella typhi* and some coliphages) are more sensitive than others (e.g. *Shigella* species) to high temperatures. *Vibrio* species (*Vibrio cholerae*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus*) are very sensitive to low temperatures; they generally become undetectable in marine environments at temperatures below 15 to 18°C (WHO, 1991).

2-15. Because of the specific toxicity of some metallic ions to numerous organisms, they can exert a negative effect on the sewage flora in even remotely low concentrations (Jones, 1971; Jones and Cobet, 1975). Because of their ability to inactivate enzymatic systems, it appears that these ions, or complexes of heavy metals, form part of the sea water's purifying process. The amount of available nutrients is another factor. Seawater is an oligotrophic substrate as a nutritive source, with a low level of nutrients, organic matter being a limiting factor in the growth of polluting microorganisms (Savage and Haines, 1971; Sinclair and Alexander, 1984). On the other hand, the presence of organic substances originating from sewage in the water stimulates bacterial growth, thus intervening in partial compensation of the bactericidal effects of other negative factors. This, of course, is not the case for strict parasites such as viruses.

2-16. In particular, solar radiation has been identified as the single most important factor responsible for microbial inactivation, and the inactivating effect of light is proportional to the intensity of, and the time exposed to, radiation (Gameson & Gould, 1975), its effect being determined by the transparency and concentration of organic matter dissolved in water (Fujioka *et al*, 1981). The lethal effect of light increases with intensity; the T_{90} values for bacteria are generally 90 to 100-fold lower in the light than in the dark (WHO, 1991). It has been indicated that the sublethal injuries induced by solar radiation in the catalase enzyme system of *Escherichia coli* render the cells sensitive to otherwise innocuous peroxide concentrations (Kapuschinski & Mitchell, 1981). In these authors' experiments, although standard cultivation techniques did not allow recovery of all the stressed microbial cells, the addition of peroxide scavengers, and particularly the catalase enzyme itself, proved capable of recovering a considerable portion of the affected *E. coli* cells. Furthermore, light acts synergistically with salinity and, to some extent, temperature. Maximal lethal action is observed in water with high solar radiation, high salinity and high temperature (WHO, 1991).

2-17. However, these physico-chemical factors, due to their large ecological influence, could have a direct or indirect effect on microbial pathogens by enhancing growth. In addition, antagonistic or protective activity by other marine organisms (bacteria, plants or animals) is possible. Extrapolation from laboratory results to natural environments is

somewhat hazardous; discrepancies observed between *in situ* and *in vitro* studies arise from the high complexity of natural conditions.

2-18. Furthermore, both *in situ* experiments and laboratory studies have shown the antagonistic activity of marine organisms on microbial pathogens. Sewage contains bacteriophages (bacterial viruses) of different microorganisms, which are also detected in seawater where the waste is discharged. Phages are strict parasites and usually kill bacterial cells, producing lysis plaques when the optimum conditions necessary for the growth of these bacteria occur. Because of these conditions, it is difficult to specify the degree of influence that phages exert on the process of seawater purification (Borrego, 1982). Another predator of bacteria, *Bdellovibrio bacteriovorus*, is widely distributed in soil, freshwater, seawater and sewage. However, it is difficult to determine its true ecological role (Starr and Seidler, 1971).

Myxobacteria have the ability to hydrolyze insoluble molecules, or to lyse bacterial cells and use them as a substrate. The purifying action of these bacteria, which act mainly on dead bacterial cells, is well established (Verstraeae and Voets, 1976). Protozoa play an efficient part, directly or indirectly, in the purification process, eliminating the organic matter and also the bacteria in the environment (Mitchell, 1971; McCambridge and McMeekin, 1980, 1981; Mallory *et al*, 1983). Naturally-occurring marine bacteria are generally better adapted than allochthonous microorganisms to the concentrations of nutrients found in their environment (Sinclair and Alexander, 1984). Thus, a competitive phenomenon for nutrients may occur in areas where the incidence of autochthonous flora is significant.

2-19. Several authors have indicated the importance of antibiotic substances in the water as inactivating agents of polluting microorganisms in the marine environment (Aubert and Aubert, 1969; Paoletti, 1970). Different microorganisms, such as *Actinomyces* species, *Streptomyces* species, *Bacillus* species and other algae, show the ability to synthesize antibiotic substances. A negative effect of these antibiotic substances on sewage flora has been observed in laboratory experiments, but its role *in situ* is of little importance, as the production of antibiotics is conditioned by the growth of the microorganisms in very rich media, conditions which are only produced with difficulty in the sea (Mitchell, 1971).

2-20. The mechanisms responsible for the evolution and disappearance of allochthonous microbial pathogens also appears to be different in other components of the marine environment, such as sediments or animal intestinal tracts. All the experiments performed in marine sediments have led to the conclusion that terrestrial bacteria can survive much longer (weeks, sometimes months) in such deposits than in the water column. This important increase in survival ability was initially attributed to the absence of light and to the presence of organic nutrients, although sediments contain many micro- and macropredators and antibiotic-producing microorganisms. Recent findings have emphasized the fact that marine sediments contain osmolytes, enabling enteric bacterial cells to regulate their turgor pressure and to restore a normal metabolism under marine conditions (WHO, 1991).

2-21. This regulatory activity can be considered an adaptive process that would help the human pathogens to survive in the marine environment. Organic osmolytes have been detected in marine deposits, sometimes at rather high concentrations. In addition, enteric bacteria are able to take up and use these osmolytes under marine conditions. Therefore, marine sediments could favour the maintenance (and probably growth) of enteric bacterial pathogens in a virulent state and thus act as a reservoir for such allochthonous contaminants. Furthermore, some eutrophicated waters could act in the same way, the overall survival capability of pathogens then depending, at least partly, on the balance between the antagonistic activity (antibiotics) and the protective action (osmolytes) of the algal population. More specifically, it is considered that some marine eutrophicated waters and sediments

could be considered as "high risk areas", as they could act as reservoirs yielding "adapted" resistant cells of enteric pathogens retaining their entire virulence. In such areas, it is assumed that "survival" would result from the balance between the antagonistic activity of several inhibitory factors (such as light, toxins and antibiotics) and the protective effect of organic matter (nutrition and osmoregulation) which would largely depend on the composition of algal and invertebrate populations (WHO, 1991). The prospective influence of marine sediments should, however, be emphasized since they prevent solar irradiation, they contain organic nutrients favouring bacterial growth, and they contain many quaternary amines and possibly osmolytes produced by microorganisms, plants and animals which could be transported through specific systems and increase osmoprotection (Gauthier *et al*, 1991). Enhanced survival of *Escherichia coli* and *Salmonella* have, in fact, been demonstrated in bottom sediments (Geldreich, 1985).

2-22. The behaviour of microbial pathogens in aquatic animal intestinal tracts and tissues is far less well known. Once taken up in fish or molluscs, they could behave differently than they do in the water. However, additional data are needed to confirm such differences and to elucidate the processes responsible for a special *in vivo* adaptation of terrestrial bacteria (WHO, 1991).

2-23. Besides these "classical" considerations, account should also be taken of recent metabolic and molecular studies of the reactions of bacteria to starvation and other environmental stresses. The ultraviolet component (wavelengths 300-400 nm) of bright sunshine is actively lethal to vegetative bacterial cells, and acts by the damaging (thymine dimerisation) of deoxyribonucleic acid (DNA). Unless this is reparable, the cell is unable to replicate. If Gram-negative bacteria are stored in suspension in water, they progressively lose the ability to be cultured. One of the first stages of starvation is an internal reorganization resulting in rapid degradation of cell protein, followed by resynthesis of new proteins, mediated by genes induced by carbon starvation and other genes, responsible for conferring resistance to stress. Mutations occur at an accelerated rate in an attempt to confer selective advantage. If the bacterial concentration is high, then metabolic products may be sufficient to maintain a small culturable population. The use of standardized studies on survival by Colwell and co-workers at the University of Maryland have suggested that bacterial pathogens can evolve toward a viable (*i.e.* a metabolically responsive) but non-cultivable state in seawater in which they could retain infectivity and virulence Colwell *et al*, 1985). This observation results from experiments in which large numbers of non-culturable but viable cells were injected into ligated rabbit ileum, resulting in an enterotoxigenic reaction and production of isolable cells. Other attempts to demonstrate retention of virulence in non-culturable gram-negative pathogens have been less successful. It is likely that the non-culturable state is a transient phase in bacterial decline in the environment. It has no significance at all for viruses, which can only multiply in their hosts.

2-24. Apart from this, any modification of environmental conditions could significantly modify the survival and evolution of microbial pathogens in the sea. The proliferation of mucilaginous algae in the Adriatic Sea in 1988 and 1989 has suggested the possibility that, under such new conditions, opportunistic pathogens may exceptionally resist or multiply. Recent studies dealing with the presence and evolution of bacterial indicators or pathogens during "mucilage" episodes have shown that the coloured tides observed along the Adriatic coast correspond to a generalized reduction of enteric microbial titres of these waters for the periods in question. However, samples containing mucilage appeared particularly enriched in marine halophilic organisms, such as *Vibrio*, *Aeromonas* and *Pseudomonas* species, indicating that opportunist autochthonous pathogens may be present in seawater and may overgrow under such abnormal conditions (WHO, 1991).

Comparative survival

2-25. Results from field studies carried out during the Coastal Water Quality Control pilot project (MED VII) between 1976 and 1980 within the framework of the MED POL Phase I Programme and elsewhere pointed out the different survival patterns of the three bacterial indicator organisms utilized for assessment of the microbiological quality of seawater. While total coliforms and faecal coliforms appear to be inactivated in seawater rather rapidly and progressively under natural conditions, faecal streptococci show a lower inactivation rate, as well as a smaller long-term percentage reduction (WHO/UNEP, 1981).

2-26. Volterra & Aulicino (1981) and Velescu (1983) have also recorded that, as is the case in circulating seawater, faecal streptococci are able to survive in sediments for longer periods than faecal coliforms, in some instances to the point of the former outnumbering the latter which is the reverse of the normal ratio observed in wastewater effluents themselves. However, the sea-bottom not being the natural environment for most of the microorganisms discharged in wastewater effluents, it can be expected that termination of the discharge or improvement in its quality, with subsequent depletion of organic substrates, the survival of these microorganisms would be highly impaired.

2-27. Considerable work on the comparative survival of pathogenic and indicator bacteria under Mediterranean conditions has been performed within the framework of the research component of MED POL Phase II. The effects of organic matter, temperature and light intensity on the survival of three *Salmonella* strains (*Salmonella enteritidis*, *Salmonella typhimurium* and *Salmonella flexneri*), *Shigella sonnei* and *Escherichia coli* were studied (Fuks 1991), both in the marine environment and in laboratory experiments with natural seawater. The survival of all *Salmonella* and *Shigella* strains tested, as well as of faecal coliforms, was higher than that of *Escherichia coli*. Results also showed that sea temperature may exert an important influence on the magnitude of decay rates in all organisms studied. However, *in situ* experiments revealed that a large amount of organic matter may overcome the bactericidal effects of sea temperature and light. It was also concluded that although the die-off rate of *Escherichia coli* did not differ significantly from that of *Salmonella typhimurium* in some experiments, their different tolerances to light and temperature should be taken into account, particularly when utilizing *Escherichia coli* as an indicator of the presence of *Salmonella typhimurium* in seawater.

2-28. A similar study was conducted to determine the comparative effects of temperature, salinity and light on the rate of die-off of *Salmonella typhi*, *Salmonella wien*, *Shigella flexneri* and *Escherichia coli* (El-Sharkawi *et al*, 1991), which were tested in natural seawater, sterilized seawater, tap water and normal saline. No great differences were found between the survival times of all test organisms at 25-35°C, and all dies off more rapidly at 40°C. The salinity of seawater did not appear to affect the survival time of the two *Salmonella* species and of *Shigella flexneri*, but *Escherichia coli* survived longer in fresh water than in seawater at temperatures between 30 and 35°C. Sunlight had a lethal effect on all test organisms. *Salmonella* and *Shigella* could only be isolated from the sewage boil, and could not be found more than 100 metres from the boil during daylight. At night, *Salmonella* could only be isolated within 400 metres, and *Shigella* within 200 metres, of the periphery of the boil.

2-29. A comprehensive study on the comparative survival of various strains of *Salmonella paratyphi*, *Salmonella thompson*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Streptococcus faecium*, *Candida albicans* and *Escherichia coli*, utilizing both strains isolated from seawater and reference strains, was performed in Malaga,

Spain (Romero and Borrego, 1991), including both *in situ* and laboratory tests. In one series of tests, the results, graphically obtained from the kinetics of inactivation, showed *Escherichia coli* as having the greatest survival rate, followed in descending order by *Salmonella* species, *Pseudomonas aeruginosa*, faecal streptococci, *Staphylococcus aureus* and *Candida albicans*. In other tests, the T_{90} values observed for *Candida albicans* and *Staphylococcus aureus* were superior to those of the indicators, thereby supposing that these pathogens can create a potential public health risk in waters in which the concentrations of indicator organisms are within normal acceptable values. A gradual process of cellular damage was observed in all the microorganisms tested. This increased in proportion to the time they were exposed to the sea. Metabolic damage was very important in the case of the enterococci and *Staphylococcus aureus*. *Escherichia coli*, *Candida albicans* and *Salmonella* species showed a significant recuperation of damaged cells with respect to initial percentages. In general terms, a more prolonged survival time of the microorganisms was found in laboratory experiments than in those tests carried out *in situ*. This has been explained as basically due to environmental factors, principally physical, which diminish concentrations in the sea while having no inactivating effects.

Survival mechanisms

2-30. The various strains of *Escherichia coli* have been the subject of a number of survival and adaptation studies, aimed at analyzing somatic, metabolic and genetic modifications of enteric bacteria placed in natural marine conditions. The structural and metabolic changes observed in thirteen strains of *Escherichia coli* cells during different periods of starvation in seawater have been described (Papapetropoulou *et al*, 1993), the conclusions of the study being that clinical *Escherichia coli* strains entering starvation in seawater face an altered physiological situation leading to different changes in the cells. The changes referred to are modification of enzymatic activity (biotyping changes), alteration of protein synthesis, loss of some factors contributing to virulence, and changes in sensitivity patterns. A number of studies have demonstrated that certain strains of *Escherichia coli* can adapt to various extents to marine conditions, thus prolonging their survival therein. In a comparative study of strains isolated from terrestrial wastewaters and those from merino samples, it was concluded that the former were more resistant to three antibiotics (novobiocin, tetracyclin and erythromycin), while the latter showed a greater tolerance to the salinity of their growth-medium, a greater resistance to certain antibiotics (cephaloridin, aminosides, nalidixic acid) and, in some cases, a greater overall resistance to antibiotics, and less tendency towards haemolysis and haemagglutination. In the case of species isolated from marine sediments, an increased sensitivity to high temperatures was recorded (Gauthier *et al*, 1991). The results suggested that the sojourn of *Escherichia coli* cells in the marine environment for a period not yet precisely defined could lead to an augmentation of their capacity to develop in saline media. In other studies, it was concluded, *inter alia*, that the survival of *Escherichia coli* cells (as well as that of other enteric bacteria such as *Salmonella paratyphi* B, *Shigella dysenteriae* and *Klebsiella pneumoniae*) in the sea depends, at least in part, on the events preceding their entry into seawater, that such survival closely depends on the possibility of the cells compensating for the osmotic shock they receive on entry into the sea as a result of the osmoprotective mechanisms at their disposal, and that cells from sediments acquire a considerable resistance to seawater and, when replaced in suspension in oligotrophic water, can survive for a comparatively long period (Gauthier, 1992a, 1992b).

2-31. The latter of the above-mentioned studies, on the influence of osmoregulatory mechanisms on the survival and adaptation of enteric bacteria, as exemplified by *Escherichia coli*, in the marine environment (Gauthier, 1992b) describes the genes responsible for the

intracellular synthesis, or acquisition from the external environment, of organic osmolytes such as betaines. Such acquisition or transport of some betaines by *Escherichia coli* is very efficient in marine sediments, which contain a considerable quantity of assimilable organic materials. This observation is considered to confer considerable sanitary importance to certain marine sediments, in the sense that, as already stated earlier in this section, these could play the role of reservoirs of enteric bacteria, and eventually pathogens, where such bacteria can develop an acquired resistance to marine conditions. It is concluded that it appears clear that enteric bacteria can survive in the marine environment according to the degree of possession of, or possibility of expressing, a certain number of genes which are directly implicated in the maintenance of homeostasis under high osmotic conditions. In this connection, the presence of nutritive organic substrates appears to play a determining role.

Experimental studies on the mechanism of gene plasmid transfer in enteric bacteria (*Escherichia coli*) in seawater, sediments and the digestive tract of marine invertebrates have been described (Gauthier, 1992c; Gauthier *et al*, 1992).

Survival of viruses

2-32. Irrespective of the source of the antagonistic processes occurring in the marine environment mentioned earlier in this chapter, both pathogenic viruses (enteric viruses, polio virus, Hepatitis virus A) and fungi (*Candida albicans*) survive for longer periods of time in seawater than bacteria (UNEP/WHO, 1991). Viruses are able to survive for extended periods of time outside an animal host (Akin *et al*, 1975) and can remain infectious for several weeks or longer after discharge into receiving waters. Enteric viruses can survive from a few days to over 130 days in marine water, survival being dependent on temperature, salinity, type of virus, bacterial antagonism, suspended solids and pollution. Factors affecting viral survival have been discussed by Gerba and Goyal (1978). Temperature appears to be the prime determining factor in viral survival in seawater, with increased inactivation in warmer waters. Little inactivation of Poliovirus 1 and Hepatitis A virus was observed at 5°C after 30 days, and at 25°C, Hepatitis A virus persisted significantly longer than Poliovirus (Bosch *et al*, 1993). In the same study, Poliovirus 1, Coxsackie virus B5, Echovirus 1 and Hepatitis A virus were shown to survive much longer in autoclaved as compared with natural seawater.

2-33. Several authors have suggested that particle association significantly extends the survival capacity of viruses, and increases their potential for interaction with local marine organisms (Shumway and Hurst, 1991). The results of a comparative study showed that the presence of marine sediment enhanced virus survival in seawater at 25°C, and the protective action of solids was particularly significant for Poliovirus after 30 days in marine waters (Bosch *et al*, 1993). Retention of viruses by gill structures is enhanced by adsorption of viruses to fine particulate matter, and this may have special significance for mussels, which are known to feed heavily on re-suspended organic matter. Solids-associated viruses transported to bathing beaches and shellfish-growing waters may therefore result in virus transmission to humans (Rao *et al*, 1986). In the 1972-1978 US Environmental Protection Agency's study on water quality and gastro-intestinal symptoms, the association between illnesses recorded and the presence of very few indicator bacteria (10 *Escherichia coli* per 100 ml) suggested that the agents responsible for the illness were highly infectious, were present in sewage in large numbers, and survived much longer than *Escherichia coli* in the marine environment. These characteristics, along with the clinical aspects of the illness, suggested a viral aetiology (Cabelli *et al*, 1982). Recent experiments carried out in the Adriatic Sea indicate that some algal species adsorb viruses and reduce their detectable infectivity. (Patti *et al*, 1990). The adsorbed viruses, however, may be infectious as free

viruses, and it is considered that unicellular algae, particularly during bloom episodes, may be a vehicle for transporting viruses to recreational and shellfish areas.

2-34. Uptake of viruses by shellfish has been clearly demonstrated (Metcalf and Stiles, 1965) and numerous studies have similarly demonstrated that shellfish can concentrate viruses in their tissues at densities that are much greater than those in the overlying waters (Geldreich, 1985). Like bacteria, the majority of viruses are concentrated in the digestive system of the host and, once inside a shellfish, the survival of viruses appears to be further prolonged (Metcalf and Stiles, 1965). Virus retention in shellfish appears to be even longer than that of bacterial pathogens (Geldreich, 1985). As an example, virus carriage for Hepatitis A in oysters has been reported to range from six to eight weeks (Portnoy *et al*, 1975; Macowiak *et al*, 1976). Carriage of enteric viruses in oysters for a period of five months with little reduction in numbers and no loss of infectious state has been reported as possible during the winter months when shellfish metabolism declined to a minimal level (Metcalf, 1982).

PART 3

PATHOGENIC MICROORGANISMS IN THE MEDITERRANEAN MARINE ENVIRONMENT

3.1 GENERAL CONSIDERATIONS

3-1. With very few exceptions, as is the case in other regions, monitoring programmes in the Mediterranean aimed at the estimation of the state of pollution of marine recreational and shellfish waters continue to rely largely on concentrations of one or more bacterial indicator organisms as an index of sanitary acceptability or otherwise, while the main risks to human health through bathing or seafood consumption depend on the presence and density of pathogenic microorganisms which are the real agents of disease. Among the exceptions are those Mediterranean EC Member States which apply the Community's 1975 Directive on bathing water Quality (European Community, 1976). This has a zero tolerance for *Salmonella* and enteroviruses, but limits sampling frequency to the discretion of national authorities by stipulating that concentrations should be checked by them when an inspection of the bathing area shows that the substance may be present or that the quality of the water has deteriorated. In the recent proposal for a new Council Directive on bathing waters (EC, 1994), the zero tolerance for enteroviruses have been retained, under conditions described in Part 4 of this document, but *Salmonella* has been removed as a monitoring parameter.

3-2. Since bacterial indicator concentrations provide a measure of the degree of faecal pollution based on total population figures, while the presence and concentration of pathogens in wastewaters depend on the incidence and amount of clinical or subclinical cases excreting the specific organisms in question, expected concentrations of pathogens in seawater or in shellfish would be much lower than those of indicators. Routine examination of wastewater or seawater for pathogenic microorganisms is rendered impracticable in most countries by the facts that:

- (a) the procedures involved in isolation and identification vary with each different pathogens;
- (b) even in the case of what is probably the most ubiquitous organism, *Salmonella*, isolation techniques involve relatively complicated procedures that exceed the routine capabilities of a number of laboratories;
- (c) negative findings for specific pathogens could only be considered provisional because state-of-the-art methodology is not sufficiently sensitive to detect a level of one pathogen in the volumes of seawater (100 ml) normally used for indicators, though this could be partially offset by the use of larger samples;
- (d) In this case of viruses, the procedures involved can only be carried out in laboratories specifically equipped for such work and, more often than not, are performed as part of special research projects and other related studies.

3-3. As a result of this, data on the presence and density of pathogenic microorganisms in seawater and shellfish is sparse in relation to that available for the commonly-used indicators of faecal pollution of the sea by sewage. Diarrhoeal diseases are prevalent throughout the Mediterranean region, and the agents concerned, usually a wide variety of enteropathogenic microorganisms, are discharged into the coastal marine environment in

sewage containing the faeces of diseased individuals or carriers. The incidence of such diseases, and the isolation of the pathogens concerned, in a coastal community will therefore provide a qualitative indication of the microbiological state of the sewage being discharged into the sea from that community, and the potential concentration of any particular microorganism in sewage will depend on its prevalence in each individual locality.

3-4. Correlation between concentrations of the more common bacterial indicators (which are found in normal human faeces) on the one hand and pathogens on the other is not possible on a general scale, as the former are determined by population density, while the latter are determined by the morbidity pattern of the pathogen in question. Studies on such correlation have been conducted in the Mediterranean (WHO/UNEP, 1995), but, as expected, results have varied considerably according to the locality in which they have been carried out.

3-5. Pathogenic microorganisms encountered in the Mediterranean marine environment include bacteria, viruses, fungi, other microparasites (mainly protozoa) and toxic algae. The more important species, are classified and described below. The data on incidence of the various microorganisms in specific areas should be taken only as examples, as it would not be possible to include all of the vast literature available on record. The information available as a whole, however, shows a geographical imbalance, and a large proportion of records are from the Northern part of the region. Information from the Southern and Eastern parts, where one would expect to find more pathogens in coastal seawater, are relatively sparse.

3.2 BACTERIA

Salmonella

3-6. *Salmonella* species, which are the agents for typhoid and paratyphoid fevers, food poisoning and gastroenteritis, have a worldwide distribution, and are abundantly represented in the Mediterranean. They are found in both untreated and treated sewage, and it has been stated (Abdussalam, 1990) that even the most effective sewage treatment only brings about reduction, rather than elimination. Over 2,200 serotypes are known though, at any given time, only a few (about 10 to 12) are prevalent in a given area or country (Abdussalam, 1990). It is considered (Brisou, 1976) that all the countries of the Mediterranean seaboard are, in general, major reservoirs of *Salmonella*. While major attention has been paid to *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella paratyphi* B, a large number of other serotypes have been isolated in the region. A total of 29 serotypes were isolated in Greece during the course of one study (Vassiliadis *et al*, 1987), 24 of these from sewage-polluted river water. Several different serotypes have also been isolated from France, Israel, Italy, Spain and Former Yugoslavia (Brisou, 1976). In a 3-year survey of *Salmonella* pollution of coastal seawater in the Gulf of Trieste, Majori *et al*, 1978), 401 of 1059 samples (37.8%) were positive for *Salmonella* strains, which were distributed among a wide range of serotypes. A total of 220 strains of *Salmonella* have been isolated from sewage effluents in Alexandria (El-Sharkawi *et al*, 1982b). 16 serotypes were isolated from coastal seawater samples from locations near a river mouth in the vicinity of Malaga, Spain, using different enrichment media (Borrego and MoriZigo, 1994).

3-7. *Salmonella* species do not survive long in seawater, and direct infection as a result of bathing or other recreational activity is not likely to occur due to the relatively high infective dose required in the case of most serotypes. On the other hand, the infective dose for *S. typhi* and *S. paratyphi* A and B is considerably lower. Consumption of seafood is a different problem, as the bacteria are concentrated either by filter-feeding shellfish or on fish

gills. In shellfish, the bacterial concentration may be 50 times that in water (UNEP/WHO/IAEA, 1988).

Vibrio

3-8. Cholera is one of the major diseases associated with the consumption of sewage-contaminated shellfish, and the causative agent, *Vibrio cholerae*, was discovered by Koch in Egypt during the 1883-1884 epidemic. The seventh pandemic wave of cholera, due to *Vibrio El Tor*, which originated in 1961 in Indonesia and is still active, spread to the Mediterranean region in the early 1970s. Cases have been reported in various parts of the region, including Algeria, France, Morocco and Spain (Brisou, 1976) and a major outbreak, in which mussels were identified as the carriers, occurred in Italy in 1973, where it caused 277 cases and 24 deaths (Baine *et al*, 1974). Brisou *et al* (1962) isolated 44 strains of *Vibrio* from the Algerian coast, Although only some of these can be considered pathogenic, there is clear evidence that some strains of naturally-resident aquatic bacteria are capable of causing gastroenteritis, systemic infections and intoxications in humans (Shumway and Hurst, 1991). An investigation in Morocco of the process and efficiency of sewage treatment by stabilization ponds under an arid Mediterranean climate showed that this treatment process appeared to be ineffective in eliminating *Vibrio cholerae* non-01, no bacterial reduction occurring during the season, and no significant reduction during the cold season.

3-9. NAG (non-agglutinable) vibrios, which cause gastroenteritis, are also frequently found in shellfish in the region. As in the case of *Vibrio cholerae*, NAG-vibrios are also discharged through sewage effluents, and infection is most likely to occur through consumption of contaminated shellfish. A total of 214 *Vibrio* serotypes were analyzed and identified during a relatively recent study in Toulon, (Martin and Bonnefont, 1990), comprising effluent, seawater and mussel samples. The *Vibrio* population in the effluent was the most diverse, including several species of public health interest, such as *Vibrio fluvialis*, *Vibrio cholerae* (non-01) and *Vibrio Metschnikovii*. these three species, however, were not found in seawater or mussels.

3-10. Two other *Vibrio* species widespread in the Mediterranean are natural to the marine environment, and no correlation exists between their presence and pollution of the sea by sewage. In the case of *Vibrio parahaemolyticus*, the main cause of infection is again shellfish (UNEP/WHO/IAEA, 1988), though wound infection by contact with seawater is another route of transmission (WHO, 1982) *Vibrio alginolyticus*, which causes otitis, sore throat and wound infections, occurs in coastal marine areas, and its main route of transmission in man is through contact with seawater and sediments. Isolations of *Vibrio parahaemolyticus* and, to a lesser extent, *Vibrio alginolyticus*, have been reported from a large number of Mediterranean countries (Boccia *et al*, 1978), the samples examined including seawater, mussels, benthic molluscs and sediments. In a study of various mollusc beds located along the Tyrrhenian coast, concentrations of *Vibrio parahaemolyticus* recorded were 10^3 in mussels (*Mytilus galloprovincialis*), 10^4 in *Ensis siliqua* minor, and 10^5 in *Chamelea gallina*, as compared to 10^1 in seawater (Volterra, 1991).

3-11. In a total of 165 samples collected in the neighbourhood of Alexandria in 1979-1980, average counts of *Vibrio parahaemolyticus* per 100 ml or 100 g were 36 for seawater, 345 for the sea urchin *Echinus*, 436 for sediments, and 534 and 1872 for the molluscs *Tapes* and *Donax trunculus* respectively. Samples collected during summer contained higher levels than those collected during winter (El-Sahn *et al*, 1982). *Vibrio parahaemolyticus* and *Vibrio alginolyticus* were also isolated from samples of seawater in North Adriatic bathing areas in

1989 (Maini *et al*, 1990), and the latter species was found to be the prevalent one in seawater and mussels during the Toulon survey (Martin and Bonnefont, 1990). Levels of *Vibrio* species recorded in seawater during an epidemiological study conducted on two beaches in Spain between 1988 and 1989 ranged from 100 to 2800 per 100 ml in the less polluted beach and from 250 to 12000 in the more polluted one (Borrego *et al*, 1991). No distinction between species was made.

3-12. Recent studies indicate that the old concept of considering *Vibrio cholerae* as a pathogenic microorganism found mainly in countries with no sanitary facilities, and that survival of this type of *Vibrio* outside the human intestine is limited, has to be radically revised. *Vibrio cholerae* and a number of other *Vibrio* species are commonly found as natural residents of aquatic environments in cholera-free areas, and their presence is not necessarily associated with faecal contamination (West, 1989).

3-13. In the last 25 years, the increasing number of epidemiological and ecological observations in many countries indicate that many environmental strains of *Vibrio* species are human pathogens. Out of 11 *Vibrio* species considered as causing illness in man (West, 1989), *Vibrio cholerae* 01, *Vibrio cholerae* non-01, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio mimicae* (previously known as *Vibrio cholerae*) and *Vibrio alginolyticus* have been found increasingly often associated with human illness, either through seafood consumption or through water contact and abrasion. More attention is being paid to *Vibrio vulnificus*, which can cause fatal septicaemia and gastroenteritis, as well as wound infections. However, the presence of vibriaceous organisms, particularly *Vibrio vulnificus*, in seawater and shellfish depends on temperature and salinity, and the species appears seasonally in the records (Kelly and Stroh, 1988; O'Neil *et al*, 1992; Kaspar and Tamplin, 1993; Cook, 1994).

Shigella

3-14. *Shigella* species, which are the agents of bacillary dysentery, are also, like *Salmonella*, widely distributed throughout the world, and enter into the marine environment in sewage effluents. The proportion of cases diarrhoea due to *Shigella* species varies in different parts of the world, but they are among the first five agents in many studies. *Shigellae* cannot be considered as one single entity, as the four main species known, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*, are different in their pathogenicity, means of spreading and resistance to environment. Epidemiological data available indicate that the incidence of *Shigella* infection has not decreased much during the last decade or so in developed countries, where *Shigella sonnei* and, to a certain extent, *Shigella flexneri*, are predominant. In developing countries, the other two species, *Shigella dysenteriae* and *Shigella boydii*, are also frequent. (Dardanoni and Nastasi, 1990). Multiresistant *Shigella dysenteriae* and *Shigella flexneri* infections show a high case-fatality rate, since both oral rehydration and antibiotic treatment may be ineffective. The importation of multiresistant *Shigella* strains carrying transmissible plasmids may be the source of spread to other enteric bacteria and to indigenous *Shigella* strains (Dardanoni and Nastasi, 1990).

3-15. *Shigella* is endemic on the eastern and southern shores of the Mediterranean (Brisou, 1976). All four species have been recorded in France and (with the exception of *Shigella dysenteriae*) in Italy (Dardanoni and Nastasi, 1990). Isolations have also been made in Egypt (El-Sharkawi *et al*, 1991) and along the Eastern Adriatic coast (Fuks, 1991). In the 1991 assessment of the state of pollution of the Mediterranean Sea by pathogenic microorganisms (UNEP/WHO, 1991), it was noted that cases of dysentery reported from

the region called for a more comprehensive assessment of the situation than had hitherto been undertaken, and that statistics on the occurrence of *Shigella* species in sewage should be the first step in correlating this with cases of dysentery. This statement still holds good.

Staphylococcus

3-16. *Staphylococcus aureus* and related species, particularly *Staphylococcus epidermidis*, are potential pathogens associated with skin, skin glands and mucous membranes of warm-blooded animals, including man. They are found in swimming pools and natural bathing waters, and coagulase-positive strains cause a wide range of infections and intoxications, including boils, abscesses, meningitis, furunculosis, pyaemia, osteomyelitis, otitis, suppuration of wounds and food poisoning. *Staphylococcus aureus* is salt-tolerant, and can survive in the marine environment (UNEP/WHO/IAEA, 1988). Ear infections due to *Staphylococcus aureus*, as well as other infections affecting the skin and naso-pharyngeal tract, are suspected of being transmitted through bathing water (WHO, 1982). The origin of the pathogen in seawater is attributed to human activity, as all strains have been found to be shed by bathers under all conditions of swimming (Robinton and Mood, 1966).

3-17. In 628 samples of coastal water monitored in Israel, 60.7% contained *Staphylococcus aureus*, including 33 samples (5.3%) with one cell per 100 ml, ranging from 49.5% in less populated beaches to 91% in beaches sampled during the highest bathing load (Yoshpe-Purer and Golderman, 1991). In a later study in the same general area, varying counts of *Staphylococcus aureus* were recorded in both seawater and sand (Ghinsberg *et al*, 1994). A comparison between concentrations of *Staphylococcus aureus* in seawater and sand of heavily-populated and slightly-populated beaches in Greece (Papadakis *et al*, 1992) showed positive readings in all samples. While concentrations of *Staphylococcus aureus* in seawater were not significantly different between the various beaches, considerably higher concentrations were found in the sand of highly-populated beaches. *Staphylococcus aureus* was not isolated from seawater and sand in the 1988-1989 study conducted in Malaga (Borrego *et al*, 1991), but the authors confirm its presence in previous surveys. Out of 265 samples of coastal seawater from bathing beaches in Greece, 6.8% were found to contain *Staphylococcus aureus*, three of the 18 positive samples having between 101 and 500 CFU per 100 ml, and the remaining 15 between 11 and 100 CFU per 100 ml (Papapetropoulou and Rodopoulou, 1994). In another study in Greece, *Staphylococcus aureus* was recorded in various concentrations from both seawater and sand samples, and a significant correlation was found between bacterial counts in both media and the number of swimmers at the beach, the correlation being clearer in a high population density beach (Papadakis, 1994).

3-18. Although *Staphylococcus aureus* is linked with food-poisoning in general, records of transmission through shellfish are relatively sparse. In Egypt, shellfish taken from highly-polluted water have been considered unacceptable in quality due to the presence of pathogenic microorganisms, including *Staphylococcus aureus* (El-Sharkawi *et al*, 1982a).

Pseudomonas

3-19. *Pseudomonas aeruginosa* causes ear, eye, wound, burn and urinary tract infections, as well as enteritis. The route of transmission to man was previously considered to be mainly infected swimming pools (WHO, 1982). but the organism is becoming increasingly implicated in ear, throat and skin infections through bathing in contaminated seawater (UNEP/WHO/IAEA, 1988). Numerous cases of folliculitis, dermatitis, ear and urinary tract

infections due to *Pseudomonas aeruginosa* that were acquired by bathing in contaminated water (mainly swimming pools, but including seawater) have been reported (Yoshpe-Purer and Golderman, 1991).

3-20. *Pseudomonas aeruginosa* can be recovered from about 10% of normal human stools, and is consequently frequently found in sewage, where concentrations may reach 10^5 per 100 ml (Rhame, 1979). Counts in excess of 1600 per 100 ml have been recorded in Spain in a polluted river near its outlet to the sea (Alonso Molina *et al*, 1984), and lower counts in seawater, also in Spain, ranging from 0 to 210 (Borrego *et al*, 1991). In Israel, out of 652 samples of seawater from various beaches collected between 1983 and 1984, nearly 50% contained *Pseudomonas aeruginosa*. In a small number of these samples, the normal indicators of faecal pollution (Faecal coliforms and *Escherichia coli*) were either low in concentration, or absent (Yoshpe-Purer and Golderman, 1991). In a later study in the same area, both seawater and sand in a number of beaches contained various levels of *Pseudomonas aeruginosa* (Ghinsberg *et al*, 1994). Out of 265 samples of coastal seawater from bathing beaches in Greece, 12.4% were found to contain *Pseudomonas aeruginosa*, counts varying from below 2 to between 101 and 500 cfu per 100 ml (Papapetropoulou and Rodopoulou, 1994). In water samples collected from several aquatic environments in Malaga, Spain, *Pseudomonas aeruginosa* counts and isolation frequencies were clearly associated with the degree of faecal pollution, the results confirming that sewage, from which it was isolated at densities of 10^5 cfu per 100 ml, was the major source (de Vicente *et al*, 1991).

3-21. High concentrations of *Pseudomonas* species have been recorded in shellfish. Concentrations as high as 110,000 per 100 ml in *Mytilus galloprovincialis*, 460,000 per 100 ml in *Donax trunculus*, 420,000 per 100 ml in *Ensis siliqua* and 34,000 per 100 ml in *Chamelea gallina* have been reported, these concentrations varying from one to four orders of magnitude greater than that of the surrounding waters (Volterra, 1991).

Campylobacter

3-22. *Campylobacter (Helicobacter)* became recognised as an important bacterial pathogen of humans about a decade ago (Geldreich, 1985). The occurrence of campylobacters in natural water is extremely variable, and it is not yet exactly known how many of the organisms isolated are pathogenic to humans (APHA, 1985). Thermophilic campylobacters have received considerable attention in recent years as a major cause of bacterial enteritis in man (Jones *et al*, 1984; Fricker, 1987; Skirrow, 1987). Two species, *Campylobacter jejuni* and *Campylobacter coli* cause diarrhoea and fever, and can both be transmitted through seafood consumption or ingestion of water. *Campylobacter jejuni* can be found worldwide and, in developed countries, is now recognised as one of the most common causes of bacterial diarrhoea (Skirrow, 1987). To a lesser extent, enteritis is also associated with *Campylobacter coli*. Enteritis is also produced by Nalidixic Acid Resistant Thermophilic *Campylobacter* (NARTC), formally described as a new species with the proposed name of *Campylobacter laridis* (Benjamin *et al*, 1983) and by a fourth thermophilic species, *Campylobacter upsaliensis* (Sandstedt and Ursing, 1986). Thermophilic campylobacters are common gastrointestinal pathogens, and they may cause more enteritis than salmonellas do (Svedhem and Kaijser, 1980; Blaser *et al*, 1983). Enteritis produced by *Campylobacter* is usually a mild to moderate self-limited illness; however, patients with severe, prolonged or relapsing enteritis should receive treatment (Lariviere *et al*, 1986).

3-23. *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter faecalis*, have been isolated from sewage outfalls and polluted seawater in various parts of the Mediterranean (UNEP/WHO, 1991). In Greece, isolations from such matrices of *Campylobacter jejuni* and, to a lesser extent, *Campylobacter faecalis*, have been reported as occurring fairly regularly, particularly during July and August (Papadakis, 1987). A total of 21 *Campylobacter* strains were subtyped during a study in Spain ((Alonso Molina *et al*, 1993). These include strains of *Campylobacter jejuni*, *Campylobacter coli*, and a number of unidentified species. Low levels of *Campylobacter jejuni* were recorded in both coastal seawater and sand (mainly in the latter matrix) in a number of Israeli beaches. Its presence in sand indicated that it may be considered a possible hazard factor for enteritis in the population bathing in the beaches in question (Ghinsberg *et al*, 1994).

Aeromonas

3-24. One of the not wholly enteric bacterial pathogens in the Mediterranean is *Aeromonas hydrophila*, which causes septicaemia in immunosuppressed hosts, diarrhoea, pneumonia, abscesses and wound infections (UNEP/WHO, 1991). It can be transmitted through contact with, or ingestion of, water or through consumption of contaminated seafood. *Aeromonas* species are known for their importance as pathogens in fish, reptiles and warm-blooded animals (Janda and Duffey, 1988). Over a decade ago,, the significance of *Aeromonas* species as human pathogens started to receive increasing attention (Burke *et al*, 1983, 1984). They are considered to be of public health significance when found in the environment in large numbers (Kaper *et al*, 1981). The aquatic environment is considered to be the major source of infection (Joseph *et al*, 1979; Burke *et al*, 1984). Reports from many parts of the world suggest that *Aeromonas* species cause an acute self-limiting diarrhoeal illness in man (Barer *et al*, 1986; Mascher *et al*, 1989). *Aeromonas hydrophila* and *Aeromonas sobria* have been more frequently associated with human infections (Daily *et al*, 1981; Diaz and Velasco, 1987), whereas *Aeromonas caviae* is less invasive (Watson *et al*, 1985). *Aeromonas* species may possess virulence factors such as proteases, enterotoxins, haemolysins, endotoxins and cytotoxins (Turnbull *et al*, 1984; Watson *et al*, 1985; Burke *et al*, 1986; Steima *et al*, 1986; Barer *et al*, 1986).

3-25. The Mediterranean appears to be an ideal environment for the proliferation of *Aeromonas* species, since the climate is temperate throughout the year, the rivers have little flow and a high load of organic matter, and many of the beaches affected by these rivers serve as bathing areas for thousands of people (Araujo *et al*, 1988). Levels of *Aeromonas hydrophila* recorded in seawater during an epidemiological study in Malaga, Spain, varied between 0 and 50 per 100 ml in non-polluted beach waters to between 80 and 11,800 in polluted beaches (Borrego *et al*, 1991). High counts of total motile aeromonads were recorded in seawater at various beaches in Valencia, Spain, levels varying considerably, but exceeding 10^6 cfu per 100 ml in many instances. Biochemical reactions were recorded for 759 motile aeromonad strains. Biotyping distinguished three species, the most frequent being *Aeromonas caviae* (79.8%, followed by *Aeromonas hydrophila* (14.9%) and *Aeromonas sobria* (5.3%) (Alonso Molina *et al*, 1993). Out of 265 samples of coastal seawater from bathing beaches in Greece, 5.6% were found to contain *Aeromonas hydrophila*, counts varying from between 2 and 10 to between 101 and 500 cfu per 100 ml (Papapetropoulou and Rodopoulou, 1994). Levels recorded in shellfish include 36,000 per 100 ml in *Mytilus galloprovincialis*, 740,000 per 100 ml in *Donax trunculus*, and 22,000 per 100 ml in *Ensis siliqua* (Volterra, 1991).

Escherichia coli

3-26. *Escherichia coli*, which falls within the faecal coliform group, is a normal inhabitant of the human alimentary tract. Some strains cause diarrhoea. Those which do can be grouped into five categories: Enterotoxigenic *Escherichia coli* (ETEC), which causes travellers' diarrhoea and childhood diarrhoea in developing countries, Enteroinvasive *Escherichia coli* (EIEC), which causes a dysentery-like illness more commonly reported from developing countries, Enteropathogenic *Escherichia coli* (EPEC), which causes infantile diarrhoea in epidemic and sporadic form in children below two years of age, Enterohaemorrhagic *Escherichia coli* (EHEC), which causes haemorrhagic colitis and haemolytic uraemic syndrome, and Enteroadherent *Escherichia coli* (EAEC), which causes diarrhoea in travellers, and possibly in others (Barua, 1990).

Other bacterial pathogens and indicators

3-27. Members of the Lancefield's Group D of streptococci (*Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus bovis* and *Streptococcus equinum*) have been incriminated in a number of outbreaks of food-borne diseases associated with mainly non-marine sources. Cases involving shellfish are less documented (WHO/UNEP, 1995). Haemolytic streptococci (Lancefield's Group A and C) have been recorded from bathing waters (WHO, 1982), and their transmission to humans by this route is suspected.

3-28. *Clostridium perfringens* is discharged in significant amounts in sewage, where it is mainly of human origin. It is more resistant than other indicators, but its detection in seawater is difficult. It does not multiply in sediments, but survives longer in the marine environment than *Escherichia coli*, and the two organisms can therefore be considered as complementary. In a study in the Barcelona area, Spain, levels of *Clostridium perfringens* were higher in naturally-occurring mussels than those of faecal coliforms and faecal streptococci (Jofre *et al*, 1994). As there are significant differences in *Clostridium perfringens* counts near to and far from sewage discharge points, interpretation of the counts appears to be feasible. The use of this species as a monitoring parameter, however, is still a matter for discussion.

3-29. *Yersinia* has been recognized as an important type of bacterium in terms of epidemiology and zoonoses (WHO/UNEP, 1995). It has been demonstrated as present in seawater, as well as in other media, and pigs and rodents are considered to be the main natural reservoirs. Its status can be considered to be similar to that of *Shigella* in that more information is required, the examination of sewage being the necessary initial step.

3-30. The sanitary significance of *Klebsiella pneumoniae* and other non-*Escherichia coli* strains within the coliform group in shellfish waters has been the subject of some controversy (Geldreich, 1985). The problem appears to be restricted to estuarine waters receiving an overlay of pulp and paper mill effluents, sugar cane processing wastes and textile manufacturing wastes. In these discharges, environmental strains of *Klebsiella pneumoniae* are the predominant coliform, representing 50 to 90% of the total coliform population, and often undergo multiplication within the warm process waters. Coliform densities may range from 10^4 to 10^6 per 100 ml upon release into the receiving waters. While these *Klebsiella* strains are not of sanitary significance, their occurrence indicates the presence of organic wastes which could seriously reduce or destroy shellfish productivity in the affected estuarine environment (Presnell and Brown, 1977). Other *Klebsiella* occurrences are of sanitary significance, as 30 to 40% of humans carry this coliform in their intestinal tract and the

Klebsiella density may range from 10^6 to 10^8 organisms per gram of faeces (Geldreich, 1985).

3.3 VIRUSES

3-31. More than 140 different viruses are known to be excreted in human faeces by infected persons, whether or not they manifest illness (Rao *et al*, 1986). These viruses belong to various families, including enteroviruses (polioviruses, coxsackieviruses and echoviruses), the new genus *Hepatovirus*, of the family Picornaviridae (Hepatitis A virus), reoviruses, adenoviruses, parvoviruses (adeno-associated viruses), and the family Calciviridae (Norwalk virus) (Schwartzbrod and Deloince, 1995). These groups, with the number of types, and the diseases caused, are outlined in Table 3.3.1

3-32. The frequency of isolation and the quantity of virus recovered from sewage depend not only on the infections caused by naturally-occurring viruses and those induced by oral poliovirus vaccine, but also on the efficiency of the recovery procedures. According to Sellwood *et al* (1981), the serotypes that can be detected at any specific time in sewage, except for polioviruses, reflect to a greater or lesser extent those viruses circulating within the community with the highest frequency. However, in countries using the Sabin vaccine to immunize against poliomyelitis, it is expected that all three strains of polioviruses would be present in urban sewage. In more than one Mediterranean country, polioviruses are detected in every sewage sample tested (Krikelis *et al*, 1985a).

Viruses in the Mediterranean Sea

3-33. Enteroviruses have been recorded in sewage effluents and various matrices (Seawater, sediments and/or mussels) of the Mediterranean marine environment. There is a geographical imbalance in the records, all of those apparently available coming from only four countries - France, Greece, Italy and Spain. According to available literature, isolates include all three serotypes of polioviruses, thirteen serotypes (1,2,3,4,5,6,11, 15,19,20,21,23,30) of echovirus, four serotypes (7,16,18,21) of Coxsackie virus A, six serotypes (1,2,3,4,5,6) of Coxsackie virus B, Hepatitis A virus, and a large number of unspecified serotypes (Crovari *et al*, 1974; De Flora *et al*, 1975; Hugues, 1994; Hugues *et al*, 1993; Krikelis, 1987; Krikelis *et al*, 1985a, 1985b, 1986; Maini *et al*, 1990). Records are summarized in Table 3.3.2.

3-34. As even a few plaque-forming units (PFU) or, as alternatively termed, cytopathogenic units (CPU) of virus may lead to infection when swallowed, provided they reach target cells in the organism, the presence of human viruses in seawater has to be taken seriously, and the danger of infection as a result of bathing in polluted waters is therefore not imaginary (Katzenelson, 1977). Although epidemiological studies have not, so far, shown any clear correlation between swimming in polluted water and viral epidemics, sporadic cases of infection cannot be ruled out. Fattal and Shuval (1991) attempted to establish the aetiology of swimming-associated gastroenteritis observed in earlier epidemiological studies correlating bathing water quality with observed health effects on exposed population groups. The response of the study population to blood donation was poor, forty individuals (4.9%) donating at least one blood sample, of which only 24 (2.9%) donated both acute and convalescent blood samples. All blood sera were positive for rotavirus in both samples, and no seroconversion was detected.

TABLE 3.3.1

HUMAN VIRUSES THAT MAY BE PRESENT IN POLLUTED WATER
(after Schwartzbrod and Deloince, 1995)

Family	Genus	Virus	Serotypes	Disease caused
Picornaviridae	<i>Enterovirus</i>	Poliovirus	3	Paralysis, meningitis, fever, poliomyelitis
		Coxsackie A virus	23	Herpangina, respiratory disease, meningitis, fever
		Coxsackie B virus	8	Myocardia, congenital heart anomalies, rash, fever, meningitis, respiratory disease, pleurodynia.
		Echovirus	32	Meningitis, respiratory disease, rash, diarrhoea, fever
	Enterovirus 68 to 71	4	Meningitis, encephalitis, respiratory disease, acute haemorrhagic conjunctivitis, fever	
	<i>Hepatovirus</i>	Hepatitis A virus	1	Viral hepatitis A
Reoviridae	<i>Reovirus</i>	Human Reovirus	3	Not clearly established
	<i>Rotavirus</i>	Human Rotavirus	6	Gastroenteritis
Calciviridae	<i>Calcivirus</i>	Human Calcivirus	3(5)	Gastroenteritis
		Norwalk virus	1	Gastroenteritis
		Small round virus	13	Gastroenteritis
		Hepatitis E virus	1	Infectious hepatitis
	<i>Astrovirus</i>	Human Astrovirus	5	Gastroenteritis
		Parvovirus-like virus (H.F.P.L.V.)	1	Gastroenteritis
Coronaviridae	<i>Coronavirus</i>	Human Coronavirus	1	Enterocolitis
Toroviridae		Coronavirus-like virus		Gastroenteritis
Adenoviridae	<i>Mastadenovirus</i>	Human Adenovirus	41	Respiratory diseases, eye infections, gastroenteritis

TABLE 3.3.2

VIRUSES ISOLATED IN THE MEDITERRANEAN MARINE ENVIRONMENT

(from WHO/UNEP, 1995)

Virus	Type	Location	Matrix
Enteroviruses			
Poliovirus	2 (vaccine-like)	Italy	sediments
	1,2,3 (vaccine-like)	Italy	seawater
	1,2,3	Greece	wastewater, seawater
Echovirus	9,11	Italy, France	seawater
	1,3,7,15,19,21,23,30	Greece	wastewater
	5,7,23	Greece	seawater
	6, unspecified	France	seawater
	5,11,20	France	seawater
	4,6,11,12,19	France	wastewater
Coxsackie virus A	7	France	seawater
	7,16,21	France	wastewater
Coxsackie virus B	1,2,3,5	Italy, France	seawater
	1,2,3,4,5,6	Greece	wastewater
	1,5,6	France	wastewater
	4,5	France	seawater
	2,4,5,6	Greece	seawater
	1,6	France	seawater
Hepatitis A virus		Spain	wastewater
unspecified, non-polio		France Italy	seawater sediments
unspecified		Greece Italy	wastewater, seawater wastewater, seawater
Other viruses			
Adenoviruses	1,2,3,4,5,7,15	Greece	wastewater
	1, unspecified	France	wastewater
	unspecified	France	wastewater
	unspecified	Italy	seawater
Rotavirus	unspecified	Spain	wastewater

3-35. Most research work performed in the Mediterranean on viruses in sewage effluents or seawater have been qualitative as distinct from quantitative. Among the results in the latter category, enterovirus concentrations recorded in sewage effluents in the Eastern Mediterranean have ranged from 10 to 90 PFU per litre, and adenovirus concentrations from 70 to 3200 PFU per litre. In coastal waters, a total virus range of 5 to 145 PFU per litre has been recorded (Krikelis *et al*, 1985a, 1987). In the Tyrrhenian Sea, the concentration of enteroviruses in coastal waters ranged between 2 and 160 TCD₅₀ per litre (Crovari *et al*, 1974).. In inshore shallow waters(0.5 to 12 metres deep), enteroviruses were shown to accumulate in clastic sediments, with concentrations ranging between 0.8 and 40.2 TCD₅₀ per 100 ml sediment eluate. Viruses were easily released into water by means of mechanical shaking and simulation of wave motion and bottom currents (De Flora *et al*, 1975). Among other records for the Western Mediterranean, mean values for total enteroviruses are 258 PFU per 10 litres in raw sewage, and 1.35 to 2.1 PFU per kg in marine sediments (Jofre, 1987). In another series of experiments, mean values for recovery of enteroviruses from 24 sediment samples varied between 200 PFU enteroviruses and 57 FF rotaviruses per kg, and 130 PFU enteroviruses and 140 FF rotaviruses per kg, depending on the specific elution procedure used (Jofre *et al*, 1989). During the years 1990 and 1991, in biologically-treated sewage effluents in the central Mediterranean (the French coast), the quantity of virus ranged from <1 to 250 MPNCU per litre (Hugues *et al*, 1993).

Viruses in Mediterranean shellfish

3-36. The role of shellfish as vectors in human enterovirus diseases is well-documented. Viruses which have been shown epidemiologically to be transmitted by shellfish are Hepatitis A and E (the latter of which, however, is not endemic to the Mediterranean region), Norwalk, Snow Mount agent, astroviruses, Coxsackie viruses and small round viruses. Of these, Hepatitis A and Norwalk viruses appear to be of chief concern to public health officials (Shumway and Hurst, 1991). There are a number of reports worldwide of gastrointestinal disease due to eating shellfish for which no causative agent has been identified, and many of these cases were believed to involve an unidentifiable viral agent, rather than a bacterial pathogen (Geldreich, 1985).

3-37. A number of epidemics of Hepatitis A have occurred in Europe, as well as worldwide, over the last 30 years, in addition to the endemic background of sporadic cases that may total several hundred per year (Shuval, 1986). It has been indicated (Stille *et al*, 1972) that consumption of contaminated molluscs accounted for an estimated 19% of the cases of Hepatitis A in Frankfurt, and that German cases were mainly attributable to consumption of oysters and mussels on the Mediterranean littoral. Poliovirus, Coxsackie virus A 18 and Echovirus 3,5,6,8,9,12 and 13 have been variously reported from France and Italy, while in Greece, Hepatitis A virus and Hepatitis A antigen have been recorded in shellfish from polluted waters (Crovari *et al*, 1974; Hugues, 1994; Hugues *et al*, 1993; Maini *et al*, 1990; Papaevangelou *et al*, 1990). Records are summarized in Table 3.3.3.

TABLE 3.3.3
VIRUSES ISOLATED FROM MEDITERRANEAN SHELLFISH
(from WHO/UNEP, 1995)

Virus	Type	Location	Matrix
Enteroviruses			
Poliovirus	3	Italy	mussels
Echovirus	3,5,6,8,9,12,13	Italy, France	mussels
Coxsackie virus A	18	Italy	mussels
	18	France	mussels
Hepatitis A virus		Greece	mussels
		France	mussels

3-38. It should be noted that clinical diagnosis of viral diseases depends on isolation of the virus and/or a specific seroconversion. The relative unavailability of the necessary specialized diagnostic facilities on a routine basis in many Mediterranean countries indicates that the extent of viral disease, particularly on an individual case basis, is still largely unknown (WHO/UNEP, 1995).

3.4 OTHER MICROPARASITES

3-39. Relatively little information is available on risks to human health arising from the presence of animal parasites in the marine environment. The eggs of *Ascaris*, *Toxoplasma*, *Oxyuris* and *Trichurus* are able to survive for months in the marine environment, and ingestion of a single egg is sufficient to cause infection (UNEP/WHO/IAEA, 1988). All the four nematode species mentioned above are prevalent in the Mediterranean region. The eggs are discharged in faeces by affected individuals, and transmission by swimming in polluted water is a possibility (WHO, 1982).

3-40. Protozoan parasites of either worldwide distribution or present in the Mediterranean region include *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli* and *Naegleria* species among those present in sewage and constituting a potential health hazard. It has been recommended that particular attention should be devoted to these and to nematode eggs when monitoring shellfish harvested in the vicinity of sewage outfalls (WHO/UNEP, 1995).

3-41. Interest in *Giardia lamblia* has increased considerably in recent years, partly with recognition of its enteropathogenicity, and partly with the publication of many reports of

episodes of giardiasis both among indigenous populations and travellers to many localities. It is now recognised as the most common intestinal parasite in developed countries which affects all ages and all socio-economic classes (Barua, 1990). Transmission of *Giardia lamblia* is mainly by contaminated water, as the cysts survive in cold waters for about two months. In the case of drinking water, the usual chlorine concentration used for treatment cannot kill the protozoal cyst.

3.5 FUNGI

3-42. A number of fungal species are pathogenic to man, causing superficial, sub-cutaneous or deep mycoses according to the eventual location of the pathogen within the host after infection. The most common one associated with infection through contact with beach sand and, to a lesser extent, seawater is *Candida albicans*, a yeast considered responsible for a number of superficial and deep mycoses. A number of other genera are also considered important, again mainly from the point of view of infection via beach sand.

3-43. *Candida albicans*, together with other *Candida* species, has been isolated from a number of sandy beaches in the Mediterranean, including the south of France (Bernard, 1985), Greece (Papadakis, 1987, Papapetropoulou and Sotiracopoulou, 1995) and Israel (Ghinsberg *et al*, 1990, 1994). In Greece, its presence in seawater has been associated with sewage pollution, as evidenced by bacterial indicator counts (Papadakis, 1987). Low counts of *Candida albicans* in seawater have been recorded on some beaches in Israel (Ghinsberg *et al*, 1994).

3-44. Work performed in the region on the identification of other fungi has included two comprehensive studies. The first, carried out along the Northern Mediterranean coast of Spain between 1983 and 1985, resulted in over 16 species of fungi isolated in both beach sand and seawater (Izquierdo *et al*, 1986). 80% of the total isolations consisted of *Penicillium*, *Aspergillus* and *Cladosporium*, the latter two of which genera contain pathogenic species. The second study, carried out on beach sand along the French Mediterranean coast between 1986 and 1987, did not result in the isolation of any pathogenic species. Eight keratinophilic and eleven non-keratinophilic species, all of which exhibit only weak pathogenic activity, were isolated (Bernard *et al*, 1988). Another study on the microbiological content of beach sand and seawater in Israel has so far revealed a considerable fungal flora, which still awaits identification (Ghinsberg *et al*, 1994). Records from Greece (Papadakis *et al*, 1990) include isolations of *Aspergillus niger*, other *Aspergillus* species, and *Mucor*, *Fusarium* and *Rhizopus* species in seawater. All of these are opportunistic pathogens, but attention is drawn to *Fusarium*, which is toxinogenic and one of the major causes of eye infections.

3.6 TOXIC ALGAE

3-45. Blooms of toxic algal species are common occurrences in shellfish-growing areas worldwide, the algal species involved, which produce potent toxins, mainly belonging to the dinoflagellate group. The shellfish accumulate the toxic cells during filter feeding, becoming vectors in various forms of shellfish poisoning (Shumway and Hurst, 1991). Of all shellfish consumed, mussels probably pose the greatest threat with regard to shellfish poisoning. Diseases (WHO, 1984; UNEP/FAO/WHO, 1995) include:

Paralytic shellfish poisoning (PSP), which is caused by a number of toxic components falling into three into three chemical groups, of which the carbamate toxins - Saxitoxin, neosaxitoxin and gonyautoxin - are the dominant component in shellfish, saxitoxin being the first component identified. They are produced by a well-defined dinoflagellate group, mainly *Gonyaulax* (also known as *Alexandrium*) and *Gymnodinium* species, occurring in both tropical and temperate sea. The toxins usually have little effect on shellfish, but are potent neurotoxins to vertebrates, including man, causing respiratory paralysis and death by asphyxia .

Neurotoxic shellfish poisoning (NSP), which is caused by *Gymnodinium breve*, with symptoms similar to, but milder than, PSP. The motile form of the dinoflagellate produces several neurotoxins, collectively called brevetoxins.

Diarrhetic shellfish poisoning (DSP), which is caused by a number of toxic components isolated from shellfish associated with human symptoms characterized by diarrhoea, nausea, vomiting and abdominal pain. The algae responsible are considered to be *Dinophysis*, *Prorocentrum* and related species.

Venerupin poisoning, which is a non-paralytic human intoxication different from DSP, is caused by consumption of oysters and clams which feed on toxic dinoflagellates of the genus *Prorocentrum*, mainly *Prorocentrum minimum*. The heat-stable toxin induces the rapid onset of nausea, vomiting, diarrhoea, headache and nervousness. In fatal poisoning, acute yellow atrophy of the liver, extreme excitation, delirium and coma occur.

Amnesic shellfish poisoning (ASP), which is caused by a toxin (Domoic acid) produced by the diatom *Nitzschia pungens*. This toxin is a mild neurological poison compared to PSP, causing gastrointestinal distress with abdominal cramps, nausea, vomiting and diarrhoea, as well as neurological symptoms involving memory loss and disorientation which can persist in severely-affected cases.

3-46. In addition to dinoflagellates, a number of chlorophytes (green algae) and rhodophytes (red algae) can also be responsible for human intoxications, pathological phenomena being present in the respiratory tract in association with neurotoxic shellfish poisoning. Other biotoxins produced by cyanophytes (blue-green algae), also called cyanobacteria, cause contact dermatitis and respiratory irritation has also been described. Comprehensive reviews of aquatic biotoxins have been compiled (WHO, 1984; UNEP/FAO/WHO, 1995). A summary of toxic and noxious algal blooms and their effects on shellfish has also been compiled (Shumway, 1990).

Algae causing PSP

3-47. Blooms of dinoflagellates producing PSP group toxins have been reported from various parts of the Mediterranean. 26 cases of algal blooms along the French Mediterranean coast in which *Gonyaulax* and *Gymnodinium* species were present have been described (Belin *et al*, 1989). *Gymnodinium catenatum* was present along the Andalusian coast of Spain in concentrations in excess of 3×10^3 cells per litre in early 1989 (Bravo *et al*, 1990), resulting in the presence of toxins in the marine bivalves *Venus verrucosa* and *Cytherea*. This was the first record in Spain's Mediterranean coast. The same species was recorded at concentrations of up to 11×10^6 cells per litre in the Bay of Volos, Greece, in July 1987 (Gotsis-Skretas, 1988) and up to 6×10^6 cells per litre in the lagoon of Fusaro, near

Naples in 1988 (Carrada *et al*, 1988). *Gymnodinium* species have also been recorded from the Northwestern Adriatic practically every year between 1976 and 1985 at maximum concentrations of 230×10^6 cells per litre (Mancini *et al*, 1986), in the Bay of Pula (Degobbi, 1990), and from Lake El-Mellah, in Eastern Algeria (Samson Kachacha and Touahria, 1992),

3-48. *Gonyaulax polyhedra* blooms have been recorded several times between 1977 and 1985 in the Northwestern Adriatic (Mancini *et al*, 1986) and in the Gulf of Trieste since 1977 (Fonda Umani, 1985). Algae responsible for red tides in the Emilia-Romagna region of the Adriatic in 1984 were identified as *Gonyaulax polyhedra*, but analysis of both algae and shellfish failed to reveal measurable quantities of saxitoxin, as compared to those contained in laboratory stock cultures of *Gonyaulax tamarensis* from Canada (Fortuna *et al*, 1985). Records of blooms of the same species in the Eastern Adriatic include Pula Bay (Degobbi, 1990), Sibenik Bay (Legovic *et al*, 1991a, 1991b) and Kastela Bay, near Split, in which last-named locality, blooms are stated to have occurred for the last 20 years (Marasovic, 1990).

Records of *Gonyaulax polyhedra* blooms from other Mediterranean areas include the Gulf of Kavala, Greece in August 1986 at concentrations of up to 10×10^6 cells per litre, and regularly between April and June along the Western coast of Turkey at up to 50×10^3 cells per litre, during May and June the blooms also containing *Gonyaulax spinifera* at up to 20×10^3 cells per litre (Koray, 1990; Koray *et al*, 1992), *Gonyaulax polyhedra* has also been recorded along the coast of Lebanon where, however, concentrations are low and no health problems are involved (Lakkis, 1991). Blooms containing *Gonyaulax* species have been recorded from Lake El-Mellah, in Eastern Algeria Samson Kachacha and Touahria, 1992). PSP toxins caused by *Gonyaulax tamarensis* in mussels have also been reported from Spain (Shumway, 1990). From a total of 128 samples of seawater in shellfish culture areas in Greece, *Gonyaulax* and *Gymnodinium* species were only found in 12 and 18 samples respectively, the former in low and the latter in relatively high numbers. Concentrations of Saxitoxin both in these samples and in shellfish collected from the market were below detection limits (Papadakis, 1991).

3-49. *Alexandrium minutum* in mussels is reported as causing the first recorded case of PSP in France in 1989 (Shumway, 1990). The same species has been observed as a large bloom (28×10^6 cells per litre) in the harbour of San Carlos de la Rapita, south of the Ebro delta in Spain in May 1989, PSP being recorded from mussels in both this and neighbouring harbours (Delgado *et al*, 1990). *Alexandrium minutum* was present in red tides inside the Eastern harbour of Alexandria, Egypt (Zaghloud and Halim, 1992), and along the Western coast of Turkey in May 1983 Koray and Buyukisik, 1988). Recurrent blooms in the last-named area are stated to contain the species between March and June (Koray, 1990; Koray *et al*, 1992), *Alexandrium minutum* has also been recorded along the coast of Lebanon where, however, concentrations are low and no health problems are involved (Lakkis, 1991).

Algae causing NSP

3-50. Apart from *Gymnodinium* blooms in which individual species were not identified (*vide* para 3-47 above), blooms containing the species responsible for causing NSP, *Gymnodinium breve*, are also mentioned as having been recorded in the North of Spain and in the Eastern Mediterranean (Steidinger, 1983; Berland and Bellan, 1990; Pagou and Ignatiades, 1990). Records of blooms in specific Mediterranean localities containing *Gymnodinium breve* include the Gulf of Saronikos, Greece, where the species was present at a concentration of 10×10^6 cells per litre in November 1977, and in Alimos Beach, in the same locality near the sewage outfall, where its concentration was 12 to 27×10^6 cells per litre (Pagou, 1990).

Algae causing DSP and Venerupin Poisoning

3-51. *Dinophysis sacculus* toxins (responsible for DSP) in shellfish were the cause of a ban on marketing affected seafood in France between 1987 and 1989 (Shumway, 1990). The species is reported as present along the southern coast of France since 1987 (Leveau *et al*, 1989; Lassus *et al*, 1991). The same species has also been recorded at a concentration of 40×10^3 cells per litre in brine lakes near Messina in Sicily (Magazzj, 1982, Magazzj *et al*, 1991), and as widespread at above 2×10^3 cells per litre in coastal waters off Syracuse, also in Sicily (Giacobbe and Maimone, 1991). DSP is reported as widespread in the Adriatic (Shumway, 1990), and *Dinophysis* species have been recorded in both the Northern and Central Adriatic, DSP intoxication being recorded in the former sub-region (Boni *et al*, 1992). A number of *Dinophysis* species are present in the Tyrrhenian Sea, but DSP was never detected in local shellfish (Innamorati *et al*, 1989b).

3-52. *Prorocentrum lima*, the species within the genus *Prorocentrum* responsible for DSP, has been recorded from the Northwestern Adriatic (Moro and Andreoli, 1991), the Gulf of Trieste (Boni *et al*, 1992) and the Tyrrhenian Sea (Innamorati *et al*, 1989a, 1989b). *Prorocentrum minimum*, responsible for Venerupin Poisoning, is described as recently increasing in occurrence in the Eastern Adriatic (Marasovic, 1986). There are numerous records of the species from other parts of the Adriatic (UNEP/FAO/WHO, 1995). It has also been recorded from lagoons along the French Mediterranean coast (Leveau *et al*, 1989).

Algae causing ASP

3-53. *Nitzschia* species, including *Nitzschia pungens*, responsible for ASP, occurs in mucilaginous aggregates from diatoms (Viviani *et al*, 1992). This mucilage phenomenon in the Adriatic has given rise to worries over health on both sides of this Sea. The presence of domoic acid, however, was not recorded during monitoring programmes (Viviani *et al*, 1992).

Cyanophyta (Cyanobacteria)

3-54. About 25 species of cyanophyta (blue-green algae), also called cyanobacteria, have been implicated in poisoning incidents worldwide, and about 75% of blooms tested have been found to produce toxins (Philipp, 1991). Ingestion of cyanophyta or body-immersion in scum-containing water have been associated with dizziness, headaches, muscle cramps, nausea, vomiting, gastroenteritis, liver damage and pneumonia (Codd *et al*, 1989; Turner *et al*, 1990). Most species are freshwater, but a number are marine. Of these latter, a filamentous species, *Lyngbya majuscula* has been identified as the causative agent of a severe contact dermatitis affecting bathers in the Pacific and Caribbean (Grauer, 1959; Moore, 1984). Seven species of *Lyngbya* have been recorded in the Mediterranean and although these do not include *Lyngbya majuscula*, another filamentous species, *Lyngbya confervoides*, has been identified (UNEP/FAO/WHO, 1995). Cyanophyte blooms have been observed in the Lake of Tunis (Kelly and Naguib, 1984), the Nile Estuary (Halim, 1989) and in the North Adriatic (Kaltenböck and Hemdl, 1992). However, no effects on human health have been described.

PART 4

MICROBIOLOGICAL CRITERIA AND STANDARDS FOR MEDITERRANEAN COASTAL AREAS

4-1. In line with global practice, recreational and shellfish water quality standards in the Mediterranean are based on acceptable concentrations of bacterial indicator organisms (mainly faecal coliforms, supplemented to a lesser extent by faecal streptococci) and, in some instances, pathogens such as *Salmonella* and enteroviruses. The use of faecal indicators for indexing the health hazards in water and seafood exposed to sewage pollution dates back to the late 1800s and early 1990s, shortly after these microorganisms were first isolated and associated with the faecal wastes of warm-blooded animals. Taking into account certain limitations based on illness rates in the discharging populations, it was recognized (WHO/UNEP, 1977b) that:

A large number of pathogenic bacteria and viruses may be present in municipal sewage, each with its own probability of illness associated with a given dose;

Routine monitoring of each of these pathogens would be a Herculean task;

Enumeration methods are not available for some of the more important pathogens, and are difficult for others;

Pathogen density data are difficult to interpret because the methodology is generally time-consuming, expensive and not always quantitative, and because in some instances dose-response data are meagre or not available; and

On theoretical grounds, the real purpose is not to index the presence of the pathogen, but rather the likelihood that it may be present in sufficient numbers to constitute an unacceptable health risk.

4-2. Since the issue of the above conclusions by the WHO/UNEP Groups of Experts in 1977, the situation has altered to the extent that available methodology for identification and enumeration of pathogenic microorganisms, including viruses, has increased significantly. Nevertheless, in many instances, such methodology is not within the reach of the majority of Mediterranean laboratories, particularly those dealing with marine pollution monitoring on a routine basis.

4-3. In their acceptance of the terms of the 1970 Athens Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources, Mediterranean States, as Contracting Parties to the Protocol, bound themselves to progressively formulate and adopt, in cooperation with the competent international organizations, common guidelines and, as appropriate, standards or criteria dealing in particular with a number of aspects, including the quality of seawater used for specific purposes that is necessary for the protection of human health, living resources and ecosystems. This eventually led to the adoption of two common measures - the Interim environmental quality criteria for bathing waters in 1985 (UNEP, 1985a), and the Environmental quality criteria for shellfish waters in 1987 (UNEP, 1987). As a matter of fact, both include standards. In both cases, the microbiological parameter selected (faecal coliforms) for evaluating the quality of the environmental matrix in question constitutes the criterion; the maximum concentration limits stipulated for acceptance constitutes the standard. In both cases, as is usual in international agreements of this

nature, the standards adopted were recognised as minimum ones, each individual country being free to adopt stricter measures if such were considered necessary in the light of national or local circumstances. The situation is rather different in the case of the common interim criteria for bathing waters, as the relevant resolution specifically provided that countries already possessing national criteria and/or standards should continue to utilize such until an adequate comparison exercise had been carried out (UNEP, 1985).

4-4. The four Mediterranean countries (France, Greece, Italy and Spain) which are Member States of the European Community are also bound by the relevant EC Directives: Council Directive 76/160/EEC of 8 December 1975 concerning the quality of bathing water (EC, 1976), in which there are five microbiological parameters, and the standard values for faecal coliforms differ from those jointly adopted by Mediterranean states in 1985; and Council Directive 79/923/EEC of 30 October 1979 on the quality of shellfish waters (EC, 1979), in which the microbiological parameters are identical with those jointly adopted for the Mediterranean. Both Directives include a number of physico-chemical parameters, in addition to microbiological. In each case, Member States are bound not to set less stringent values than the ones stipulated as mandatory standards, but are free to set more stringent ones. In their national legislation, all four countries concerned have set microbiological standards for bathing water which are stricter than the EC mandatory values. Greece and Spain have adopted the EC values in their national microbiological standards for shellfish waters, France and Italy have done the same (EC, 1995b), but both countries also have a classification system for shellfish waters with stricter standards (WHO/UNEP, 1987).

4-5. Standards and criteria for both recreational and shellfish waters exist in practically all countries of the region but, particularly in the case of recreational waters, differ to a large extent both as to the particular microorganism(s) monitored, and the acceptable levels of each (WHO, 1989). Apart from this, even were the same standards to be prevalent throughout the Mediterranean, comparison would still be difficult, owing to differences in sampling techniques, analytical methodologies and interpretation of results (Saliba and Helmer, 1990). As far as analytical methodologies are concerned, approximately half the countries in the region utilize the Most Probable Number (MPN) method for determination of the major bacterial indicator organisms in seawater, while the rest utilize the Membrane Filtration (MF) method. The two methods are not fully comparable, and there is a degree of controversy regarding the respective advantages and disadvantages of each under different circumstances. Apart from the technique itself, culture media recommended can produce unexpected results. Methods used for seawater analysis have been developed from those used for potable water which, in the unpolluted state, is bacteriologically pure. Components of the natural marine bacterial flora often interfere with readings and as, within the Mediterranean itself, the natural marine bacterial species vary according to the particular area, it has been found difficult to develop standard methodology guaranteed to produce the same results wherever applied. Comparison of the state of microbiological pollution in coastal recreational and shellfish waters in different areas of the region can therefore be quite misleading if restricted solely to counts obtained, without taking sampling and analytical methodology, not to mention quality control, into account (Saliba and Helmer, 1990).

4.1 EXISTING INTERNATIONAL PROVISIONS FOR COASTAL RECREATIONAL AREAS

4-6. The earliest international guidelines of relevance to the Mediterranean concerning recreational water quality were the recommendations of the WHO Working Group on guides and criteria for recreational quality of beaches and coastal waters, convened in Bilthoven in the Netherlands in October/November 1974 (WHO, 1975), which are commonly considered as constituting the first pronouncement of the Organization on this issue. The guidelines and criteria recommended by this group covered microbiological, chemical and aesthetic characteristics. From the first-named viewpoint, acting on the basic assumption that the variability pattern within the marine environment called for classification of bacterial concentrations by orders of magnitude rather than by detailed numerical figures, the working group came up with specific suggested guidelines based on the presence of bacterial indicator organisms in coastal bathing waters: consistently less than 100 *Escherichia coli* per 100 ml for highly satisfactory bathing areas, and not consistently greater than 1000 *Escherichia coli* per 100 ml for bathing waters to be considered acceptable. These suggested guidelines were eventually generally followed in a number of European and Mediterranean countries, a number of which incorporated the suggested standards in their legislation, and equally generally, referred to as the "WHO guidelines" or "WHO standards even though, strictly speaking, they had no formal status (Saliba, 1993).

Common Mediterranean criteria and standards

4-7. During the course of MED POL Phase I, WHO and UNEP convened a group of experts in Athens in 1977 within the framework of the MED VII pilot project on coastal water quality control to initiate a scientific study concerning health criteria and epidemiological studies related to coastal water pollution. One of the purposes of the meeting was to review the epidemiological factors on which quality standards for recreational and other coastal waters were based. After examining the available evidence, the group concluded that there was still not enough epidemiological evidence to justify any recommendation for altering the guidelines proposed by the 1974 WHO Bilthoven working group. It was considered, however that, while there might be sufficient justification for water quality control at existing recreational facilities in the Mediterranean to continue to be based on the more lenient standard of 1000 *Escherichia coli* per 100 ml, the stricter standard of 100 *Escherichia coli* per 100 ml should be considered for new recreational facilities, as well as for long-term decisions on water quality management involving large investments. The group also considered that the above numerical limitation of 1000 *Escherichia coli* per 100 ml should be more closely defined statistically, and recommended the term "no more than 10% of at least ten consecutive samples collected during the bathing season should exceed 1000 *Escherichia coli* per 100 ml (WHO/UNEP, 1977).

4-8. Following a number of further expert meetings organized within the framework of the MED VII pilot project, it was considered that, under the climatic and other conditions relating to bathing in the Mediterranean, the stricter Bilthoven value would be a more reasonable standard. It was similarly considered that more than one bacterial indicator was necessary.

After a comprehensive review of both scientific evidence and prevailing standards in the various countries, it was concluded that the coastal bathing waters of the Mediterranean could be considered as satisfactory and safe for use by the general public if the concentrations of faecal coliforms (FC) and of faecal streptococci (FS) in at least ten representative water samples collected during the bathing season at intervals of not more than 14 days did not exceed 100 faecal coliforms or 100 faecal streptococci per 100 ml in

50% of the samples and 1000 faecal coliforms or faecal streptococci in 90% of the samples. It was also concluded that the concentrations of faecal coliforms and faecal streptococci should be determined by agreed-on reference methods, or by methods yielding comparable results, proved through intercalibration with the relevant reference methods.

4-9. In terms of the above, WHO and UNEP formally proposed interim environmental quality criteria for recreational waters to the Contracting Parties for adoption on a joint Mediterranean basis. The proposals were designed to be of an interim nature, pending the acquisition of sufficient hard epidemiological evidence on the correlation between water quality and health effects, on which "permanent" standards would be proposed. The interim proposals are given in Table 4.1.1. The Contracting Parties accepted only one bacterial indicator organism, faecal coliforms, as a criterion. The analytical method was also altered to include both the membrane filtration (MF) and the Most Probable Number (MPN) methods. The interim criteria as adopted by Contracting Parties are shown in Table 4.1.2. The relevant resolution regarding the measure adopted referred to it as a measure for a transitory period that would assure, as a minimum common requirement, that the quality of bathing waters would conform with the proposed WHO/UNEP environmental quality criteria concerning faecal coliforms. In effect, the criteria adopted could no longer be termed the WHO/UNEP criteria, as these were essentially based on two parameters, not one. The resolution also stated during the transition period, the Contracting Parties which already had standards would continue to apply them without modifying their legislation, and would perform comparative studies between their own standards and the WHO/UNEP criteria. To date, there is no information from any country to the effect that such comparative studies have been carried out.

European Community standards

4-10. The European Community's Directive on the quality of bathing water (EC, 1976), which affects four Mediterranean countries, contains both microbiological and physico-chemical parameters. The former are reproduced in Table 4.1.3. There are two sets of standards: mandatory or imperative (I) values, representing the minimum Member States can use in setting their national standards, and stricter guide (G) values, which Member States shall endeavour to observe as guidelines. There is a provision in Article 7 of the Directive to prevent the downgrading of already-existing standards to the mandatory values. Paragraph 1 of the Article states that implementation of the measures taken pursuant to the Directive may under no circumstances lead either directly or indirectly to deterioration of the current quality of bathing water. In the case of the mandatory parameters, bathing waters shall be deemed to conform to each relevant parameter if 95% of the samples taken comply with the standard value. In the case of the guide parameters, the degree of compliance with these higher standards required is 80% for total coliforms and faecal coliforms in addition to 95% compliance with the mandatory values, and 90% for faecal streptococci.

TABLE 4.1.1

INTERIM ENVIRONMENTAL QUALITY CRITERIA FOR RECREATIONAL WATERS
PROPOSED BY WHO AND UNEP IN 1985

Parameter	Concentrations per 100 ml not to be exceeded in		Minimum number of samples	Analytical method	Interpretation method
	50% of the samples	90% of the samples			
Faecal coliforms	100	1000	10	Membrane filtration, m-FC broth or agar incubated at 44.5±0.2°C for 24h	Graphical or Analytical adjustment to a log-normal probability distribution
Faecal streptococci	100	1000	10	Membrane filtration, M-enterococcus agar incubated at 35±0.5°C for 48h	

TABLE 4.1.2

INTERIM ENVIRONMENTAL QUALITY CRITERIA FOR BATHING WATERS
ADOPTED BY THE CONTRACTING PARTIES IN 1985

Parameter	Concentrations per 100 ml not to be exceeded in		Minimum number of samples	Analytical method	Interpretation method
	50% of the samples	90% of the samples			
Faecal coliforms	100	1000	10	WHO/UNEP Reference Method No. 3. "Determination of Faecal Coliforms in sea water by the Membrane Filtration Culture Method" or WHO/UNEP Reference Method No. 22. "Determination of Faecal Coliforms in sea water by the Multiple Test Tube Method"	Graphical or Analytical adjustment to a log-normal probability distribution

TABLE 4.1.3

EC MICROBIOLOGICAL QUALITY REQUIREMENTS FOR BATHING WATERS
AS PER COUNCIL DIRECTIVE 76/160/EEC OF 8 DECEMBER 1975

(From EC, 1976)

Parameters	Guide value	Mandatory value	Minimum sampling frequency	Method of analysis and inspection
Total coliforms per 100 ml	500	10 000	Fortnightly (1)	Fermentation in multiple tubes. Sub-culturing of the positive tubes on a confirmation medium. Count according to MPN (most probable number) or membrane filtration and culture on an appropriate medium such as Tergitol lactose agar, endo-agar, 0.4% Teepol broth, subculturing and identification of the suspect colonies.
Faecal coliforms per 100 ml	100	2 000	Fortnightly (1)	In the case of coliforms, the incubation temperature is variable, according to whether total or faecal coliforms are being investigated.
Faecal streptococci per 100 ml	100	--	(2)	Litsky method. Count according to MPN (most probable number) or filtration on membrane. Culture on an appropriate medium.
<i>Salmonella</i> per 1 litre	--	0	(2)	Concentration by membrane filtration. Inoculation on a standard medium. Enrichment -- subculturing or isolating agar -- identification.
Enteroviruses PFU per 10 litres	--	0	(2)	Concentration by filtration, flocculation or centrifuging and confirmation.

- (1) When a sampling taken in previous years produced results which are appreciably better than those in the annex and when no new factor likely to lower the quality of the water has appeared, the competent authorities may reduce the sampling frequency by a factor of 2.
- (2) Concentrations to be checked by the competent authorities when there is a tendency towards the eutrophication of the water.

4-11. A proposal for a Council Directive concerning the quality of bathing water, with the aim of replacing the 1975 Directive, has recently been published (EC, 1994b). The new proposed microbiological parameters are given in Table 4.1.4. This constitutes part of Table 1 of the Annex to the proposed Directive. The main points to note are (a) total coliforms have been removed as a parameter, which is understandable, considering their doubtful value in this regard, and (b) Faecal coliforms have been replaced by *Escherichia coli*, with the guide and mandatory standards remaining at 100 and 2000 respectively per 100 ml, as they were for faecal coliforms. In effect, however, as *Escherichia coli* falls within the faecal coliform group, retention of the same figures represents a degree of relaxation, as the upper limits now apply to one species, as opposed to the whole group. (c) The guide value for faecal streptococci remains the same, but a mandatory standard of 400 per 100 ml has been introduced, (d) *Salmonella* has also been removed as a parameter from the technical annex, and (e) bacteriophages are being introduced as a new parameter with values, however, still to be decided. Such standards (for bacteriophages) are expected to be introduced as soon as the necessary scientific information becomes available.

4-12. Tables 2 and 3 in the annex to the proposed new Directive give the number of samples which need not comply with the mandatory and guide standards respectively, *i.e.* the degree of compliance. In essence, compliance requirements for mandatory values has remained at 95% only where more than 59 samples are taken. Otherwise the tolerance is zero for 1 to 19 samples, 1 for 20 to 39 samples, and 2 for 40 to 50 samples. In practice, therefore, as the bathing stations are hardly ever sampled more than 19 times in one year, 100% compliance will be required for the mandatory standards. In the case of the guide standards, the number of samples allowed to exceed the standard rises by one for every additional five samples taken. There is a zero tolerance for up to 4 samples taken from a station in one year, 1 for 5 to 9 samples, 2 for 10 to 14 samples, and so on up to 11 for 50 to 59 samples. Beyond this figure, it is 20% (*i.e.* 80% compliance). In practice compliance remains at 80% for stations where samplings are in multiples of five, otherwise, it is stricter. In the case of faecal streptococci, which also fall into this category, compliance requirements with the guide value have gone down from the 90% stipulated in the 1976 Directive, although this is offset by the requirement of 95% compliance with the new mandatory value. The two tables showing compliance requirements are reproduced in Table 4.1.5.

4-13. There are therefore two different international standards for recreational waters in the Mediterranean: the 1985 common adopted measure which applies to the whole region, and the 1976 EC Directive, which applies to four countries, which between them make up a considerable part of the Northern seaboard of the Mediterranean. The provision in the former that countries already having standards of their own need not alter their legislation results in a trichotomy with different states (a) observing the EC standards, while at the same time enforcing variably stricter measures through their national legislation, (b) observing the 1985 Mediterranean standards, and (c) with independent legislation based on or other of the former two, or in at least one case, on the 1975 WHO Bilthoven recommendations.

TABLE 4.1.4

EC MICROBIOLOGICAL QUALITY REQUIREMENTS FOR BATHING WATERS
AS PER PROPOSED COUNCIL DIRECTIVE, 1994

(From EC, 1994b)

Parameters	Guide value	Mandatory value	Minimum sampling frequency	Method of analysis and inspection
<i>Escherichia coli</i> per 100 ml	100	2 000	Fortnightly	Incubation at 44°C. Fermentation in multiple tubes. Subculturing of the positive tubes on a confirmation medium. Count according to MPN (most probable number) or membrane filtration and culture on an appropriate medium such as Tergitol lactose agar, endo-agar, 0.4% Teepol broth, subculturing and identification of the suspect colonies.
Faecal streptococci per 100 ml	100	400 (1)	Fortnightly	Litsky method with incubation at 37°C. Count according to MPN (most probable number) or filtration on membrane. Culture on an appropriate medium.
Enteroviruses PFU per 10 litres (2)	--	0	Monthly	Concentration by filtration, flocculation or centrifuging and confirmation.
Bacteriophages No. per 100 ml				

- (1) In case of abnormal peak value, Member States can within 2 working days re-test this parameter. If following re-testing a normal value is recorded, the peak value can be disregarded. The Commission shall be informed of the number of peak values disregarded for each bathing zone.
- (2) This parameter must be checked once in the fortnight before the start of the bathing season. If during the two preceding bathing seasons the bathing water complied with the G value for *Escherichia coli* and the I value for faecal streptococci, on the basis of Tables 3 and 2 respectively, and the bathing water does not receive discharges of chemically treated sewage, then the parameter needs to be measured only once more. This measurement should be made in the middle of the bathing season.

TABLE 4.1.5

EC MICROBIOLOGICAL QUALITY COMPLIANCE REQUIREMENTS FOR BATHING WATERS AS PER PROPOSED COUNCIL DIRECTIVE, 1994

(From EC, 1994b)

Number of samples taken and analyzed	Maximum number which need not conform to the I value
up to 19 inclusive	0
20 to 39 inclusive	1
40 to 59 inclusive	2
Greater than 59	5% of number of samples

Number of samples taken and analyzed	Maximum number which need not conform to the G value
up to 4 inclusive	0
5 to 9 inclusive	1
10 to 14 inclusive	2
15 to 19 inclusive	3
20 to 24 inclusive	4
25 to 29 inclusive	5
30 to 34 inclusive	6
35 to 39 inclusive	7
40 to 44 inclusive	8
45 to 49 inclusive	9
50 to 54 inclusive	10
55 to 59 inclusive	11
Greater than 59	20% of number of samples

4.2 EXISTING NATIONAL PROVISIONS FOR COASTAL RECREATIONAL AREAS

4-14. Although all Mediterranean countries have long-standing comprehensive regulatory mechanisms for ensuring strict quality control for drinking water, the situation has still not reached the same level, at least in the matter of enforcement, in the case of recreational waters. In addition, apart from the diversity of quality standards adopted, there is a similar degree of variation in the particular position such standards occupy within national legislative frameworks: *i.e.* whether they are incorporated in principal or in subsidiary legislation or, alternatively, are fixed and enforced through administrative procedures stipulated in umbrella-type covering legislation. The last practice, which has now practically disappeared as a result of adherence to international legal instruments which normally demand that standards be fixed by law, obviously provided a greater degree of flexibility in the response to change. Apart from this, in what is fast becoming the norm in national laws, not only the standards themselves, but also all related aspects, including frequency of sampling, methods of analysis and interpretation of results, are prescribed by the law itself. While this is desirable from a number of aspects, it raises problems when legislation requires updating or amendment, particularly when the national mechanism through which such alterations have to be made happens to be (as is not unusual) variously cumbersome (Saliba, 1993).

4-15. The salient features of national legislation and related measures concerning the quality of recreational waters in the various Mediterranean countries are summarized in the following paragraphs. The picture is not quite complete, as information from some countries was not made available by the time of finalisation of this document.

Albania

4-16. Up to 1988, Albania used the 1974 WHO Bilthoven guidelines as a basis for evaluating recreational water quality as part of its Public Health legislation. Specific standards regarding the quality of bathing water were established by Ministerial Regulation in 1989. Under these regulations:

Pathogenic microorganisms should be absent

Escherichia coli should not generally be present in concentrations above 200-400 per 100 ml water.

Croatia

4-17. Coastal seawater quality in Croatia is performed under the terms of the Bathing and Recreational Sea Waters Ordinance (Croatian Official Gazette 48/86), which regulates the manner of controlling seawater for bathing and recreation, whereas local authorities have to specify the areas of the sea intended for such use, as well as provide all site details and sampling points. The Ordinance prescribes sampling throughout the bathing season (May through October) twice a month.

4-18. The standards, which are identical with the guide values in the EC 1975 bathing water Directive, except for the zero tolerance for total coliforms, are as follows:

100% of samples tested must not exceed 500 total coliforms per 100 ml;
80% of samples tested must not exceed 100 faecal coliforms per 100 ml;
80% of samples tested must not exceed 100 faecal streptococci per 100 ml.

Cyprus

4-19. There are no standards prescribed by law for bathing water quality. The standards adopted by Contracting Parties in 1985 are applied. Since January 1995, Cyprus commenced participation in a pilot project of the European Blue Flag programme. The standards in the EC 1975 Directive are used for evaluation of bathing waters in the case of those bathing beaches applying for the Flag.

France

4-20. French Law is based on the 1975 EC bathing water Directive. On the basis of the limit values therein, the French authorities assign four categories to bathing water:

- A. High quality
- B. Moderate quality
- C. Temporary pollution possible
- D. Poor quality.

4-21. Category A is assigned to stations monitored at the required frequency, and complying with the EC guide values for all three indicator parameters (total coliforms, faecal coliforms and streptococci). Category B is assigned to stations complying with frequency requirements and with the EC mandatory values for total coliforms and faecal coliforms. Category C is assigned to those stations which exceed the EC mandatory values 5% to 33% of the time. Category D is assigned to stations in which at least one of the mandatory parameter values is exceeded over 33% of the time. Any area classified under Category D for two consecutive years must be closed to bathers.

Greece

4-22. Greek legislation has standards based on the 1975 EC Directive, but samples have to conform with both mandatory and guide standards, which are identical with those in the Directive, except for faecal coliforms, where the mandatory value in Greece is set at 500 per 100 ml. as against 2000 per 100 ml in the Directive. In the case of faecal streptococci, the EC guide value of 100 CFU per 100 ml is the mandatory value under Greek legislation.

Israel

4-23. According to Israeli law, the quality of sea water at bathing beaches shall be in accordance with, inter alia, the following provisions:

The geometric mean of seawater tests taken during the season at the bathing beach shall not exceed 200 faecal coliforms per 100 ml of seawater, and individual samples shall not exceed 400 faecal coliforms in more than 20% of all the samples;

If more than 400 faecal coliforms are discovered in a single sample, a follow-up test shall be performed 24 to 48 hours after results of the sample exceeding 400 faecal coliforms are known. The follow-up test shall include three seawater samples taken from three different locations along the bathing beach in question.

If one or more of the follow-up samples also shows a result on excess of 400 faecal coliform bacteria, a sanitation inspection shall be performed to locate the source of contamination.

If the results of the follow-up test indicate a danger to public health, the Ministry of Health shall prohibit bathing at that beach until completion of the sanitary inspection and cessation of contamination. The sanitation inspection may include tests as directed by the Ministry of Health.

4-24. The law also defines the circumstances under which public bathing shall be prohibited. these include failure of the seawater to meet stipulated quality standards.

Italy

4-25. Italian legislation is based on the 1975 EC bathing water directive, but is stricter in the sense that national standards are set at 2,000 total coliforms (as compared to the mandatory limit of 10,000) per ml, and are equivalent to the EC guide values for faecal coliforms and faecal streptococci (100 per 100 ml in each case).

Libya

4-26 National standards for recreational waters have been in force in Libya since 1977 and are based on upper limits of 100 total coliforms per 100 ml, and 100 faecal coliforms per 100 ml, with a 100% compliance requirement in both cases. These standards are now being revised, and in the meantime, Libya is following the standards adopted by the Contracting Parties in 1985, pending finalisation of new national standards.

Malta

4-27. There are no standards specifically established by Maltese regarding bathing water quality. Control is exercised by the Department of Health under overall Public Health legislation, under which bathing in any area can be prohibited if, in the opinion of the Superintendent of Public Health, this would constitute a health risk. In assessing bathing water quality, the standards adopted by the Contracting Parties are used as a basis. Stations are also categorized as follows:

First class: Stations with faecal coliform counts of less than 100 per 100 ml in 95% or more of the samples;

Second class: Stations complying with the 1985 standards (50% of samples less than 100 faecal coliforms per 100 ml, and 90% less than 1000 per 100 ml) throughout the whole of the bathing season;

Third class: Stations complying with the 1985 standards on a seasonal basis, but not on a monthly basis;

Fourth class: Stations failing to conform over the whole of the whole 4-month bathing season.

Bathing at any beach is temporarily prohibited when faecal coliform counts exceed 1000 faecal coliforms per ml for any period.

Morocco

4-28. National standards in force are those adopted by Mediterranean Governments in 1985 (50% of samples not to exceed 100 faecal coliforms per 100 ml, and 90% not to exceed 1000 per 100 ml). Beaches are classified into five categories based on total coliform and faecal coliform levels per 100 ml:

Class I	Good quality	< 500 TC	+	< 100 FC
Class II	Medium quality	500 < 2500 TC	+	100 < 500 FC
Class III	Poor quality	2500 < 5000 TC	+	500 < 1000 FC
Class IV	Bad quality	5000 < 10000 TC	+	1000 < 2000 FC
Class V	Unsuitable	> 10000 TC	+	> 2000 FC

Slovenia

4-29. Bathing water quality control in Slovenia is carried out under the terms of the Bathing Waters Ordinance of 1988. Standards are generally based on the 1975 EC Directive.

Spain

4-30. Spanish law is based on the 1975 EC Directive, and classifies bathing water into three quality categories:

- A. Stations complying with both the mandatory values and guide values for each parameter measured or assessed;
- B. Stations complying with the mandatory values for each parameter measured or assessed;
- C. Stations not complying with the mandatory values for one or more of the parameters measured or assessed;

Tunisia

4-31. Tunisian legislation on bathing water quality incorporates standards based on the 1975 EC guide values, and mandatory limits are 500 total coliforms per 100 ml, 100 faecal coliforms per 100 ml, and 100 faecal streptococci per 100 ml. The following classification is applied: Stations classified as of good quality are those where, on the basis of at least ten

samples per year, 80% of the samples conform with total coliform and faecal coliform limits, and 90% of the samples conform with the faecal streptococci limit.

Turkey

4-32. Under the terms of Turkish law, the standards for sea water used for recreational purposes are generally modeled on the 1975 EC Directive, and contain both microbiological and physico-chemical parameters. The former are:

Total coliforms (MPN/100 ml) 1000
Faecal coliforms (MPN/100 ml) 200

Turkey also has Blue Flag water quality criteria, the microbiological parameters of which have standards to the 1975 EC guide values (500 total coliforms per 100 ml, 100 faecal coliforms per 100 ml and 100 faecal streptococci per 100 ml).

4.3 EXISTING INTERNATIONAL PROVISIONS FOR SHELLFISH AREAS

4-33. At the beginning of the first phase of the MED POL programme, no international agreement on the quality of shellfish-growing waters covering the Mediterranean area existed (UNEP/WHO, 1985). A proposed draft code of hygiene practice for molluscan shellfish was prepared by the Codex Alimentarius Commission (1978). Appendix III to the draft code provided general recommendations on environmental sanitation, including the classification, control and re-classification of shellfish-growing areas. In the same appendix, a list of microbiological standards and methods currently employed in several developed countries included only two Mediterranean countries; France and Italy. The situation prevailing at the time was that successful shellfish control programmes had been in operation in a number of countries for many years, using a wide range of bacteriological standards and methods, but at the same time, it was virtually impossible to reach agreement on any specific set of standards and methods (UNEP/WHO, 1985).

Common Mediterranean standards

4-34. During the course of the pilot project on Coastal water quality control (MED VII), attempts were made to harmonize methodology through the development of microbiological methods suitable for use in the Mediterranean marine environment. A number of these methods had been completed by the end of the pilot project, including specific ones on the determination of concentrations of the major bacterial indicator organisms in seawater and shellfish (WHO/UNEP, 1981).

4-35. Within the framework of the same pilot project, a seminar on the monitoring of recreational coastal water quality and shellfish culture areas was convened by WHO and UNEP in Rome in April 1978 (WHO/UNEP, 1978). With regard to monitoring of shellfish and shellfish-growing waters, it was considered by the seminar that the first two phases in assessing shellfish quality (the culture area and the shellfish in its natural surroundings), should comply with appropriate microbiological limits, it being understood that for a full quality assessment of shellfish as a food product, these should also be examined at subsequent phases of handling (transport, processing and marketing). The group also endorsed the recommendations made by a previous WHO/UNEP working group earlier that

year (WHO/UNEP, 1977a) that in shellfish, the flesh alone, as opposed to the flesh and intervalvular fluid, should be utilized for microbiological analysis. The seminar recommended interim standards for shellfish waters, based on concentrations of faecal coliforms in the water itself, and for shellfish, based on concentrations of faecal coliforms in the flesh. These recommendations were renewed by a meeting of principal investigators of the MED VII pilot project convened by WHO and UNEP in Rome in November 1979, in which one of the subjects for discussion was the development of interim microbiological criteria (WHO/UNEP, 1980).

4-36. On the basis of the above recommendations, and taking into account the current situation regarding national criteria for shellfish and shellfish-growing waters in Mediterranean countries, WHO and UNEP made formal proposals to the Contracting Parties on interim microbiological criteria for (a) shellfish-growing waters and (b) shellfish to be adopted on a common basis. As in the case of recreational waters, since they contained numerical limits, these were actually proposed standards. The proposals are reproduced in Table 4.3.1. As already stated earlier in this document, these proposals were not endorsed by Contracting Parties at their 1985 ordinary meeting. The whole matter was comprehensively reviewed by a consultation on environmental quality criteria for shellfish-growing waters and shellfish in the Mediterranean, convened by WHO and UNEP in Athens in March 1987 (WHO/UNEP, 1987). On taking every aspect of the situation into consideration, the meeting recommended that for the assessment of the microbiological quality of the waters, the shellfish themselves should be taken into account, and that for the determination of microbiological parameters, preference should be given to analysis of shellfish flesh and intervalvular fluid, rather than flesh alone. The faecal coliform criterion was retained, and the standard proposed was 300 per 100 ml in at least 75% of the samples. The question of shellfish standards for consumption was dropped. This proposal was adopted by the Contracting Parties in September 1987 (UNEP, 1987), the operative parts of the relevant resolution being reproduced in Table 4.3.2. In effect, the criteria and standards adopted are practically identical to the microbiological component of the 1979 EC Directive, the only difference being that the EC Directive specified analysis of shellfish flesh plus intervalvular fluid. The approved Mediterranean measure, while expressing a preference for this, also allows for analysis of the flesh alone.

European Community standards

4-37. Council Directive 79/923/EEC of 30 October 1979 (EC, 1979) was introduced to protect and improve the quality of shellfish waters within the Community, and is applicable to the four Mediterranean Member States (France, Greece, Italy and Spain). The aim of defining quality objectives for shellfish waters is to protect the development of shellfish populations from the principal sources of pollution. The Directive stresses that it cannot, by itself, ensure protection of consumers of shellfish products, and that it is therefore necessary to take other measures to this effect. With this in mind, the Council adopted Directive 91/492/EEC of 15 July 1991, which lays down the health conditions for the production and the placing on the market of live bivalve molluscs. The microbiological and related components of the 1979 Directive are reproduced in Table 4.3.3.

4-38. Unlike the case with bathing waters, the two international agreements on shellfish waters covering the Mediterranean (the 1979 EC Directive, which applies directly to four countries and serves as a model for others, and the 1987 common Mediterranean measure, which applies to all) are practically identical insofar as the microbiological components are concerned.

Algal biotoxins in shellfish

4-39. To date, there appears to be no specific legislation in the Mediterranean relating to algal biotoxins in shellfish. The 1979 EC Directive contains saxitoxin as a parameter, but no values have been set. Control is exercised in a number of countries through general public health legislation, whereby contaminated shellfish are declared unfit for consumption. Both international and national legislation covering various aspects of marine pollution prevention and control contain the means to restrict discharges which actually or potentially contribute to the development of algal blooms.

TABLE 4.3.1

INTERIM QUALITY CRITERIA FOR SHELLFISH AND SHELLFISH-GROWING WATERS IN THE MEDITERRANEAN SEA PROPOSED BY WHO AND UNEP IN 1985

SHELLFISH-GROWING WATERS

Parameter	Concentrations per 100 ml not to be exceeded in 50% 100% of the time	Minimum sampling frequency	Analytical method	Interpretation method
Faecal coliforms	10 100	in winter: monthly in summer: fortnightly	Membrane filtration, m-FC broth or agar incubated at $44.5 \pm 0.2^\circ\text{C}$ for 24h	Graphical or Analytical adjustment to a log-normal probability distribution

SHELLFISH FLESH

Parameter	Concentration per gram of flesh	Minimum sampling frequency	Analytical method	Interpretation method
Faecal coliforms	2 Sale permitted Between 3 and 10 temporary prohibition of sale Above 10 Sale prohibited	in winter: monthly in summer: fortnightly	Multiple tube fermentation and counting according to MPN (Most Probable Number) McConkey broth incubated at $35 \pm 0.5^\circ\text{C}$ for 24h and then at $44.5 \pm 0.2^\circ\text{C}$ for 24h	By individual results, histograms or graphical; adjustment of a log-normal probability distribution

TABLE 4.3.2

CRITERIA FOR SHELLFISH WATERS PROPOSED BY WHO AND UNEP AND
ADOPTED BY THE CONTRACTING PARTIES IN 1987

OPERATIVE SECTIONS

1. Adoption, as a minimum common requirement for the quality of shellfish waters, the proposed WHO/UNEP interim environmental quality criteria, as detailed in 2 and 3 below and in the accompanying table.
2. For the purpose of such criteria, consideration of the term "shellfish waters" to mean those coastal and brackish waters in which shellfish (bivalve and gastropod molluscs) live.
3. Utilization of the following in the application of such criteria:
 - for the assessment of the microbiological quality of shellfish waters, the shellfish themselves shall be taken into account;
 - for the determination of microbiological parameters, preference shall be given to analysis of shellfish flesh and intervalvular fluid, rather than flesh alone;
 - the results of analysis of microbiological quality shall be expressed by the number of faecal coliforms recorded in 100 ml (FC/100 ml);
 - the method of analysis utilized shall be incubation at $37 \pm 0.5^\circ\text{C}$ with fermentation on a liquid substrate for a period of 24 to 48 hours, followed by a confirmation test at $44 \pm 0.2^\circ\text{C}$ for 24 hours. Enumeration shall be effected according to the Most Probable Number (MPN) method;
 - the concentration of faecal coliforms should be less than 300 per 100 ml of shellfish flesh and intervalvular fluid, or of flesh alone, in at least 75% of the samples, based on a minimum sampling frequency of once every three months.

SUMMARY TABLE

Matrix	Shellfish
Parameter	Faecal coliforms
Concentration	Less than 300 per 100 ml flesh + inter-valvular fluid or flesh, in at least 75% of the samples
Minimum sampling frequency	Every 3 months (more frequently whenever local circumstances so demand)
Analytical method	Multiple tube fermentation and counting according to MPN (most probable number) method. Incubation period: $37 \pm 0.5^\circ\text{C}$ for 24 h or 48 h, followed by $44 \pm 0.2^\circ\text{C}$ for 24 h.
Interpretation method	By individual results, histograms, or graphical adjustment of a lognormal -probability distribution.

TABLE 4.3.3
EUROPEAN COMMUNITY MICROBIOLOGICAL AND RELATED QUALITY REQUIREMENTS FOR SHELLFISH WATERS
 (From EC, 1979)

Parameter	Guide value (G)	Imperative value (I) (mandatory)	Reference methods of analysis	Minimum sampling and measuring frequency
Water 100 ml	< 300 in shellfish flesh and intervalvular fluid		Method of dilution with fermentation in liquid substrates in at least 3 tubes in 3 dilutions. Subculturing of the positive tubes on a confirmation medium. Count according to MPN (Most Probable Number). Incubation temperature 44 ± 0.5°C	Quarterly
Total suspended matter		% discharge affecting shellfish waters must not cause the suspended solid content of the waters to exceed by more than 30% the content of waters not so affected	<ul style="list-style-type: none"> - filtration through a 0.45µm membrane, drying at 105°C and weighing. - centrifuging (for at least 5 minutes with mean acceleration 2800 to 3200 g), drying at 105°C and weighing. 	Quarterly
Bacterial contamination %	> 80%	<ul style="list-style-type: none"> -> 70% longer (average value) -should an individual measurement indicate a value lower than 70%, measurements shall be repeated. -an individual measurement may not indicate a value of less than 60% unless there are no harmful consequences for the development of shellfish colonies 	<ul style="list-style-type: none"> - Winkler's method - Electrochemical method 	Monthly, with a minimum of 1 sample representative of low oxygen conditions on the day of sampling. However, when major daily variations are suspected, a minimum of 2 samples in one day shall be taken

Israel

4-52. There are no standards for shellfish growing, as shellfish are not grown or harvested in Israel.

Italy

4-53. Although Italy has not yet set values for the parameters listed in the 1979 EC Directive, the microbiological standards prescribed by Italian law for shellfish waters and shellfish are probably the most comprehensive. Shellfish waters are classified into approved zones and conditioned zones, with the following standards:

Approved zones: Sea water should not contain more than 2 *Escherichia coli* per 100 ml. Up to 7 per 100 ml seawater is tolerated in not more than 10% of the samples, provided that the shellfish themselves come up to the required standards.

Shellfish should not contain more than 4 *Escherichia coli* per ml of flesh plus intervalvular fluid, and *Salmonella* must be absent in 25 ml flesh plus intervalvular fluid.

Conditioned zones: Seawater should not contain more than 34 *Escherichia coli* per 100 ml. Up to 49 per 100 ml are tolerated in not more than 10% of the samples.

Shellfish should not contain more than 39 *Escherichia coli* per ml of flesh plus intervalvular fluid.

4-54. Depurable species are only cleared for direct consumption if they originate from culture areas in an approved zone. Depurable species originating from (a) natural breeding grounds in approved zones and (b) culture areas in conditioned zones are subject to mandatory depuration prior to consumption. Those originating from natural breeding grounds in conditioned zones must be cooked prior to consumption. Non-depurable species are cleared for direct consumption if they originate from approved zones, or from culture areas in conditioned zones, otherwise they are subject to mandatory cooking.

4-55. An expert commission convened by the Italian Ministry of Health has produced regulations aimed at harmonization of existing legislation with the provisions of EC Directive 91/492/EEC of 1991. The zones and parameters are the same as for France and Greece, but under Italian Law, Class A zones also have a requirement for *Salmonella* (0 in 25 g flesh plus intervalvular fluid). Stabilization zones are also included, with the same standards as for Class A zones.

4-56. Italy also possesses standards for algal biotoxins in shellfish. For DSP, waters must contain less than 1000 *Dinophysis* per litre, and shellfish must conform to a Death time of more than five hours. The limit value for PSP is 40 µg per 100 g flesh.

Libya

4-57. There are no national standards for shellfish waters in force in Libya. Pending the development and adoption of new standards, which are currently being finalized, Libya is observing the standards adopted by the Contracting Parties in 1987.

Malta

4-58. There are no standards fixed by law in Malta for shellfish water quality. The quality of shellfish for consumption comes under general Public Health legislation, and the sale of shellfish consignments is prohibited unless the person concerned is in possession of a special permit from the Superintendent of Public Health. There are currently no valid permits for the sale of fresh (as distinct from imported) shellfish.

Morocco

4-59. Microbiological quality standards and criteria for shellfish waters are generally based on French legislation. For acceptability of shellfish waters, the concentration of faecal coliforms in the flesh of shellfish therein must not exceed 300 per 100 ml, in line with the 1979 EC Directive and the 1987 Mediterranean standards.

Slovenia

4-60. Water quality control instructions for shellfish breeding are contained in the 1988 Slovenian Decree of 1988 on Preventive Vaccination, Diagnostics and Research in the Relevant Field. The standard for acceptable shellfish waters is 10 faecal coliforms per 100 ml flesh, based on a fortnightly sampling frequency.

Spain

4-61. Under Spanish law, the limits regarding the acceptability of shellfish waters from the point of view of their microbiological quality were that concentrations of *Escherichia coli* should not exceed 15 per 100 ml of seawater in more than 50% of the samples and 50 per 100 ml of seawater in more than 10% of the samples. By Royal Decree 38/1989, Spain set values for the parameters listed in the annex to the 1979 EC Directive. The standards are the same as those in the Directive.

4-62. Shellfish areas are now also classified into three zones according to the terms of the 1991 EC Directive, the parameters and limit values being the same as described for France and Greece. Depurated shellfish destined for consumption must comply with the following microbiological standards:

Aerobic microorganisms:	up to 100,000 per gram
<i>Escherichia coli</i>	up to 500 per litre
<i>Salmonella</i>	absent in 25 ml
Streptococci (Group D)	up to 100 per gram
<i>Vibrio parahaemolyticus</i>	up to 100 per gram.

Tunisia

4-63. Shellfish-growing waters are classified into three categories:

Sanitary zones:	Shellfish flesh	up to 300 faecal coliforms per 100 ml Salmonella absent in 25 g
	Water	up to 2 faecal coliforms per 100 ml
Conditioned zones:	Shellfish flesh	up to 3900 faecal coliforms per 100 ml
	Water	up to 34 faecal coliforms per 100 m
Unsanitary zones:	Shellfish flesh	above 3900 faecal coliforms per 100 ml
	Water	above 34 faecal coliforms per 100 m

Turkey

4-64. The Aquatic Products Law, which came into force in 1971, contains general conditions and regulations for coastal protection and production of aquatic products. The Aquatic Products regulations entered into force in March 1995. This law regulates, *inter alia*, the discharges to fish and shellfish production areas and tolerable values in receiving waters. The Ministry of Health is responsible for the coordination of activities related to aquatic products at both national and international levels. The annex to the regulations defines limits on activities and substances. The microbiological limits for receiving waters are:

Total coliforms	not to exceed 70 per 100 ml
Faecal coliforms	not to exceed 10 per 100 ml
<i>Escherichia coli</i>	not to exceed 2 per 100 ml (extendable to 7 per 100 ml).

PART 5

THE STATE OF MICROBIOLOGICAL POLLUTION OF MEDITERRANEAN SENSITIVE COASTAL AREAS

5.1 THE STATE OF COASTAL RECREATIONAL AREAS

The MED VII Pilot Project (1976-1981)

5-1. During the first or pilot phase of the MED POL Programme, the recreational water component of the pilot project on Coastal Water Quality Control was executed by 30 collaborating national laboratories from 14 Mediterranean countries under the overall technical coordination of WHO. This was the first time that the microbiological quality of Mediterranean recreational waters was studied on such a large scale. Monitoring was performed between 1976 and 1981. During this period, a total of 12,500 water samples from a number of sampling stations varying in number from 25 (1981) to 288 (1979) were analyzed. The sampling areas were selected primarily by taking into account their importance as public recreational beaches. However, road access to the area, distance to the analytical laboratory, and local administrative requirements resulted in a considerable diversity of sampling locations and sampling strategies between the different laboratories and, occasionally, between different annual periods for the same laboratory (WHO/UNEP, 1981).

5-2. An analysis of MED VII data was carried out as the basis for the first assessment of microbial pollution of the Mediterranean Sea, carried out in 1983 (UNEP/WHO, 1985). Summary results for those stations which satisfied the frequency requirement of at least 10 samples per year in terms of the interim criteria for bathing waters adopted by the Contracting Parties to the Barcelona Convention and Protocols in 1985 (50% of the samples not exceeding 100 faecal coliforms per 100 ml, and 90% not exceeding 1000 faecal coliforms per 100 ml), ranging in number from 21 (1976) to 133 (1979), are given in Table 5.1.1 (A). In an effort to broaden the basis for evaluation of the microbiological quality of Mediterranean recreational waters, an additional analysis was carried out as part of the 1985 assessment, also taking into account the results from all the other stations in which sampling was undertaken at least six times per year (the average sampling frequency in MED VII). The results are summarized in Table 5.1.1 (B). (UNEP/WHO, 1985).

5-3. As can be seen from these results, there was no evident trend in quality change during the period in question. In analyzing the results of the MED VII pilot project, it was concluded (UNEP/WHO, 1985) that although the selection of sampling stations in recreational coastal waters was not fully random, and consequently the conclusions derived from the quality of such stations could not be considered as universally applicable, the number and spatial distribution of the sampling stations and water samples analyzed provided a reliable assessment of the microbiological quality of coastal recreational waters in the Mediterranean. It should also be considered, however, that the number of stations was small as compared to the overall number of bathing beaches in the Mediterranean, and that there was a geographical imbalance in the distribution of the stations, the eastern and southern parts of the region being rather poorly represented.

TABLE 5.1.1

FIRST SUMMARY ASSESSMENT OF THE MICROBIOLOGICAL QUALITY OF RECREATIONAL WATERS IN THE MEDITERRANEAN ACCORDING TO THE INTERIM CRITERIA ADOPTED BY THE CONTRACTING PARTIES IN 1985

(From UNEP/WHO. 1985)

A

MED POL VII SAMPLING STATIONS WITH AT LEAST 10 SAMPLES PER YEAR

Year	Number of stations			
	surveyed	in accordance with		satisfactory (both limits)
		50% limit	90% limit	
1976	21	16 (76%)	14 (67%)	14 (67%)
1977	40	38 (95%)	34 (85%)	34 (85%)
1978	33	30 (91%)	30 (91%)	28 (85%)
1979	133	124 (93%)	104 (78%)	104 (78%)
1980	86	79 (92%)	72 (84%)	69 (80%)

B

MED POL VII SAMPLING STATIONS WITH AT LEAST 6 SAMPLES PER YEAR

Year	Number of stations			
	surveyed	in accordance with		satisfactory (both limits)
		50% limit	90% limit	
1976	26	16 (62%)	15 (58%)	14 (54%)
1977	55	50 (91%)	46 (84%)	46 (84%)
1978	193	181 (94%)	164 (85%)	161 (83%)
1979	288	251 (87%)	201 (70%)	200 (69%)
1980	118	110 (93%)	100 (85%)	97 (82%)
1981	25	19 (76%)	20 (80%)	19 (76%)

Note: The stations in Table 5.1.1. (A) are also included in Table 5.1.1. (B).

National monitoring programmes - MED POL Phase II

5-4. The strategy adopted in implementation of the monitoring component of MED POL Phase II was radically different from the initial phase in that, instead of constituting an international programme in which institutions participated on an *ad hoc* basis independently of similar programmes at national level, the component now aimed at strengthening already-existing national monitoring programmes, and assisting in the establishment of such programmes where these did not yet exist. Such assistance was provided through overall agreements between UNEP as the coordinating body for MED POL on one side, and the relevant national authorities on the other, whereby the country concerned provided details of existing and planned monitoring programmes on an annual basis, and submitted the results to UNEP in the form of raw data. These agreements were not signed with the more developed countries, which were considered as not in need of such assistance, as a result of which no raw data was obtained.

5-5. This means that, in the case of Mediterranean countries submitting raw monitoring data to MED POL (Albania, Algeria, Croatia, Cyprus, Egypt, Israel, Lebanon, Malta, Morocco, Slovenia, Syria, Tunisia, Turkey and (up to 1990) Yugoslavia), assessment of the microbiological quality of coastal recreational waters is based on compliance or otherwise with the provisions of the interim criteria for bathing waters adopted by Contracting Parties to the Barcelona Convention and Protocols in 1985 (UNEP, 1095a). On the other hand, a similar assessment in the case of the four EC Mediterranean Member States (France, Greece, Italy and Spain) has to be made on the basis of reports submitted by these countries to the European Community in terms of the 1976 Directive on the quality of bathing waters (European Community, 1976), in which the standards are not the same. It is not possible, therefore, to make a direct comparison between the two sets of processed data.

Interim evaluation of MED POL data

5-6. A preliminary evaluation of the microbiological quality of recreational beaches in countries submitting data to MED POL (UNEP, 1989) was made on the basis of data obtained covering the years 1983 to 1987. These data totalled 9682 water samples obtained from 289 sampling stations in seven countries (Algeria, Cyprus, Israel, Lebanon, Malta, Morocco and Yugoslavia), the details being summarized in Table 5.1.2. It should be noted that for each country, the number of stations represent the maximum in any one year. There were annual fluctuations in most countries both in the number and location of stations, and in the number of water samples analyzed during the 5-year period under review. The results of the evaluation, again made on the basis of the 1985 interim criteria adopted by the Contracting Parties, are reproduced in Table 5.1.3. It should be noted that in this particular evaluation, all sampling stations with at least six samples per year were taken into consideration, *i.e.* a number of stations considered as satisfactory from the viewpoint of compliance with faecal coliform limits did not satisfy the minimum frequency requirement of ten samples per year.

TABLE 5.1.2

SUMMARY OF MONITORING DATA ON THE MICROBIOLOGICAL QUALITY OF COASTAL RECREATIONAL WATERS SUBMITTED BY SEVEN MEDITERRANEAN COUNTRIES WITHIN THE FRAMEWORK OF THE MONITORING COMPONENT OF MED POL PHASE ii, 1983-1987

(From UNEP, 1989)

Country	Period	Number of stations surveyed	Number of water samples analyzed
Algeria	1986 - 1987	19	60
Cyprus	1983 - 1987	125	3182
Israel	1983 - 1987	43	2667
Lebanon	1984 - 1987	8	243
Malta	1983 - 1987	11	241
Morocco	1983 - 1987	2	54
Yugoslavia	1983 - 1987	81	3235
Total		289	9682

TABLE 5.1.3

INTERIM EVALUATION OF THE MICROBIOLOGICAL QUALITY OF RECREATIONAL WATERS IN SEVEN MEDITERRANEAN COUNTRIES, 1983 - 1987, ACCORDING TO THE INTERIM CRITERIA ADOPTED BY THE CONTRACTING PARTIES IN 1985

SAMPLING STATIONS WITH AT LEAST 6 SAMPLES PER YEAR

(From UNEP, 1989)

Year	Stations monitored	Samples analyzed	Average sampling frequency	Stations complying with		Satisfactory stations FC50+FC90
				FC50	FC90	
1983	50	524	10	43 (86%)	39 (78%)	39 (78%)
1984	133	1755	13	120 (90%)	111 (83%)	108 (81%)
1985	128	2178	17	115 (90%)	104 (81%)	102 (80%)
1986	238	3048	13	216 (92%)	200 (84%)	200 (84%)
1987	150	1908	13	145 (97%)	145 (97%)	144 (96%)

5-7. In the 1989 interim evaluation of these results, it was concluded that the figures indicated that the proportion of sampling stations conforming to the interim criteria was slightly higher than the corresponding value for MED POL Phase I (Tables 5.1.1. A and B). It was also stated that, however, the uneven distribution of sampling stations considered in the interim evaluation and, particularly, the lack of information from Mediterranean states with large coastal water quality monitoring programmes, did not allow the reaching of definite conclusions on possible trends observed in coastal water quality over the two phases of MED POL.

5-8. In fact, the figure of 96% compliance recorded during 1987 should be viewed in the light of the fact that this value was based on a relatively limited number of sampling stations, most of which were located in a single Mediterranean state. If this figure is ignored, the general situation during the period 1983-1987 does not appear to differ significantly from that obtaining during 1976-1981. In any case, reliable comparisons cannot be made between the two groups of data, as both countries and station locations differed to a large extent.

Present assessment

5-9. The present assessment was made on the basis of MED POL monitoring data collected from the beginning of MED POL Phase II to date. This covers the period 1983 to 1992. Data for 1993 and 1994 were not included as, in each case, they were only available from two countries. Data were obtained from the same seven countries contributing to the 1989 interim evaluation, together with another four countries (Albania, Egypt, Syria and Tunisia) which started to send results after the interim evaluation. Monitoring results from Yugoslavia were partially replaced by corresponding ones from Croatia, specific station locations remaining practically the same. Details of country data submitted are given in (a) global and (b) country form in Table 5.1.4. The data were computerized and processed by the Coordinating Unit for the Mediterranean Action Plan in Athens. In evaluating the data, stations were classified as satisfactory (*i.e.* complying fully with the interim criteria) only if they satisfied the three requirements: frequency (at least 10 samples in one year), FC50 (50% of the samples not exceeding 100 faecal coliforms per 100 ml, and FC90 (90% of the samples not exceeding 1000 faecal coliforms per 100 ml. Data for 1993 and 1994 were not evaluated as, in each case, those available were limited to two countries.

5-10. Table 5.1.5 shows the results. The number of stations satisfying each individual parameter are shown in separate columns, followed by the number of stations satisfying the microbiological parameters only (irrespective of frequency) and, finally, the number of stations satisfying all parameters, classified as satisfactory. All percentages are in terms of all stations actually monitored. The major limiting factor in compliance is the frequency, which explains the difference between the last two columns in the table. On the basis of total compliance with all three parameters in the interim criteria, the percentage of satisfactory stations ranged between 28.8% in 1985 and 71.2% in 1991. Considering compliance with only the microbiological requirements, and omitting frequency, such compliance, as shown in the penultimate column of the table, varied between 84.4% in 1986 and 91.4% in 1983. This latter figure, however, must be viewed in the light of the fact that the bulk of data for 1983 were from two countries with relatively high-quality beaches.

TABLE 5.1.4

**SUMMARY OF MONITORING DATA ON THE MICROBIOLOGICAL QUALITY OF
COASTAL RECREATIONAL WATERS SUBMITTED BY THIRTEEN
MEDITERRANEAN COUNTRIES WITHIN THE FRAMEWORK
OF THE MONITORING COMPONENT OF
MED POL PHASE II, 1983-1994**

A. GLOBAL DATA

Year	Number of countries	Countries submitting monitoring data	Number of sampling stations	Number of samples analyzed
1983	5	ISR, MAT, MOR, TUR, YUG	139	1358
1984	6	ISR, LEB, MAT, MOR, TUR, YUG	183	2035
1985	7	CYP, ISR, LEB, MAT, MOR, TUR, YUG	344	2717
1986	7	ALG, CYP, ISR, LEB, MAT, MOR, YUG	352	3903
1987	7	ALG, CYP, ISR, LEB, MAT, MOR, YUG	353	3908
1988	7	ALG, CYP, ISR, LEB, MAT, MOR, YUG	354	4326
1989	9	ALG, CYP, EGY, ISR, LEB, MAT, MOR, TUN, YUG	414	5041
1990	7	ALG, CYP, EGY, ISR, MAT, MOR, YUG	376	4725
1991	6	CYP, EGY, ISR, MAT, SYR, YUG	389	5112
1992	5	ALB, CRO, CYP, SYR, TUN	404	5006
1993	2	ALB, CYP	195	2786
1994	2	ALB, SYR	43	149
	13			41065

B. COUNTRY DATA

Country	Period	Number of stations surveyed	Number of water samples analyzed
Albania	1992 - 1994	44	718
Algeria	1986 - 1990	19	162
Croatia	1992	59	634
Cyprus	1983 - 1992	154	17816
Egypt	1989 - 1991	14	61
Israel	1983 - 1991	70	7849
Lebanon	1984 - 1989	8	309
Malta	1983 - 1991	12	656
Morocco	1983 - 1990	24	456
Syria	1992, 1994	32	388
Tunisia	1989, 1992	111	2100
Turkey	1983 - 1985	16	201
Yugoslavia	1983 - 1991	138	9715
Total		642 *	41065

* total does not include 59 Croatian stations, as already counted under Yugoslavia

TABLE 5.1.5
COMPARATIVE EVALUATION OF THE MICROBIOLOGICAL QUALITY OF RECREATIONAL WATERS IN THIRTEEN MEDITERRANEAN COUNTRIES, 1983 - 1992, ACCORDING TO THE INTERIM CRITERIA ADOPTED BY THE CONTRACTING PARTIES IN 1985, SHOWING COMPLIANCE WITH INDIVIDUAL PARAMETERS, EXPRESSED AS PERCENTAGES OF TOTAL NUMBER OF STATIONS MONITORED

Year	Number of countries submitting data	Number of Stations monitored	Number of stations complying with			Number of stations complying with FC50 + FC90 only	Number of satisfactory stations
			Frequency	FC50	FC90		
1983	5	139	56 (40.3%)	128 (92.1%)	128 (92.1%)	127 (91.4%)	50 (36.0%)
1984	6	183	97 (53.0%)	165 (90.2%)	165 (90.2%)	159 (86.9%)	85 (46.4%)
1985	7	344	118 (34.3%)	316 (92.0%)	311 (90.4%)	307 (89.2%)	99 (28.8%)
1986	7	352	220 (62.5%)	304 (86.4%)	304 (86.4%)	297 (84.4%)	191 (54.3%)
1987	7	353	241 (68.3%)	314 (89.0%)	318 (90.1%)	300 (85.0%)	221 (62.6%)
1988	7	354	256 (72.3%)	322 (91.0%)	315 (89.0%)	308 (87.0%)	227 (64.1%)
1989	9	414	275 (66.4%)	369 (89.1%)	362 (87.4%)	353 (85.3%)	248 (59.9%)
1990	7	376	286 (76.1%)	366 (97.3%)	367 (97.6%)	354 (94.1%)	263 (69.9%)
1991	6	389	285 (73.3%)	348 (89.5%)	342 (87.9%)	331 (85.1%)	277 (71.2%)
1992	5	404	269 (66.6%)	346 (85.6%)	289 (71.5%)	279 (69.1%)	208 (51.5%)

5-11. It is not possible to arrive at any accurate interpretation of results from the 1983-1992 MED POL data for a number of reasons. One major factor is the global evaluation method which has had to be adopted to preserve individual country confidentiality, as has been the case ever since the commencement of the MED POL programme in 1975. Interpretation of these global results are affected by the following:

A number of countries submitted data covering different periods between 1983 and 1992. The pooling of data has resulted in both the number of countries, and the individual countries concerned, differing from year to year;

The amount of data submitted by individual countries showed considerable variation;

The number and locality of stations sampled by a certain proportion of countries also differed from year to year:

There was a large variation in sampling frequency between individual stations, a considerable proportion of which were not sampled at the required frequency.

5-12. The number and percentage of satisfactory stations as shown in the last column of Table 5.1.5 can be considered to reflect the situation according to the strict requirements of the interim standards adopted by the Contracting Parties in 1985. As has already been observed, the major factor limiting complete compliance was monitoring frequency. It might be considered that if this particular requirement is not taken into account, the percentages shown in the penultimate column of Table 5.1.5 might be taken as a better indication of the overall microbiological quality of the coastal areas pertaining to the group of countries in question. However, the frequency requirement is of considerable importance, as the quality of stations cannot be assessed at all reasonably unless a certain number of samples are taken throughout the year, and particularly throughout the bathing season. The actual situation probably lies somewhere between the two sets of percentages. One other factor to be considered is that all percentages refer to officially-designated monitoring stations. The difference between the number of these and the total number of bathing areas, including those which are not monitored, is not known.

5-13. The percentages of bathing beaches complying fully with the terms of the 1985 interim criteria (shown in the last column of Table 5.1.5) appear to show a slightly upward trend when viewed over the whole of the 1983-1992 period. This, however, is difficult to confirm on the basis of the current data, as already explained in paragraph 5-11 above.

5-14. The conclusion that, from the overall point of view, a fairly large proportion of the beaches in the thirteen Mediterranean countries supplying their data to the Coordinating Unit for the Mediterranean Action Plan in Athens within the framework of MED POL agreements do not satisfy the 1985 interim quality criteria adopted by the Contracting Parties should be seen in the light of the fact that a significant number of such beaches evaluated as non-conforming have been so evaluated because they have not been monitored at the required frequency. Compliance with the frequency requirement varied between 34.3% in 1985 and 76.1% in 1990, with an average overall frequency of 61.1% over the whole 10-year period. This highlights the need for improvement in this aspect of monitoring in order to reach a proper evaluation.

Bathing water quality in EC Mediterranean Member States

5-15. The four Mediterranean EC Member States (France, Greece, Italy and Spain) submit annual reports on the microbiological quality of their coastal recreational waters to the Community in terms of the 1975 bathing water quality Directive. Information supplied covering the period 1983 to 1987 (which, in a number of instances was only partial and cannot be meaningfully tabulated) show that the number of monitoring stations in France with high to acceptable quality water (A, AB or B) rose from 76.4% in 1983 to 83% in 1987, with a corresponding reduction in lower water quality stations (23.5% in 1983 to 16.7% in 1987). In the case of 606 coastal marine stations sampled at least ten times annually, the ratio of acceptable (A, B) to lower quality (C, D) stations changed from 55.8% - 44.2% in 1980 to 81.0 - 19.0% in 1988 (France, 1989). Results from Greece during this period are only available for 1987 and are restricted to Attica, where 77.7% of stations with at least five samplings per year were found to be in conformity with requirements. In Italy, the number of stations conforming with Italian criteria (based on, but stricter than, the Directive) showed a steady increase from 68% in 1984 to 89.3% in 1989 (Italy, 1990). The only negative trend recorded was in Spain, where the number of stations with high quality water (A2) decreased from 65.2% in 1986 to 51.0% in 1987.

5-16 Records are more complete from 1988 onwards, and an evaluation of the compliance of bathing beaches in France, Greece, Italy and Spain with the mandatory values of the microbiological parameters of the 1976 Directive (95% of the samples not to exceed 10,000 total coliforms and 2000 faecal coliforms per 100 ml) for the period 1988 to 1994 shows that compliance with the limit values for both parameters, expressed as percentages of the total number of identified beaches) varied between 79% and 91% in France, between 88% and 97% in Greece (where the total amount of identified beaches was only provided from 1991 onwards and percentages for 1988 -1990 are based on stations actually monitored), between 70% and 92% in Italy, and between 80% and 96% in Spain. In general, a definite positive trend emerged over this seven-year period. The number of stations monitored also rose steadily between 1988 and 1994, from 1663 to 1853 in France, from 247 to 1259 in Greece, from 3115 to 4173 in Italy, and from 985 to 1479 in Spain. It is not possible to convert these individual country results into global figures, as during the first three years of the period, the total number of identified beaches is available for only three countries.

5-17. The EC standard which is the closest approach to the 1985 Mediterranean criteria is the guide standard for faecal coliforms (80% of the samples not to exceed 100 per 100 ml). Compliance with this standard also implies compliance with the mandatory standard (95% of the samples not to exceed 2000 per 100 ml). Records of compliance with this standard are available from 1991 onwards, and apart from results for individual years given in EC annual reports, those for the period 1991-1994 are also summarized in the report covering the 1994 season (EC, 1995). An evaluation for this period, showing compliance with the EC guide values for total and faecal coliforms, is given in Table 5.1.6. Stations complying are shown as percentages of the total number of identified beaches. Compliance varied between 58.4% and 69.4% in France, between 85.0% and 94.9% in Greece, between 81.0% and 85.4% in Italy, and between 68.3% and 83.4% in Spain. From the overall point of view, compliance with the guide values showed a slight but steady increase during the four-year period, rising from 78.1% in 1991 to 80.4% in 1994.

TABLE 5.1.6

COMPARATIVE EVALUATION OF THE MICROBIOLOGICAL QUALITY OF COASTAL RECREATIONAL WATERS IN EC MEDITERRANEAN MEMBER STATES, 1991 - 1994, ACCORDING TO THE GUIDE STANDARDS OF THE 1976 EC BATHING WATER DIRECTIVE FOR (A) COLIFORMS AND (B) FAECAL STREPTOCOCCI, EXPRESSED AS PERCENTAGES OF TOTAL NUMBERS OF IDENTIFIED BATHING AREAS

(Compiled from European Commission report, 1995a)

Country	Year	Identified bathing areas	Stations conforming with coliform guide standards TC80 + FC80	Stations conforming with streptococci guide standards FS90
France	1991	1556	1053 (67.7%)	1145 (73.6%)
	1992	1934	1129 (58.4%)	1184 (61.2%)
	1993	1856	1201 (64.7%)	1305 (70.3%)
	1994	1870	1298 (69.4%)	1285 (68.7%)
Greece	1991	1097	932 (85.0%)	-
	1992	1203	1142 (94.9%)	1166 (96.9%)
	1993	1250	1170 (93.6%)	1246 (99.7%)
	1994	1282	1167 (91.0%)	1258 (98.1%)
Italy	1991	3824	3205 (83.8%)	3425 (89.6%)
	1992	4033	3444 (85.4%)	3690 (91.5%)
	1993	4288	3516 (82.0%)	3964 (92.4%)
	1994	4543	3680 (81.0%)	4229 (93.1%)
Spain	1991	1303	890 (68.3%)	574 (44.1%)
	1992	1335	980 (73.4%)	743 (55.7%)
	1993	1405	1121 (79.8%)	1091 (77.7%)
	1994	1490	1243 (83.4%)	1153 (78.0%)

5-18. The EC guide parameters also include faecal streptococci (90% of the samples not to exceed 100 per 100 ml). In the new Proposal for a Council Directive concerning the quality of bathing water (EC, 1994b) which, in its final form, will eventually replace the 1976 Directive, the guide value of 100 faecal streptococci per 100 ml has been retained, but the limit of compliance is now 80% (instead of 90%). In addition, a new imperative value of 400 per 100 ml, with a compliance limit of 95% has been introduced. Tables 5.1.6 also includes

percentages of compliance with the guide value for this parameter. from the comparative point of view, there is no definite conclusion one can reach on the data, compliance recorded for faecal streptococci being better than for total and faecal coliforms in some cases, and the reverse in others.

5-19 An overall evaluation of comparative compliance with (a) coliform mandatory standards (TC95 + FC95), (b) coliform guide standards (TC80 + FC80, and (c) streptococci guide standards (FS90) for the period 1991 - 1994 is given in Table 5.1.7. Because of the difference in the number of stations monitored for the various parameters, compliance has again been worked out as a percentage of the total number of identified beaches, rather than for stations actually monitored for each parameter in question. Apart from compliance with the coliform mandatory value alone, which remained virtually static at just above the 89% level, compliance with the guide values for both coliforms and faecal streptococci showed a rise, which was more pronounced in the case of faecal streptococci, during the four-year period under review.

TABLE 5.1.7

OVERALL COMPARATIVE EVALUATION OF THE MICROBIOLOGICAL QUALITY OF COASTAL RECREATIONAL WATERS OF EC MEDITERRANEAN MEMBER STATES, 1991 - 1994, IN TERMS OF COMPLIANCE WITH EC 1976 BATHING WATER DIRECTIVE (A) MANDATORY STANDARDS FOR COLIFORMS, (B) GUIDE STANDARDS FOR COLIFORMS AND (C) GUIDE STANDARDS FOR FAECAL STREPTOCOCCI, EXPRESSED AS PERCENTAGES OF TOTAL NUMBER OF IDENTIFIED BATHING AREAS

(Compiled from European Commission report, 1995a)

Year	Identified bathing areas	Stations conforming with coliform mandatory standards TC95 + FC95	Stations conforming with coliform guide standards TC80 + FC80	Stations conforming with streptococci guide standards FS90
1991	7780	6938 (89.2%)	6080 (78.1%)	5144 (66.1%)*
1992	8505	7623 (89.6%)	6695 (78.7%)	6783 (79.8%)
1993	8799	7836 (89.1%)	7008 (79.6%)	7606 (86.4%)
1994	9185	8261 (89.9%)	7388 (80.4%)	7925 (86.3%)

* One country (Greece) did not submit results for faecal streptococci in 1991. The degree of compliance for the other three countries combined would be 5144 stations out of a total of 6683 identified bathing areas, *i.e.* 77.0%.

5-20. In all EC reports on the quality of bathing waters, data from France and Spain are not subdivided into Mediterranean and non-Mediterranean coasts, and the data presented in the relevant tables (5.1.6 and 5.1.7) reflect this. The total number of identified bathing areas and the stations monitored in respect of these two countries therefore include parts of the Atlantic and North Sea coastlines. In the case of France, the Mediterranean coastline contains approximately one third of the country's total number of bathing beaches, but the overall standard is very similar, so that the general state of all beaches in France is a fairly accurate representation of that of the Mediterranean beaches. It is not, therefore, considered that such inclusion of a proportion of non-Mediterranean data affects an appraisal of the bathing water quality in the part of the Mediterranean bounded by the coastlines of the four EC Member States to any significant extent.

5-21. Because of the differences in parameters, numerical standards and compliance limits prevailing between the 1985 Mediterranean interim criteria and the 1976 EC Directive, no direct comparisons can be drawn between the two groups of data. As has been already stated, the EC standard which provides the nearest approach to the 1985 joint Mediterranean interim standard (50% of the samples not to exceed 100 faecal coliforms per 100 ml, and 90% not to exceed 1000 per 100 ml) is the guide standard for faecal coliforms. This, however, specifies that a minimum of 80% of the samples should not exceed 100 colonies per 100 ml and, (as it also incorporates the mandatory value, 95% should not exceed 2000, so that the two standards are not directly comparable from the numerical viewpoint.. Comparability is rendered even more difficult by the fact that complying stations could be expressed as percentages of *either* stations actually sampled at the regulatory minimum frequency, *or* the overall number of identified bathing beaches. In the case of the four EC countries, stations monitored at or beyond minimum frequency requirements form the vast majority (approximately 95%) of the total number of identified beaches. In the case of the thirteen countries submitting their data to MED POL, there is no reference to identified bathing beaches. The only distinction which can be drawn is between the total number of officially-designated monitoring stations (which is not quite the same as identified bathing beaches), and those stations monitored at or beyond the minimum frequency. Between 1983 and 1992, the highest percentage of designated stations monitored at minimum frequency was 76.1% in 1990, and the general average works out at just over 61%. In addition, the frequency requirements are not quite the same, The EC Directive stipulates fortnightly sampling during the bathing season, while the Mediterranean 1985 standard stipulates a minimum of ten samplings per year. Any attempt, therefore, at collation of the two sets of faecal coliform data would not achieve any practical purpose, and could only lead to misinterpretation.

5-22. For this reason, evaluation of the microbiological quality of bathing water in the Mediterranean has to be performed on a dual basis. The area covered by the coastlines of the four EC Member States comprises the whole of the Northwestern region from the Straits of Gibraltar right up to the Gulf of Trieste in the Northeastern Adriatic, and the Greek coastline from the Ionian to the Aegean Sea, including the islands. The area covered by what can be termed the MED POL group covers the rest of the Mediterranean, including the Eastern Adriatic, the Eastern coastline of the Aegean, the Eastern and Southern coastlines, and the islands of Cyprus and Malta.

5-23. Nevertheless, a number of general conclusions can be drawn. As has already been stated earlier when referring to individual tables, it is evident that, in terms of compliance with set standards, the quality of bathing waters in the areas covered by the four EC Member States has shown a positive trend during recent years. This is particularly illustrated by the percentages of stations complying with the faecal coliform and faecal streptococci guide

values (Tables 5.1.6 and 5.1.7). Furthermore, the number of beaches monitored at or beyond minimum frequency, in relation to the total number of identified bathing areas, is very high, as is the absolute number of stations monitored (even allowing for the proportion French and Spanish non-Mediterranean beaches contribute to the overall total.

5-24. On the other hand, the picture appears to be somewhat different for the rest of the Mediterranean. While compliance with the 1985 standard might appear to be of a reasonably high proportion on microbiological grounds alone (*vide* the penultimate column of Table 5.1.5), the percentages in question are severely affected by the fact that a large proportion of the stations were not monitored at the minimum frequency, and on the basis of strict compliance with all requirements, the picture emerging is quite different (*vide* the last column of Table 5.1.5). In both cases, no general trends can be confirmed. Lack of observation of the minimum frequency requirement is therefore one of the major failings. Furthermore, the total number of officially-designated monitoring areas is small, as compared to the situation in the EC area.

5-25. It should be stressed that the remarks in the preceding paragraph apply solely to the total data pooled from thirteen countries. It does not in any way reflect the actual situation in any one of the countries concerned, some of which have high-quality bathing water in their beaches, most of which are sampled at or beyond minimum frequency. One other major consideration is that the data submitted by the various countries to MED POL, although submitted within the framework of agreements on what are termed national monitoring programmes, does not, in every case, necessarily constitute the official or legally-recognised national bathing water quality monitoring programme in the country in question. There is at least one country in which a comprehensive national bathing water quality programme has been operational since well before the commencement of the Mediterranean Action Plan. The only data submitted to MED POL, however, are from a much smaller *ad hoc* programme run by different national authorities. In another country, independent water quality programmes are implemented by the various municipal authorities, and data from these are not available to the central government and, in turn, to MED POL. These, and other instances, which are mainly due to lack of liaison between different national authorities in individual countries, confirm the impracticality of drawing any definite conclusions on bathing water quality in the parts of the Mediterranean coastline bordered by non-EC Member States solely on the basis of the data submitted and the way it has to be presented.

5.2 THE STATE OF SHELLFISH AREAS

The MED VII Pilot Project (1976-1981)

5-26 During the course of the Pilot Project on Coastal Water Quality Control (MED VII), six collaborating laboratories in four Mediterranean States monitored shellfish waters. Monitoring started in late 1976, simultaneously with monitoring of recreational waters, and ended in March 1981. A minimum programme was applied by all participating laboratories to enable comparability of results. In actual fact, monitoring programmes were generally above those required, by the inclusion of parameters other than those specified (WHO/UNEP, 1981). The selection of sampling areas, as well as the number of sampling stations, was mainly determined by the location and organization of already-existing shellfish-growing areas. As a result, the conclusions from the monitoring programme carried out on shellfish-growing areas in the Mediterranean could not be considered as of general application. However, considering the number and spatial distribution of the collaborating laboratories, the conclusions derived therefrom could be reasonably considered as a valuable indication of the current situation of shellfish-growing areas in the Mediterranean (UNEP/WHO, 1985).

5-27. During the course of the project, 2300 water and shellfish samples from fifty sampling stations were analyzed. The average number of water samples analyzed at each sampling station was estimated at 10 per year, although the sampling frequencies varied widely among sampling stations, both within the area monitored by a given laboratory and within areas of different laboratories. The basic parameters used in assessing the microbiological quality of shellfish-growing waters were four indicators of sewage pollution: total coliforms, faecal coliforms, faecal streptococci and total heterotrophic bacteria. In addition, other microbiological parameters, including qualitative and quantitative analysis of vibrios (including *Vibrio parahaemolyticus*), *Salmonella* and viruses were determined (WHO/UNEP, 1981).

5-28. In the assessment of microbial pollution in the Mediterranean Sea (UNEP/WHO, 1985), the proposed UNEP/WHO interim criteria (*vide* Part 4 of this document) were used as a basis for evaluation of the microbiological quality of shellfish-growing waters and shellfish. The results of this assessment is summarized in Table 5.2.1 (A). Specifically, the 2 faecal coliform per gram of shellfish flesh was considered the limiting factor in the evaluation process. Consequently, any sampling stations complying with this standard, as well as with the corresponding one for the quality of shellfish-growing waters, was considered as satisfactory for direct sale of shellfish, without additional purification or depuration, during the yearly period considered. Only those stations with ten or more shellfish analyses per year were considered in this particular evaluation (UNEP/WHO, 1985).

5-29. An analysis of Table 5.2.1 (A) clearly shows the marked influence that the microbiological limitation on shellfish flesh had in the evaluation of a sampling station. While the percentage of sampling stations considered satisfactory from the point of view of the microbiological quality of their water varied between 47% and 76%, depending on the year, those satisfactory from the point of view of shellfish flesh ranged from 0% to 21% during the period in question. The stations satisfying both standards (water and shellfish) varied between 0% and 10%. (UNEP/WHO, 1985). An additional evaluation, based on the same microbiological criteria, considered all sampling stations with six or more samples of both water and shellfish per year (Table 5.2.1 (B)). This showed a similar pattern, between 52% and 86% complying with the water standard, and between 0% and 9% with the shellfish standard.

5-30. The data were also analyzed on the basis of conformity with the EC criteria (EC, 1979), which stipulates that, as a guide value, the number of faecal coliforms in 100 ml of shellfish flesh plus intervalvular fluid should not exceed 300 in 75% of the samples collected at quarterly intervals (*i.e.* a minimum of four times per year). The results of this evaluation (UNEP/WHO, 1985) are reproduced in Tables 5.2.2 (A) and 5.2.2 (B). Both tables show an almost identical pattern, the percentage of satisfactory stations from the point of view of water quality, both types (with at least ten and at least six samplings per year respectively) varying between 0% and 71%.

5-31. A comparison of the two standards (1985 Proposed Mediterranean and 1979 EC) for shellfish waters through compliance of 1976-1981 MED POL stations with each shows that the EC standard appears to be the stricter of the two. Of the MED POL stations with at least ten samplings per year, the rate of compliance with the proposed Mediterranean standards varied between 47% and 76% during the period under review, with an average compliance of 63%, while compliance of the same stations with the EC standards varied between 0% and 71% with an average of 40%. In the case of stations with at least six samplings per year, the rate of compliance with the Mediterranean standards varied between 52% and 86%, with an average of 64%, while compliance with the EC standards varied between 0% and 71%, with an average of 40%. The value of the averages should be viewed with some reservation, as the number (and in some cases, the locality) of the stations varied from year to year. The differences in compliance should be viewed in the light of the fact that the proposed Mediterranean standard stipulated analysis of shellfish flesh only, while the EC Directive stipulates analysis of the flesh and intervalvular fluid. In spite of this difference, however, the inference to be drawn is that analysis of the shellfish themselves constitutes a stricter standard for the acceptability of shellfish-growing waters than analysis of the water itself. One cannot compare compliance of the stations with the Mediterranean standards for shellfish on the one hand and with the EC standards on the other, as the former were intended to evaluate shellfish for human consumption, while the latter constituted an index of acceptability of the waters for shellfish growing.

MED POL Phase II data

5-32. While shellfish waters were included in the monitoring component of MED POL Phase II, none of the countries signing national monitoring agreements with UNEP included this matrix in their programme of activities during the period under review. No data on shellfish waters in the Mediterranean are therefore available from 1982 onwards. EC Member States do not submit reports on shellfish waters to the Commission, as they do in the case of bathing waters. The information in the preceding paragraphs and in Tables 5.2.1 and 5.2.2, while of historical interest, only provide at best a partial indication of the situation existing fifteen years ago, and can in no way be considered an indication of the present situation, which remains largely unknown until such time as data from regular monitoring is made available.

TABLE 5.2.1

**SUMMARY ASSESSMENT OF THE MICROBIOLOGICAL QUALITY
OF SHELLFISH WATERS AND SHELLFISH IN THE
MEDITERRANEAN, 1976-1981, ACCORDING TO THE
PROPOSED WHO/UNEP INTERIM CRITERIA, 1985**

(Adapted from UNEP/WHO, 1985)

A

MED POL VII SAMPLING STATIONS WITH AT LEAST 10 SAMPLES PER YEAR

Year	Stations surveyed	Stations with satisfactory		Satisfactory stations
		water	shellfish	
1976	15	10 (67%)	0 (0%)	0 (0%)
1977	12	7 (58%)	0 (0%)	0 (0%)
1978	21	14 (67%)	2 (10%)	3 (10%)
1979	19	9 (47%)	4 (5%)	1 (5%)
1980	21	16 (76%)	0 (0%)	0 (0%)

B

MED POL VII SAMPLING STATIONS WITH AT LEAST 6 SAMPLES PER YEAR

Year	Stations surveyed	Stations with satisfactory		Satisfactory stations
		water	shellfish	
1976	18	12 (67%)	0 (0%)	0 (0%)
1977	13	8 (62%)	0 (0%)	0 (0%)
1978	24	17 (71%)	3 (13%)	2 (8%)
1979	33	17 (52%)	6 (18%)	3 (9%)
1980	21	14 (67%)	0 (0%)	0 (0%)
1981	7	6 (86%)	0 (0%)	0 (0%)

TABLE 5.2.2

SUMMARY ASSESSMENT OF THE MICROBIOLOGICAL QUALITY OF SHELLFISH WATERS IN THE MEDITERRANEAN, 1976-1981, ACCORDING TO THE STANDARDS IN THE 1979 EC DIRECTIVE

(Adapted from UNEP/WHO, 1985) .

A

MED POL VII SAMPLING STATIONS WITH AT LEAST 10 SAMPLES PER YEAR

Year	Stations surveyed	Satisfactory stations
1976	15	4 (27%)
1977	12	0 (0%)
1978	21	11 (52%)
1979	20	6 (30%)
1980	21	15 (71%)

B

MED POL VII SAMPLING STATIONS WITH AT LEAST 6 SAMPLES PER YEAR

Year	Stations surveyed	Satisfactory stations
1976	18	4 (22%)
1977	13	0 (0%)
1978	24	14 (58%)
1979	34	11 (32%)
1980	21	15 (71%)
1981	7	4 (57%)

PART 6

HEALTH RISKS FROM POLLUTED RECREATIONAL AND SHELLFISH AREAS IN THE MEDITERRANEAN

6.1 GENERAL HEALTH RISKS

6-1. The main types of human exposure to pathogenic microorganisms in the marine environment are through direct contact with polluted seawater, sand or sediment, including ingestion of seawater, while engaging in swimming or other coastal marine recreational activities, and through consumption of contaminated seafood.

6-2. Concern about actual and potential health effects arising out of such exposure has been expressed worldwide, particularly during the last three decades. In the case of shellfish, this has led to the progressive development of various quality criteria and standards, not only for the shellfish themselves within the framework of public health and food quality legislation, but also for the growing and harvesting waters. Apart from the variation between statutory requirements for acceptability of shellfish for human consumption in different countries, doubts have also been expressed regarding the efficiency of shellfish depuration techniques in flushing out microbiological hazards and making the product safe for human consumption. (Geldreich, 1985). In the case of recreational waters, more fundamental problems have been encountered. Attempts at quantifying health hazards from polluted recreational waters have been made in several countries through the conduction of microbiological/epidemiological studies aimed at establishing a direct correlation between the microbiological quality of the water and health effects on exposed population groups. In general, these studies have produced different results, leading to a wide variation in recreational water quality criteria and standards applied, and to considerable controversy regarding their implementation (Jones and Kay, 1989).

6-3. Health hazards arising from the presence of pathogenic microorganisms in the Mediterranean marine environment can be considered as particularly significant as a result of a heterogeneous variety of factors. A WHO document on Microbiological quality control in coastal recreational and shellfish areas in the Mediterranean (WHO, 1989) included the following factors, the influence of which on increased health risks from marine pollution in the Mediterranean was confirmed by a WHO/UNEP consultation on microbiological pollution of the Mediterranean Sea, held in Valletta, Malta, in December 1989 (WHO/UNEP, 1990):

Apart from the 130 million inhabitants estimated to live permanently along the Mediterranean coastline, over 100 million tourists visit the area annually. During the summer months, the sea constitutes the main recreational amenity for local and tourist populations alike, as a result of which most beaches, especially those in the vicinity of cities and tourist resorts, are heavily overcrowded, particularly on weekends. The heterogeneous nature of beach populations further facilitates the spread of infections;

Prevailing warm climatic conditions not only result in a relatively long bathing season, but are also responsible for longer periods of exposure to seawater and/or beach sand, as compared to the situation in other, more temperate, countries;

Considerable amounts of shellfish are grown or harvested in the Mediterranean area, and consumed by both local and tourist populations. The total consumption of shellfish has been estimated at over 12,000 metric tons annually. The larger part of this can be considered to be consumed in coastal areas;

Although the general situation is progressively improving through the establishment of sewage treatment facilities and the construction of submarine outfall structures, the bulk of municipal sewage in most parts of the region is still being discharged untreated into the immediate marine coastal zone, in many instances in the vicinity of recreational and shellfish areas;

Water and seafood quality control measures vary from country to country. In many cases, control measures in terms of quality criteria or standards are practically wholly based on "acceptable" concentrations of bacterial indicator organisms. While such organisms can provide a reasonable estimate of the degree of sewage pollution, and perhaps a relatively satisfactory correlation with concentrations of bacterial intestinal pathogens, they have not so far been accepted as providing any clear correlation with the presence and density of either viruses or non-gastrointestinal pathogens, as well as with algal biotoxins such as PSP and DSP. In general, there is very little control over the quality of beach sand, which has only recently commenced to be recognised as a factor to be considered in the transmission of a number of skin and other contact infections, including fungal ones.

6-4. The first three factors are climatic and socio-economic, and more or less permanent in nature. Their significance is increasing as a result of increases in tourist numbers and extension of the length of coastline utilized for marine recreation. The impact of the penultimate factor, regarding the general situation, still essentially hold good, although improvements in wastewater management, as evidenced by the growing number of sewage treatment plants and submarine outfall structures, particularly along the northern part of the coastline. The last factor is global in character, rather than specific to the Mediterranean. It does however constitute an accentuated risk factor in the region when considered in combination with the other specifically-Mediterranean factors.

6-5. In this context, quality criteria and standards are normally based on the results of epidemiological studies correlating water and shellfish quality with health effects on exposed population groups. In the case of bathing waters, such studies have been conducted in various countries of the region over the past two decades. Details of these studies are provided further on in this part of the present document. Although generally based on internationally-recognised protocols developed for studies outside the region, they varied as to specific scope and detail, and were all relatively small-scale. To a variable extent, all were limited, particularly in the interpretation of results, by a number of confounding factors over which satisfactory control has been found difficult to achieve. Control measures, therefore, apart from the degree of their inter-country variation, have not, in the main, been the end-result of health risk assessments based on hard epidemiological evidence.

6.2 DISEASES AND DISORDERS

6-6. On a very general level, diseases and disorders caused by pathogenic microorganisms in the various matrices of the marine environment can be broadly divided into two categories: those that affect the gastrointestinal tract, and those that affect other parts of the body. With regard to the former category, potentially, all the diseases which are spread by the faecal-oral route, and whose aetiological agents are shed in the faeces of diseased individuals or carriers, could be contracted by swimming in sewage-polluted waters (Cabelli, 1983). The same author has reported such diseases to include (a) bacterial diseases, such as salmonellosis (including typhoid and paratyphoid fevers), shigellosis (bacillary dysentery), cholera and gastroenteritis caused by enteropathogenic *Escherichia coli*, *Yersinia enterocolitica*, etc., (b) viral diseases, such as infectious hepatitis, illnesses caused by enteroviruses (polioviruses, coxsackie viruses A and B, echoviruses, reoviruses and adenoviruses), and "non-specific" gastroenteritis caused by the human rotavirus and parvo-like viruses, and (c) diseases caused by a variety of protozoan and metazoan parasites, such as Amoebic dysentery, giardiasis, ascariasis, etc. The pathogenic microorganisms present in the Mediterranean which cause such diseases (which include the above) have been described in Part 3 of this document.

6-7. Insofar as the ingestion of water during swimming or bathing is necessarily limited, with the exception of pathogens having a relatively low infective dose, the diseases mentioned above can be contracted much more easily through the consumption of raw or partially-cooked shellfish. Other pathogens reported as causing human infections through this latter route include *Vibrio parahaemolyticus* and *Clostridium botulinum* (Type E), whose native habitat is the sea. There is, in fact, extensive evidence of the spread of diseases to man following the consumption of polluted shellfish. A wide range of diseases has been described (Shuval, 1986), with the main ones being typhoid and paratyphoid fevers, salmonellosis, *Vibrio parahaemolyticus* infections, viral hepatitis type A (infectious hepatitis), paralytic shellfish poisoning and cholera (UNEP/WHO, 1991).

6-8. Apart from diseases affecting the gastrointestinal tract, a number of diseases or disorders affecting the eye, ear, upper respiratory tract and other areas have been associated with bathing. This particular category of infective conditions may be caused by microorganisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Clostridium welchii* and *Candida albicans*, and infection may be caused as a result of the microorganisms being forced into breaks or tears in the skin, or into ruptures in delicate membranes in the ear or nose resulting from the trauma associated with diving into the water (Shuval, 1986). These microorganisms have been described (Mood and Moore, 1976) as often present in man, but giving rise to disease only when, for one reason or other, the resistance of the individual who harbours them is lowered. Although all the four species mentioned above may also be found in polluted waters, the same authors consider the suggestion that an individual suffering from infection has acquired it from polluted water as one to be treated with reserve, as the bather is very likely to have been carrying the microorganism beforehand, and the disease may be largely determined by the individual's susceptibility, rather than by exposure to the microorganisms in the marine environment.

6.3 CORRELATION BETWEEN RECREATIONAL WATER QUALITY AND HEALTH EFFECTS

6-9. The basic aim of investigations into the health effects of swimming or bathing have always been as originally designed by Stevenson (1953), *i.e.* to determine what difference in illness incidence might be expected from swimming in waters containing varying degrees of bacterial pollution. The establishment of a dose-response curve, based on epidemiological research, should be the ultimate goal of research in this area. This dose-response curve does not depend on indices of pollution based on pathogens themselves; indicators of sewage pollution are more appropriate, since they have more predictable distributions and are likely to be used as the basis for standards. Moreover, it is not necessary to define disease risk with reference to specific pathogens; symptoms or groups of symptoms are more appropriate where the risk is due to a variety of agents with unknown seasonal and spatial distribution (Wheeler, 1990). Since the first study by Stevenson in 1953, a number of studies have been carried out in an attempt to define the levels of risk following exposure to different concentrations of bacteria in bathing waters. Most studies have been of the prospective epidemiological type, now generally termed as "Cabelli-style" after the leader of the first large-scale study conducted by the U.S. Environmental Protection Agency between 1972 and 1979. A number of studies of this type have been conducted worldwide, including some in the Mediterranean.

6-10. The relevant literature, including details of the design and results of the studies performed, have been reviewed by Shuval (1986), Jones and Kay (1989), Lightfoot (1989), Saliba and Helmer (1990), Wheeler (1990) and Pike (1994). Most studies performed in the Mediterranean involved marine beaches, and the micro-organisms employed to assess water quality varied from one to a combination of total coliforms, faecal coliforms, faecal streptococci, enterococci, *Escherichia coli* and staphylococci. Practically all of them obtained results in the form of higher morbidity among bathers as compared to non-bathers, the best correlation with water quality being with one or other microorganism where different beaches were compared.

Mediterranean studies

6-11. A study in Alexandria in 1980 was sponsored by USEPA, who were interested in locating some more heavily polluted beaches than those available for study in the United States, so as to be able to determine the nature of the dose-response curve at the higher dose levels (Shuval, 1986). Some of the beaches included in the study were exposed to nearby sewage outfalls and heavily polluted, enterococci and *Escherichia coli* densities reaching 10^4 per 100 ml. Other beaches were acceptable according to USEPA guidelines. The study population included both Alexandria residents and summer visitors coming from Cairo. It was assumed that these latter had lower levels of immunity to the diseases endemic in Alexandria. The main findings showed a strong association of highly-credible gastrointestinal symptoms and enterococcus densities, as well as for *Escherichia coli* densities. A comparison with previous studies of a similar nature in New York showed that at equivalent enterococci densities, disease rates were lower in Egypt, and more markedly so, among the Alexandria local population. This was attributed to the endemic status of enteric diseases in the area, and the resultant relative immunity of older children and adults (Shuval, 1986). A report on certain aspects of the study (El-Sharkawi *et al*, 1982b) stated that there was a significant risk of contracting typhoid from bathing in polluted seawater, and that the young age group was found to be the most susceptible.

6-12. A 1983 Israeli epidemiological study was carried out at three bathing beaches in the Tel-Aviv area, having varying degrees of exposure to sewage pollution. The study encompassed 2,231 swimmers and non-swimmers (32% in the 0-4 age group, and the indicators used were faecal coliforms, enterococci, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The main finding was a significant excess of enteric symptoms (gastrointestinal diseases) among swimmers in the 0-4 age group in water with higher concentrations of enterococci or *Escherichia coli*. Faecal coliforms and *Pseudomonas aeruginosa* did not appear to be of equivalent value as indicators of swimming-associated enteric disease (Fattal and Shuval, 1988).

6-13. A second prospective epidemiological study in Israel was conducted on four beaches in the Tel-Aviv area and to the South (Fattal and Shuval, 1991). Apart from the primary objective of studying the swimming-associated morbidity with regard to water quality in terms of bacterial indicator concentrations, a second objective was to determine the feasibility of extending the project to establish the aetiology of swimming-associated gastroenteritis. A total of 233 families, comprising 784 persons were interviewed for the study, 23% of the study population were in the 0-4 age group. A total of 42 seawater samples were tested for bacterial indicator concentrations (faecal coliforms and enterococci), the concentrations of both varied significantly between the cleanest and most polluted beach. No significant difference in the incidence of swimming-associated enteric symptoms were found between these two beaches, both for all ages and for the 0-4 age group, although the overall morbidity results were similar to those found in earlier studies. Forty individuals, including both swimmers and non-swimmers, donated blood samples, all of which were found to be positive for Rotavirus. No seroconversion was detected. It was also noted that swimming-associated rates for total and highly-credible enteric and respiratory symptoms were markedly higher during "high bather effect" days than during "low bather effect" days. Two of the conclusions drawn by the authors were (a) that the bathers themselves can be an important source of the agents responsible for swimming-associated illness at marine beaches under conditions of heavy beach usage and poor water exchange, and (b) that lower microbiological indicator standards should be applied in beaches with low water exchange (i.e. due to surf-breakers, etc.) than those applied in beaches with relatively high water exchange (open beaches).

6-14. Three studies were conducted in Spain. The first (Mujeriego *et al*, 1983) involved a large-scale cross-sectional study utilizing over 20,000 respondents on various beaches in Malaga and Tarragona, where interviews were carried out. The highest morbidity rates observed concerned skin infections (2%) followed by ear and eye infections (nearly 1.5%), the latter group being considered by the authors as significantly associated with immersion of the head in seawater. Concentrations of faecal streptococci gave better correlation with morbidity rates than faecal coliforms. The second study (MariZo *et al*, 1982), carried out on a number of Mediterranean and Atlantic beaches, was roughly similar in design, and again gave higher morbidity rates for ear, eye and skin infections. The best correlation with such morbidity was obtained with faecal coliforms, threshold concentrations for increases in morbidity being calculated in the range of 400 FC per 100 ml.

6-15. The third study was carried out on two beaches in Malaga, determining water quality in terms of total coliforms, faecal coliforms, faecal streptococci, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Vibrio* species and *Candida albicans*. During the study (Borrego *et al*, 1991), surveys of test population groups were performed by a modified Cabelli-type questionnaire. Preliminary conclusions so far have been that: (a) enteric symptomatology was higher among swimmers than non-swimmers on the more polluted beach; (b) there was a significant excess in dermatological and respiratory symptoms among swimmers as compared to non-swimmers, irrespective of recorded

bacterial densities in sea waters; (c) no statistical significance was evident between swimming-associated symptom rates and faecal indicators; and (d) fungal density in beach sand was the same for both the polluted and non-polluted beach, and no relation was found between sand-associated dermatitis symptoms and dermal pathogens. One other problem associated with this study was the fact that, out of 1,447 individuals surveyed, only 4.6% could be defined as non-swimmers, and the control group was therefore very small.

6-16. Studies carried out in France included one (Foulon, 1983) on five beaches, the water quality of which was tested for concentrations of total coliforms, faecal coliforms and faecal streptococci. Nearly 5,000 individuals were interviewed on the beach and just over 1,500 followed up on the basis of a completed answer-card questionnaire. The results indicated differences between the incidence of conjunctivitis and skin infections between bathers and non-bathers, as well as in the incidence of abdominal discomfort, nausea and pruritis between those immersing their head in the water and those refraining from doing so. There was, however, no correlation between morbidity and water quality.

6-17. A later study in France (CAREPS, 1987) carried out in the Bassin de l'Ardèche on freshwater beaches, concerned over 5,700 subjects in the summers of 1985 and 1986. The water-quality indicators selected, apart from total coliforms, faecal coliforms and faecal streptococci, were *Aeromonas* species and *Pseudomonas aeruginosa*. Subjects were kept under constant observation for periods of between 3 and 8 days and symptoms recorded. Higher morbidity rates were found among bathers, as opposed to non-bathers, for each type of disease observed (gastrointestinal, skin, eye and otorhinolaryngeal). Total coliform and faecal coliform concentrations gave the best correlation with overall morbidity. When taking each type of symptom separately, correlation varied with the different indicators, faecal streptococci providing the best correlation with gastrointestinal symptoms. Analysis of results produced the conclusion that a concentration of 100 faecal coliforms per 100 ml (the EEC "guide" standard) corresponded to an incidence of 15.3 cases of overall gastrointestinal morbidity per 1,000 person-days, and 2,000 per 100 ml (the EEC "mandatory" standard) to an incidence of 20.4 cases per 1,000 person-days. The guide standard for faecal streptococci (100 per 100 ml) was calculated as corresponding to 23 cases per 1,000 person-days. The calculation on expected incidence of gastrointestinal morbidity in non-bathers was not made but, on the basis of the authors' results, this would come to 8.5 cases per 1,000 person-days. The authors' conclusions include the statement that the results cannot be safely extrapolated to seawater.

6-18. Other studies performed in the Mediterranean included one on the relationship between coastal tourism, sea pollution and public health (Kocasoy, 1989). The study, on 15 beaches on the western coast of Turkey, involved only visitors, and the local population was excluded. The study aimed at correlating total coliform density in the various beaches with symptoms reported. 8399 questionnaires were distributed, of which 4068 were returned, and 3407 classified as valid. 3240 questionnaires (95% of the valid ones) were from swimmers. Morbidity rates for gastrointestinal diseases reported by swimmers ranged from 7.2% in the less polluted beaches to 9.5% in the more polluted, the corresponding figures for non-swimmers being 2.9% and 3.1%. Between 66% and 71% of affected subjects were children of under 12 years of age. Another study was carried out on three beaches near Palermo, Sicily (Torregrossa *et al*, 1994). Beaches were classified as polluted or non-polluted. Of the microbiological parameters determined at all beaches, levels of total coliforms, faecal coliforms, faecal streptococci, *Staphylococcus aureus* and other *Staphylococcus* species and *Candida albicans* were variably higher in the polluted beaches. Levels of *Pseudomonas aeruginosa* and other *Pseudomonas* species, as well as *Vibrio* species were approximately the same in both polluted and non-polluted beaches. *Salmonella*

was absent in all beaches. A total of 581 subjects were interviewed, of which 372 (64%) were swimmers. Morbidity for enteric symptoms were higher in polluted beaches. No significant differences for other symptoms (respiratory and general) were found between beaches. Levels of *Pseudomonas aeruginosa* and *Candida albicans* in beach sand were the same as those recorded for seawater.

Recent non-Mediterranean studies

6-19. Apart from the USEPA study, a number of studies aimed at the correlation between recreational water quality and health effects have been conducted in a number of countries. Two of the largest held outside the European region were in Hong Kong Cheung *et al*, 1991) and in Australia (Corbett *et al*, 1993). In the former study, nine bacterial indicators were tested, *Escherichia coli* and staphylococci being recorded as good indexes of different health effects due to swimming. The levels of *Escherichia coli* at a number of beaches was found to be influenced by tide, and those of staphylococci by bather numbers. In the latter study, carried out in twelve beaches in Sydney, 24% of participants reported experiencing symptoms in the ten days following initial interview. Almost two-thirds of these symptoms were respiratory. The two main bacterial indicators tested were faecal coliforms and faecal streptococci, the geometric mean density of which varied between 591.4 (morning) and 389.1 (afternoon) per 100 ml for the former, and between 65.9 (morning) and 42.7 (afternoon) per 100 ml for the latter. Swimmers were twice as likely to report symptoms than non-swimmers, and with the exception of gastro-intestinal complaints, there was a linear relationship between water pollution and all reported symptoms.

6.20. During the last five years, comprehensive studies were held in the United Kingdom. In August 1989, the UK Department of the Environment commissioned two pilot studies on the health effects of sea bathing. One of the aims of these studies was to assess the feasibility of the methods tested on a large-scale basis (Pike, 1990). Two methods were tried - a prospective design adapted from the USEPA Cabelli-style study, and a "healthy volunteer design. These two complementary methods utilized followed the protocols for prospective cohort-type studies and for randomized controlled clinical studies as finalized by a WHO/UNEP meeting on microbiological quality of coastal recreational waters, held in Athens in June 1993 (WHO/UNEP., 1994). In the first study, the reports of illness varied by activity, and surfers had higher morbidity than waders. Ear and throat symptoms were most frequently reported (1 in 13 bathers vs. 1 in 32 non-bathers). The quality of the water met EC mandatory standards. In the second study, healthy volunteers were recruited and divided into bathing and non-bathing cohorts. Clinical tests were held both before and after exposure. While significantly higher rates of sore throat, ear and eye symptoms were reported by the bather cohort three days after the study, and diarrhoea three weeks after the study, there was no correlation between the results of the clinical tests and the perceived symptoms reported (Pike, 1990).

6-21. The second phase of the study was commissioned in 1990, again using both methods. In the first, involving over 2000 beach users, participants aged 15 to 24 years had the highest age-associated risk. Bathers were 47% more likely to develop gastrointestinal illness and had an 85% greater risk of suffering from diarrhoea as compared with non-bathers. Illness was associated with exposure, and surfers and divers were significantly more likely to suffer from respiratory and eye symptoms (Pike, 1991). In the second, involving over 800 adults, clinical results of throat and ear swabs were not significantly different between bathers and non-bathers, although there was some evidence to suggest that the concentration of faecal streptococci in the bathing water was related to

the isolation of *Escherichia coli* from throat swabs of bathers. While children were excluded from the study, 131 children under 18 years of age accompanied the adults to the beach, and 64 of them swam. These children suffered significantly more stomach upsets than those who had not bathed.

6-22. Studies in the United Kingdom continued on a larger scale after the two-year pilot phases. The prospective cohort studies ("beach surveys") continued at eight beaches varying widely in quality, from those regularly meeting the guide standards of the EC bathing water Directive to those regularly failing to meet the imperative standards (Pike, 1994). The results were combined with those of the previous studies at two beaches to give usable results from 16,596 subjects. Those entering the sea reported more of all symptoms than those who did not. The relative increases were related to the degree of water contact and age, being greatest in surfers and divers and in the 15-24 age group. Relative increases in eye, ear, nose, throat, skin and respiratory symptoms were not related to microbiological quality of the water. Relative increases in diarrhoea in those entering the water were related to geometric mean counts of coliform bacteria and enteroviruses. These findings have been described as preliminary, and data are being examined in greater detail. The results of a 4-year study in four different resorts involving 1216 adults were published in 1994 (Kay *et al*, 1994). This study was conducted along the lines of the randomized controlled exposure method. Detailed interviews were used to collect data on potential confounding factors, and intensive water quality monitoring was used to provide more precise indices of exposure. 548 individuals were randomized to bathing, exposure including total immersion of the head. Crude rates of gastroenteritis were significantly ($P=0.01$) higher in the exposed group (14.8 per 100) than in the unexposed group (9.7 per 100). Linear trend and multiple logistic regression techniques were used to establish relationships between gastroenteritis and microbiological water quality. Of a range of microbiological indicators assayed, only faecal streptococci concentrations, measured at chest level, showed a significant dose-response relation with gastroenteritis. Adverse health effects were identified when faecal streptococci concentrations exceeded 32 per 100 ml. This relation was independent of non-water-related predictors of gastroenteritis.

Harmonization of methodology

6-23. In their review of the literature on results of the main Cabelli-type prospective epidemiological studies, Jones and Kay (1989) reached the conclusion that there are no good epidemiological data on which to base the implementation of scientifically justified quality standards for recreational waters which would control for a high level of risk. In quoting such conclusions, attention has been drawn (Colley, 1990) to some general problems in the conducting of epidemiological studies carried out so far, including:

- Difficulties in defining exposed and non-exposed population groups;
- Uncertainty in the degree and duration of bathing exposure;
- Availability of only limited information on pollutants present in the bathing water;
- Inadequate identification of illnesses and their relationship to bathing;
- Difficulties in the interpretation of any associations between illnesses and exposure to polluted water, and in ascribing a cause-effect relationship.

6-24. The same author has also pointed out that a number of issues remain to be addressed in future epidemiological studies, including subgroups that may need to be identified both in terms of estimating risk and in addressing of preventive measures, the adequate follow-up of exposed and non-exposed persons, the obtaining of some measure of the "dose" bathers receive in estimating risk of illness, and the differentiation between marine and non-marine bathing exposures. Similarly important are non-bathing exposures, particularly consumption of contaminated food, as illness which has no relation to bathing may be present in a population, and must be recognized in order to prevent confusion with bathing-acquired illness, as well as sufficient evidence that the pathogen or agent causing a disease is also present in the water.

6-25. It has been concluded (Saliba and Helmer, 1990) that on the basis of evidence available so far, it would be difficult to attempt to quantify the actual health risks arising from bathing in sewage-polluted coastal bathing waters, as well as to correlate such risks to specific levels of water pollution as expressed in terms of the more routinely-measured bacterial indicator organisms. From the qualitative viewpoint, however, the evidence clearly indicates that health risks do exist, and are most pronounced in areas directly exposed to pollution by untreated sewage. While this conclusion is valid from the global viewpoint, it applies specifically to the Mediterranean region. A similar conclusion was also reached as a result of a review of the results of a multi-year programme of microbiological/epidemiological studies conducted by USEPA (Calderon, 1990), which confirmed that for marine beaches, a good correlation with swimming-associated gastrointestinal symptoms was only obtained by concentrations of enterococci densities in the water. In these studies, the source of pollution was an identified non-point sewage pollution source. A USEPA study examined the relationship between faecal indicators from non-point sources and swimming-associated illness. The results of this study found no such illness associated with faecal water quality indicators.

6-26. Within the framework of the MED POL programme, active steps were taken by WHO to harmonize methodologies through development of appropriate study protocols which would serve not only to determine the degree of health risk, but also to identify the best possible microbiological indicators correlating water quality with adverse health effects. The WHO/UNEP Group of experts on health criteria and epidemiological studies relating to coastal water pollution agreed on an outline protocol, based on the USEPA Cabelli methodology, for microbiological/epidemiological studies in the region (WHO/UNEP, 1977b). This outline was expanded into a more detailed protocol by a WHO/UNEP consultation on correlation of coastal water quality with health effects, held in Follonica, Italy, in October 1985 (WHO/UNEP, 1986). Following the development outside the region of new methodologies to cover both bathers and other types of exposure groups, the whole matter was fully reviewed by a WHO/UNEP consultation on health risks from bathing in marine waters, held in Athens in May 1991 (WHO/UNEP, 1992), during which part of a comprehensive set of guidelines for prospective microbiological/epidemiological studies on the association between natural recreational water quality and health effects on exposed population groups was prepared. A further WHO/UNEP meeting on microbiological quality of coastal recreational waters, held in Athens in June 1993, finalized the complete guidelines, including parameters to be determined, cohort-type studies, studies on special bather groups, randomized controlled clinical studies, and small-scale studies on different water-exposure groups, which were published during the following year (WHO/UNEP, 1994).

6.4 CORRELATION BETWEEN SHELLFISH QUALITY AND HEALTH EFFECTS

6-27. The situation regarding shellfish has two distinct aspects: the quality of the waters in which the shellfish are grown or harvested (which can be measured either by analysis of the water, or by that of the shellfish themselves while still in this environment), and the quality of the shellfish when they reach the market. The microbiological content of shellfish taken from polluted waters can be considerably increased as a result of incorrect treatment or storage (UNEP/WHO, 1991).

Epidemiological studies on shellfish poisoning

6-28. Two approaches are commonly used in the epidemiological investigation of disease aetiology. In a retrospective (or case-control) study, individuals who have developed the disease are compared with a group of similar individuals who did not, with respect to exposure to the aetiological factor in question. In a prospective (or cohort) study, groups differing only in their exposure to a certain aetiological factor are followed up and disease incidence is compared in relation to such exposure. While most epidemiological studies on the correlation between bathing water quality and health effects have utilized the prospective approach, studies linking disease incidence with shellfish quality have invariably utilized the retrospective one. In a study of this nature, all the relevant events (causes and effects) have already occurred when the study is initiated, and fundamentally, such study constitutes an investigation of the past histories of affected persons, whether sporadic cases, or cases occurring during an outbreak or an epidemic of a disease. The distinction between outbreaks and sporadic cases is a real one in terms of the probability of obtaining an association between a given disease and some suspected cause (e.g. consumption of contaminated shellfish). This is especially true when other routes of transmission account for the large majority of cases of the disease concerned (WHO/UNEP, 1977).

6-29. Apart from the two studies on Typhoid fever in Egypt (El-Sharkawi *et al*, 1982a) and the 1973 Cholera outbreak in Italy (Baine *et al*, 1974), current review literature available contains very little information on epidemiological evidence in the form of retrospective studies on shellfish-induced illness, particularly as regards large outbreaks, in the Mediterranean. Two recent outbreaks are on record, both in France. The first, in 1992, involved 10,000 cases of gastroenteritis, the pathogen being Norwalk virus. Contaminated oysters were identified as the source. The second (much smaller) outbreak in 1994 involved 21 cases, also suffering from gastroenteritis, the pathogen in this case being Small round virus and the source identified as contaminated clams (WHO/UNEP, 1995). In Italy, the possible connection between Hepatitis A and consumption of raw seafood was demonstrated by a radical reduction of total incidence which dropped from 15 per 100,000 in 1984-85 to 5 per 100,000 in 1986 after a rigorous campaign on control of seafood gathering, storage and distribution. In the same period, evidence of a direct connection was also obtained during a Hepatitis A epidemic in Leghorn, where a case-control investigation demonstrated a highly significant relationship between the consumption of raw seafood (mussels and clams) and the disease, as compared to controls (Zampieri, 1989). The relatively high frequency of typhoid fever incidence in the region of Puglia during the months of January and February, in contrast to the traditional summer epidemic curve shown by other southern regions of Italy, has been related to the eating habits in this region, consisting in the indiscriminate consumption of raw seafood during Christmas and the New Year. This is a further confirmation of the direct correlation between infection and seafood consumption, independently of bathing (Zampieri, 1989).

6-30. The large number of sporadic cases, particularly among tourists, is evidenced by the estimate (Stille *et al*, 1972) that as many as 19% of Infectious Hepatitis occurring in Frankfurt were attributable to the consumption of contaminated mussels and oysters in the Mediterranean by German tourists. In addition, recent epidemiological evidence in a number of countries had provided clear evidence that a major contributing factor in the contraction of viral hepatitis was the consumption of raw shellfish harvested from sewage-contaminated coastal waters (WHO/UNEP, 1990), although no specific association with the Mediterranean was recorded in this case. Several other retrospective studies, the results of a selection of which are outlined later on in this chapter, were made on groups of tourists returning from the Mediterranean to their homes in Northern Europe, but in most cases, the source of infection was not identified.

6-31. The link between shellfish water quality and health effects on man as the final consumer is slightly more complex than that applying to bathing water quality. The extent of faecal contamination that can be tolerated in the growing waters is a complex problem because of a variety of factors. There is no constant level of pathogens in sewage, the ratio between indicators and most pathogens varying with every unit volume of waste flowing from the outfall, and a specific level of faeces in wastewater may be relatively free of pathogens at one moment and have a high potential for pathogen transmission through shellfish a moment or so later (Geldreich, 1985). To date, no satisfactory correlation has been established, either between bacterial indicator levels in shellfish and those in the growing waters, or between indicators and pathogens in the shellfish themselves.

6-32. The first problem has been overcome to some extent by assessing the acceptability of marine areas for shellfish cultivation or harvesting by microbiological quality of the shellfish themselves, as distinct from the surrounding water body. Such acceptability of the growing area, as has been stated earlier, does not automatically result in the shellfish themselves being acceptable for human consumption in the raw state, and other filtering mechanisms such as public health and food safety practices come into play. In addition, current epidemiological methods are still not sensitive enough to effectively detect virus disease transmission through the seawater-shellfish route, as clinically-observable illness only occurs in a small number of people who become infected and because of the widely-varying incubation periods before the onset of symptoms (Geldreich, 1985). Furthermore, virus methods are not currently available for all suspect aetiologic agents of gastroenteritis. All this could partially explain the apparently sparse epidemiological evidence on shellfish-transmitted diseases, particularly those caused by viruses, in the Mediterranean.

6-33. One major confounding factor in the interpretation of morbidity data and its association with shellfish contamination which is significant in the Mediterranean is the concurrent exposure of populations to a combination of risk-factors such as contamination of food of non-marine origin and, in certain instances, contamination of drinking water. As a result of this, it can be difficult, in very general terms, to ascribe any particular illness of potential multi-source origin to any specific cause (WHO/UNEP, 1995).

The microbiological quality of shellfish

6-34. The situation regarding shellfish varies throughout the Mediterranean region. At general level, potential health risks arising from shellfish consumption are alleviated by the fact that the three main countries producing shellfish on a large commercial scale (France, Italy and Spain) have strict legislation regarding shellfish destined for human consumption in the raw state, which generally makes depuration mandatory prior to marketing. Artificial

purification of mussels is widely practiced in Europe. One method involves the use of chlorine to disinfect seawater (which must then be dechlorinated before it can be used to depurate contaminated shellfish). Although this method is relatively costly, it is still the method of choice in many depuration facilities in France and Spain (Shumway and Hurst, 1991). Disinfection by ozone is now the depuration method of choice in major shellfish-cleaning stations in France. On the other hand, however, a considerable amount of shellfish all over the Mediterranean are still not subject to strict depuration procedures or proper control of storage after harvest (WHO/UNEP, 1995).

6-35. Drawbacks associated with depuration include variable efficacy, unfeasibility in the case of very heavy bacterial loads, virtual uselessness in reducing heavy metal and hydrocarbon contaminants, lack of control over viral contaminants and, in some cases, economic unfeasibility (Canzonier, 1988). It has also been recorded that during depuration, the water in which shellfish are being decontaminated may show a progressive die-off of released faecal coliforms, but it does not necessarily follow that the rate of releases for all pathogens will follow the same uniform pattern (Geldreich, 1985). Little appears to be known of the depuration of viruses by mussels, and the relatively-sparse data available have (a) demonstrated that elimination of viruses from non-digestive tract tissues is slow, (b) indicated that conventional depuration practices are inappropriate for efficient elimination of viruses from mussels and (c) showed no significant correlation between viral and faecal coliform numbers, supporting the contention of many authors that faecal coliform numbers are unreliable indicators of the presence of human enteroviruses, i.e. the absence of faecal coliforms is not sufficient to ensure the safe consumption of shellfish (Shumway and Hurst, 1991).

6-36. These conclusions are particularly relevant to the Mediterranean situation, as the main bacterial indicator utilized for the acceptability of shellfish waters and, in a number of cases, acceptability of shellfish for consumption, is faecal coliforms. This accentuates the contention which has, in fact, been expressed in relevant meetings of Mediterranean experts both before and since the adoption of common quality criteria for shellfish waters in 1987, that viruses in shellfish represent a particular point of concern in the region (WHO/UNEP, 1995).

6.5 PUBLIC HEALTH IMPLICATIONS

6-37. It is clear from the record that many pathogenic microorganisms (bacterial, fungal and viral) which are the recognized causes of human disease are prevalent in the coastal marine areas of the Mediterranean, with a number of species endemic in various geographical zones. WHO 1985 estimates cite 12 million diarrhoeal cases in the Mediterranean countries of the European region out of a total population of approximately 300 million. In contrast to this, in other European countries, the total was about one million cases out of a total population of 500 million. In the same year, in the Eastern Mediterranean region of WHO, cases of typhoid and paratyphoid fever were estimated at 19.3 million out of a total population of nearly 99 million, comparative estimates for the rest of the region (the Middle East, Pakistan and Afghanistan) being just over 26 million cases out of a total population of 206.5 million (Cvjetanovic, 1989). It is stated that the main reservoir of typhoid infection is a large number of carriers, of some are food handlers in tourist establishments, and the prevalent mode of spread is by food and beverages. The percentages of these cases attributable to seawater ingestion and/or consumption of contaminated seafood is not known, but the potential mode of spread by sewage-polluted seawater and beaches should not be disregarded (Cvjetanovic, 1990).

6-38. As stated earlier in this document, the incidence of the various pathogens cited should not be taken as anything approaching a complete survey of the literature, less so as a comprehensive description of the situation as it actually exists, as much of this situation has still to be recorded. It is equally clear that the current situation is resulting in adverse health effects on both local and tourist populations. Regarding the latter, apart from the attribution of Infectious Hepatitis cases in Germany to consumption of contaminated Mediterranean shellfish (Stille *et al*, 1972), which was mentioned in Part 3 of this document, participants at a 1989 WHO/UNEP consultation on the microbiological pollution of the Mediterranean Sea (WHO/UNEP, 1990) reported a number of adverse health effects on European tourists traveling to the Mediterranean. A Swedish study had revealed that 63% of the *Salmonella* cases reported in that country were the result of infections contracted overseas, mainly in Mediterranean countries. Another Swedish report showed that 90 to 95% of giardiasis, 10% to 16% of viral hepatitis, 34% to 53% of shigellosis and 92% to 95% of Amoebic dysentery were imported cases. In addition, European tourist authorities had estimated that some 40% of tourists on vacation at Mediterranean coastal resorts became ill at some time during or immediately after their visit, one third of these reporting their having been bedridden as a result, and one fifth having been forced to cut their vacation short as a result of such illness.

6-39. It was considered by the meeting referred to above that while, undoubtedly, a portion of such tourist illness was associated with the consumption of unsanitary food or unsafe drinking water, as well as other types of exposure, there was ample evidence that a major source of illness in areas where the sea was polluted resulted from consumption of sewage-contaminated shellfish and/or bathing at sewage-contaminated beaches.

6-40. There are a number of other records linking gastrointestinal diseases contracted by Northern European tourists with travel to Mediterranean countries. The bulk of Swedish tourists go to the Northern shores of the Mediterranean. Since 1978, there has been a considerable increase in the number of salmonellosis and shigellosis cases recorded, 80% of which were infected abroad (Andersson and Böttiger, 1989). More than 75% of the reported cases of salmonellosis in Norway are positively stated to be contracted abroad (Aasen 1989). The whole increase in incidence since 1983 appears to be due to the increase in *Salmonella enteritidis* alone. Data in the Norwegian surveillance system for infectious diseases revealed an outbreak in tourists returning from Mediterranean countries during this period. The same problem has been reported from Finland, where *Salmonella enteritidis* is rarely found in domestic animals or food of animal origin. At least 80% of human infections caused by *Salmonella enteritidis* occur during or shortly after a trip abroad and, since 1883, when the current boom of tourism from Finland to the Mediterranean started, *Salmonella enteritidis* infections caused there have outnumbered infections caused by other serotypes (Jahkola, 1990). The total number of *Salmonella* cases imported into Finland from Mediterranean countries rose from 394 in 1980 to approximately 5000 in 1988. Of these, 97 and 3000 respectively were caused by *Salmonella enteritidis*.

6-41. The results of a 1986 survey for acute diarrhoea among over 19,000 European travellers returning to Switzerland and Germany showed incidence rates varying between 16.0% and 56.2% for those visiting a number of Mediterranean countries (Barua, 1990). A series of four time-independent studies was held between 1986 and 1988 on nearly 3700 Austrian travellers visiting various developing regions of the world. The incidence of travellers' diarrhoea in those visiting the Southern part of the Mediterranean, varying between nearly 25% in Winter to over 50% in summer, was on a par with most other developing regions (Kollaritsch and Wiedermann, 1990). The incidence of traveller's diarrhoea in British package holiday tourists has been monitored since 1983. An analysis of nearly 150,000

questionnaires from tourists visiting Mediterranean countries between April and October 1988 gave an average overall incidence of 3% in April rising to 15% in August and September, then falling to 11% in October. The incidence varied by country and resort, peaks of 64%, 52% and 40% during particular months being recorded in one Northern and two Southern Mediterranean countries respectively.

6-42. While such records provide evidence of occurrence and indications of magnitude, the extent of overall damage to health on a Mediterranean-wide basis, and the proportion contributed by marine pollution to such damage, still have to be determined. The same conclusion can be applied to the situation regarding pathogen incidence and prevalence. In this regard, while the references cited in the present document can be considered as a fairly representative cross-section of relevant literature in the region, the fact that such records, many from different specific locations have a chronological span of one and a half decades, makes it difficult to arrive at any accurate assessment of the overall situation at the present time, although it is obvious that a health risk, the degree of which varies with the particular sub-region, is present. Furthermore, there are still large stretches of the Mediterranean coastal zone mainly in the Southern and Eastern parts, from which records are relatively sparse.

PART 7

CONCLUSIONS

7.1 ANALYSIS OF THE CURRENT MEDITERRANEAN SITUATION

7-1. It is evident that, however much the situation may have improved during the last decade, there are still public health problems regarding both coastal recreational and shellfish areas in the Mediterranean in general. These problems are not uniformly distributed within the region. In this context, while the amount of both microbiological data on seawater and shellfish quality and epidemiological data on health effects has increased considerably, much more data is required, particularly from the Eastern and Southern parts of the Mediterranean, to provide the basis for satisfactory prevention control measures at region-wide level.

Bacterial indicator organisms

7-2. One particular problem concerns the validity or otherwise of the bacterial indicator organisms in current use as an accurate index of water quality. The main aim of quality criteria and standards for recreational waters is to protect the health of the user. In the case of shellfish waters, the aim is to ensure a satisfactory first stage in a multi-stage quality control mechanism designed to protect the health of the consumer of the relative produce. Any index of water quality should therefore be directly health-related, rather than simply concerned with the degree of general pollution by sewage, *i.e.* water quality should be more directly linked to those components of sewage (pathogenic microorganisms), which are responsible for adverse health effects. In this context, bacterial and other indicators (often defined as health effects water quality indicators) are expected to meet certain requirements, which have been outlined by the WHO/UNEP Group of Experts on Health Criteria and Epidemiological studies relating to coastal water pollution (WHO/UNEP, 1977b). Satisfactory indicators must:

be consistently and exclusively associated with the sources of pathogens or noxious substances;

be present in sufficient numbers or quantities to provide an "accurate" density estimate whenever the level of each of the pathogens is such that the risk of illness is unacceptable;

approach the resistance to disinfectants and environmental stress, including that resulting from toxic materials deposited in the aquatic environment, of the most persistent pathogen potentially present at significant levels in the source; and

be quantifiable in recreational waters by reasonably easy and inexpensive methods, and with considerable accuracy, precision and specificity.

7-3. The technical and economic reasons whereby it is difficult to measure pathogenic microorganisms directly on a routine basis, thereby having to assess their presence indirectly through measurements of indicator concentrations, have been described in Part 4 of this document. It is, however, extremely doubtful, to say the least, whether faecal coliforms alone as indicators comply with all, or even most, of the requirements listed in the preceding paragraph. In most epidemiological studies, both within and outside the Mediterranean,

concentrations of faecal streptococci were more closely linked with observed health effects. It would also be relevant to view the conclusions of the two most extensive epidemiological studies, conducted in the USA and, more recently, in the United Kingdom. As a result of the first study, including the recommended health effects criterion therein (Cabelli, 1983), the proposed USEPA standard for marine bathing waters, based on an acceptable swimming-associated gastroenteritis rate of 19 per 1000 swimmers was a limit of 33 enterococci (which form part of the faecal streptococci group) per 100 ml on the geometric mean of not less than 5 samples equally spaced over a 30-day period (USEPA, 1986). On the basis of the criterion, one would expect to find 25-40 cases of gastrointestinal symptoms per 1,000 persons exposed to seawater containing a concentration of 100 enterococci per 100 ml. This figure is not very far removed from the percentages of European tourists with gastrointestinal ailments following visits to the Mediterranean area (as described in Part 6 of this document), although such tourists were also exposed to pollution of non-marine origin. The results of the second study showed that adverse health effects were identified when faecal streptococci concentrations exceeded 32 per 100 ml, this relation being independent of non-water-related predictors of gastroenteritis (Jones *et al*, 1994).

7-4. It has always been acknowledged that no individual indicator is ideal and no indicator system perfect. As has been described earlier in this document, extensive work has been performed in the Mediterranean (and elsewhere) in an attempt to correlate the presence (and density, wherever possible) of pathogenic microorganisms, particularly bacteria and, in some cases, fungi, with concentrations of one or more standard bacterial indicator organisms in the same sample. In practically all the references cited in this document with regard to records of the presence and density of bacterial and fungal pathogens in Mediterranean recreational and shellfish waters, such parallel determination on bacterial indicators were carried out. While a certain number of comparative results obtained have indicated some form of pathogen/indicator correlation, in many cases statistically significant within the framework of individual studies, there is a wide range of variation between the results of different studies, both regarding the question of which particular indicator corresponded best with one or more specific pathogens, and as to what particular concentration-range of the indicator in question represented a minimum level at which the presence of the pathogen was detected.

7-5. With regard to recreational waters, two major difficulties have been pointed out in assessing the risk to health (Pike, 1993). Firstly, apart from bacterial diseases, which are rare, those which are commonly reported by swimmers and appear to be related to faecal contamination are symptomatic and not related to an identifiable pathogen, although appearing to be viral in aetiology, and secondly, currently-used microbial indicators of faecal pollution only indicate that waterborne pathogens may be present and are therefore poorly correlated with the risk to the health of the swimmers. Individual risk will vary with innate and acquired immunity, as well as with pathogenicity. Therefore no constancy of risk can be expected for any given microbiological standard.

7-6. Viruses present a major problem. Apart from the fact that they may survive for considerable periods of time, particularly in association with sediments, it has been shown (Vasi *et al*, 1981) that they can travel substantial distances in the marine environment. The comparative survival of viruses and indicator bacteria in the marine environment has been summarized (Wheeler, 1990) at the request of the United Kingdom House of Commons Environment Committee Inquiry into Pollution of Beaches. The principal conclusion of this summary was that whereas bacteria discharged from a typical long sea outfall might be 'undetectable' in the environment within a few days of their release, enteric viruses might present in an infective state at detectable levels for several months. While this conclusion

was based primarily on conditions prevailing in Northern European waters, it has been confirmed by a number of workers that it also holds good under Mediterranean conditions (*vide* Part 2 of this document), and accentuates the inadequacy of current bacterial indicators as a monitoring tool for pollution by viruses.

7-7. It should be noted that in the recent EC proposal for a new Council Directive on the quality of bathing waters (EEC, 1994b), faecal streptococci have been "upgraded" from a guide to a mandatory parameter. While enteroviruses have been retained, bacteriophages, which are being increasingly considered as a good indicator of the presence of viruses, have been introduced as a new parameter, although numerical standards have still to be decided upon.

Recreational water quality monitoring programmes

7-8. The purpose of monitoring programmes is to provide data for use as a tool in management and decision-making; More specifically, in the case of recreational waters, their results indicate whether beaches are safe for swimming through compliance with established criteria and standards, and, in the case of polluted beaches, contribute towards the identification of actual and potential pollution sources, thus enabling appropriate rehabilitation measures to be implemented. As shown in Part 5 of this document, monitoring results show that the quality of bathing beaches in the four EC Mediterranean Member States has shown a positive trend over the last few years. On the other hand, the pooled monitoring data from thirteen other Mediterranean States submitting their monitoring results to MED POL fail to show any definite trend when taken as a whole. The progress shown in the former group of countries is accentuated by the fact that results are based on three indicator organisms, including both faecal coliforms and faecal streptococci. In addition, although not included in the assessment, determination of the presence of *Salmonella* and enteroviruses is also performed, though to not quite the same degree. Regarding the latter group, a country-by-country assessment, which was precluded in the present document, would have provided a more accurate picture. It is nevertheless evident that bathing water quality in a number of countries has not improved significantly over the last decade, mainly due to lack of control measures at source. In order to safeguard human health, and prevent bathing in unsatisfactory areas until such time as the necessary measures have been implemented, monitoring programmes require a varying degree of upgrading in a substantial part of the Mediterranean.

7-9. In the case of recreational areas, apart from being normally limited to the water body itself, monitoring is generally confined to what can be termed the bathing area proper. This matter now has to be re-considered. Technological developments in the exploitation of the aquatic environment for recreational purposes has also expanded available use options from the traditional bathing and swimming activities to include others such as scuba diving, underwater fishing and wind-surfing, which have both increased the quality coverage necessary in terms of area and depth, and added new dimensions to risk assessment procedures to cope with different exposure patterns among the various recreational groups (Saliba, 1993). Several microbiological hazards have been associated with recreational water sports, although so far, there is only limited evidence of any substantial health risks (Philipp, 1991b). Comparatively little work, however has been done on this subject, and the increasing popularity of water sports along the Mediterranean littoral provides sufficient justification for investigation.

Data quality assurance

7-10. Data quality assurance forms an integral part of any monitoring programme, as decisions have to be taken on the basis of accurate data. This aspect has received particular attention during the course of MED POL Phase II. Because of logistic problems caused by the instability of bacterial samples over extended time-frames, it was not possible to carry out intercalibration exercises in the way normally practiced with chemical samples: *i.e.* to distribute standard samples to Mediterranean laboratories, and then evaluate analytical quality in terms of results obtained. Instead, WHO organized a series of intercalibration exercises in microbiological methods between 1982 and 1993. Each exercise was held in a selected host laboratory, with a number of participants (normally averaging twenty) from different Mediterranean laboratories engaged in seawater and shellfish analysis. Participants determined bacterial concentrations in the same sample under the same laboratory conditions, and interpreted results according to specific instructions. Results obtained were generally variable, much of this variation being attributed to differences in individual experience. The need for training was recognized, and apart from arrangements for such training at individual level, the intercalibration exercises were combined with training courses from 1988 onwards. Guidelines for microbiological monitoring were issued by WHO as early as 1977, and recommended methods for determination of concentrations of individual microbiological parameters in seawater, shellfish and sewage were prepared and issued from 1982 onwards. All this material was recently updated and expanded, and re-issued in the form of comprehensive guidelines for health-related monitoring of coastal recreational and shellfish areas, comprising general guidelines, recommended methods for determination of bacterial indicator organisms and selected bacterial pathogens, statistical methods for interpretation of results, and guidelines for laboratory quality control (WHO/UNEP, 1994c). Widespread use of this publication throughout the region would contribute significantly towards the production of better monitoring results. Although the general quality of Mediterranean laboratories has improved significantly during the past two decades, personnel still require training, and the general efficiency of a number of laboratories calls for improvement, in order to achieve the necessary high quality in results.

Beach quality

7-11. Examination of the water alone, which is the system practiced both in the Mediterranean and elsewhere, is not sufficient to assess actual or potential health hazards. In Mediterranean resorts and bathing beaches, a considerable amount of time is spent on the sand - this is particularly the case with young children. The presence of pathogens in beach sand has been extensively demonstrated (*vide* Part 3 of this document), and a number of non-gastrointestinal infections can be contracted through contact with this particular medium which, so far, is not monitored. In shallow waters, where children normally play, any bacteria contained in bottom sand or sediments will be transferred to the water column immediately above by normal human activity. Apart from this, the whole beach area should be viewed as an integral complex, as quality deficiencies in beach amenities, including catering facilities, showers and toilets may contribute to the overall health hazards to the same degree as poor quality water.

7.12. The aesthetic quality of recreational water and the surrounding environment is extremely important for the psychology and well-being of users. Poor aesthetic quality of recreational waters may also imply poor microbiological/chemical quality. Visible and aesthetically displeasing pollutants include discarded food and its wrappings, domestic garbage, dead fish, birds or other animals, algal scums, chemicals, oil, grease, detergents,

floating objects made of wood or plastic, polythene film, plastic bags, foam products, rubber, glass, and medical and sanitary waste. For this reason, the aesthetic value of recreational waters has been defined as freedom from (a) visible materials that will settle to form objectionable deposits, (b) floating debris, oil, scum and other matter, (c) substances producing objectionable colour, odour, taste or turbidity, and (d) substances and conditions, or combinations thereof, which produce undesirable aquatic life (WHO/UNEP, 1995).

Criteria and standards

7-13. As shown in Part 4 of this document, microbiological standards for both bathing and shellfish waters, apart from being based mainly on concentrations of one or more bacterial indicator organisms, vary to a considerable degree in the different countries. While some can easily be seen as stricter than others, a number are not so comparable. Apart from the standards themselves, as already stated, analytical methods also differ to varying extents, both between the two main methods (Membrane filtration and Most Probable Number) employed, and between the various culture media used. There is also the all-important question of quality control, which also varies between individual laboratories.

7-14. There is, therefore, an obvious need not only to update current monitoring parameters, but also to pursue a more positive trend towards minimizing the present differences in microbiological quality criteria and standards existing between the various countries. Such Harmonization should also be extended to the way the standards are applied and enforced. Complete harmonization would naturally not be possible for a variety of reasons, nor would it be absolutely necessary from the viewpoint of health protection, since two or more different systems may be equally satisfactory. However, a minimization of differences combined with the maximum degree of upgrading possible wherever appropriate would go a long way towards the prevention of undesirable intra-regional and inter-regional comparisons, particularly by tourist organizations.

Shellfish waters and shellfish

7-15. Shellfish waters deserve particular attention, and it is unfortunate that lack of monitoring data on them precludes an assessment both of the current situation and of any progress effected since the 1985 assessment, which apart from only reflecting the position between 1976 and 1981, was based on an insufficient number of areas to justify regional generalization. Although the divergence in standards here is not as pronounced as is the case with recreational waters, reliance on one single bacterial indicator on the basis of only four samplings per year, as is the practice in a number of countries, would not appear to be sufficient for acceptability of a marine area for shellfish-growing, and certainly not for acceptability of shellfish for consumption. This deficiency is offset in many countries by the strict quality standards imposed on shellfish destined for human consumption through public health and related legislation. The general situation should be viewed in the light of the incidence of gastrointestinal diseases and disorders, both among local coastal populations and tourists, which provides cause for concern. Admittedly, overall morbidity statistics in themselves are insufficient, as practically all diseases caused by pathogens are capable of being contracted through media other than the marine environment, and most, in fact, are probably so caused. As an example, during the 14-year period between 1973 and 1986, 3144 *Salmonella* isolates from food were examined by the National Centre for Enterobacteria in Rome and the three Inter-regional Centres in Milan, Palermo and Pisa (Fantasia *et al*, 1989). Foods most frequently contaminated were fresh and processed meat, accounting for

51.3% and 20.4% of the samples respectively. The report does not state what type of food the remainder of the samples represented. Most statistical reports on morbidity are of this overall nature, making it difficult to establish clear cause-effect relationships. There are, however, a number of cases on record, as described earlier in this document, where positive links between disease outbreaks and incidence on the one hand, and contaminated shellfish on the other, were conclusively established.

7-16. The problem of algal biotoxins in shellfish is a relatively recent one in the Mediterranean, and apart from the records mentioned earlier in this document, the general situation still appears to be largely unknown. In this context, more information is required, particularly from areas known to be subject to regular or sporadic eutrophication phenomena. The draft of a comprehensive document on the state of eutrophication of the Mediterranean Sea (UNEP/FAO/WHO, 1995), which has been recently prepared, contains an abundance of material on toxic algae, parts of which have been cited in this document. This, however, only serves to demonstrate the need for further comprehensive field studies in the appropriate areas.

Internal country legislation and administrative organization

7-17. The 1976-1977 survey of Pollutants from land-based sources in the Mediterranean (UNEP/ECE/UNIDO/FAO/UNESCO/WHO/IAEA, 1984) included a review of legislative practices within the region. It was concluded that legal enactments controlling various aspects of marine pollution tended to be dispersed among laws and regulations intended primarily for other purposes, and sometimes the only effective and enforceable control was that available under planning laws. Where, as in some countries, comprehensive water legislation existed, provision for the protection of coastal waters was usually included, the method of control varying largely according to the degree of decentralization practiced. In some countries, the central government laid down fairly detailed standards which were applicable at national level. Alternatively, there could be detailed classification of receiving waters with corresponding effluent standards permitting a restricted measure of local decision. Among the countries with a long history of water resources management, there was a movement away from national standards and a delegation of authority to local agencies, with legislation tending to become more of an enabling nature, leaving the detailed execution as a local responsibility.

7-18. Since that time, a significant degree of consolidation has occurred in environmental pollution prevention and control legislation in most countries, both Mediterranean and otherwise. In the case of recreational and shellfish water quality, this has generally, but not always, resulted in a transfer of responsibility from ministries of health to newly-created ministries of environment, and to recreational and shellfish water quality control becoming an integral part of overall environmental legislation, instead of, as was generally the case previously, public health legislation. This dichotomy, whereby one ministry assumed responsibility for enforcement of such standards, while another obviously had to retain that part of its former responsibility concerning the public health considerations of such enforcement, has resulted, at least in some countries, in a number of inter-ministerial misunderstandings (Saliba, 1993). The fact that control of sewage pollution at source, which is the only effective measure to protect coastal recreational and shellfish waters from microbiological pollution, necessitates the establishment of sewage treatment plants and the construction of submarine outfall structures, which normally fall within the purview of ministries other than those connected with environment or health, only adds to the organizational problem. At the same time, the trend towards harmonization of standards

at international level (as is occurring within the European Community and, independently, in the Mediterranean and other regional seas) has brought about a re-centralization of standards establishment at national level, limiting the role of local bodies to execution.

7-19. One of the consequences of what can be termed organizational misunderstandings in a number of Mediterranean countries is the relative lack of information and data obtained by the Coordinating Unit of the Mediterranean Plan on a number of aspects of marine pollution control organization and programmes which, in turn, is leading to an incomplete, and possibly inaccurate, regional base for both overall and country assessments of coastal recreational and shellfish water quality in the region, and the measures being implemented to protect and enhance it.

7.2 RECOMMENDED ACTION

7-20. A number of immediate measures should be taken by the countries of the region. These actions concern (a) the adoption of more realistic microbiological criteria and standards for coastal recreational water quality, (b) a review of the current criteria and standards for shellfish water quality, with the aim of approving and adopting a revised version more concomitant with health requirements, (c) continuation of the studies necessary to fill gaps in existing essential information, and (d) acceleration of implementation of that part of the 1985 Genoa Declaration concerning sewage treatment plants and outfall structures for cities and towns, to enable the control of sewage pollution at source.

Criteria and standards for coastal recreational areas

7-21. The 1991 assessment on the state of pollution of the Mediterranean Sea by pathogenic microorganisms (UNEP/WHO, 1991), which was adopted by the Contracting Parties (UNEP, 1991) specified in its conclusions that the interim environmental quality criteria for bathing waters jointly adopted by Contracting Parties in 1985 should be clearly recognized as what it actually constituted - a temporary minimum palliative measure based on the immediate capabilities of the lowest common denominator - and that it was important that long-term planning, particularly in the design of treatment and disposal facilities, should not be performed solely on the basis of such criteria. The assessment similarly specified that the eventual requirement would be the development of modified criteria and standards, based on more reliable indicators. It was not recommended that any formal action be taken to amend the current criteria at that particular stage in time, even on a further interim basis, as the global situation regarding the validity of the several bacterial indicators currently in use was in a state of flux, although it did recommend that attempts should be made wherever possible to measure faecal streptococci as well as faecal coliforms, on the basis of either the criteria originally proposed by WHO/UNEP in 1985, or the Guide concentrations in the EEC 1975 bathing water quality Directive, if this could be done without major legislative modifications.

7-22. The current situation is not quite the same. There is now sufficient evidence that the solitary bacterial indicator in use is not satisfactory for the assessment of recreational water quality with a view to avoiding health risks. New quality standards should therefore be considered, based on concentrations of both faecal coliforms *and* faecal streptococci. Both standards should be set at values corresponding either to the present one used for faecal coliforms, or to the EC guide (*not only* the mandatory) values. Selection of the former would mean that in the case of each parameter, 50% of the samples should not exceed 100 colony

forming units (CFU) per 100 ml, and 90% should not exceed 1000 CFU per 100 ml. This would be identical to the original WHO/UNEP proposal in 1985. Selection of the latter as a basis, and translating the lists of non-conformable samples per total number analyzed into a common percentage, would mean that, again in each case, 80% of the samples should not exceed 100 CFU per 100 ml, and 95% should not exceed 2000 CFU (faecal coliforms) and 400 CFU (faecal streptococci) per 100 ml. There is no overall effective difference between the two alternatives, although specific local circumstances could make either the one or the other more stringent for particular beaches. Considering the fact that two major epidemiological studies conducted in different areas and time-periods, both resulted in adverse health effects being observed when the concentration of faecal streptococci rose slightly above 30 CFU per 100 ml, neither alternative could in any way be described as unnecessarily strict. The frequency should be more fully specified, and set at a fortnightly minimum throughout the bathing season, possibly coupled with an annual minimum of ten samplings.

7-23. Enteroviruses should really be introduced as a parameter on the lines of the EC Directive (zero in 10 litres, sampled at monthly intervals). This could raise problems of a technical nature in a number of countries whose laboratories are not equipped for such work. Viruses, however, represent the major current risk in bathing waters due to their persistence and low infective dose, and their presence does not correlate with that of bacterial indicators.. Their importance in other matrices (*i.e.* outside the marine environment) warrants the presence of at least one fully-equipped laboratory, per country, and monthly samples throughout the bathing season would entail four to five samples per year per sampling point. Initial difficulties could be overcome either by specifying a later entry into force of the enterovirus component of the new standards, or by limiting enterovirus analysis to those circumstances when and where their presence is suspected. Monitoring would therefore be at the discretion of national or local health authorities.

7-24. The status of *Salmonella* in the Mediterranean region as a whole is more than sufficient to justify its introduction as a mandatory monitoring parameter, possibly on the lines of the 1975 EC Directive (zero in 1 litre when there are indications that it may be present). It would be up to national authorities to decide on the specific times such analysis should be carried out. This should not present any major technical difficulties, as *Salmonella* is the pathogen most routinely monitored in other media, such as food and drinking water, in all Mediterranean countries. Alternatively, the procedure proposed for adoption in the new EC Directive could be considered as a base. This obliges national competent authorities to identify all discharges which might lead to *Salmonella* reaching bathing areas, and to take appropriate action to avoid pollution from such sources. In this context, it should be pointed out that the EC decision to change the status of *Salmonella* was based on arguments that, in common with enteroviruses, it can enter bathing waters by a number of routes, not all of which are controllable by Member States, and the ubiquitous nature of the parameter precluded any action which could guarantee compliance. It is considered that the status of *Salmonella* in the Mediterranean is not quite the same as that prevailing in Europe (talking each region globally), and it is therefore doubtful whether the latter alternative would solve the problem, even if the general proviso on prohibition of bathing when there is a threat to public health (which is contained in Article 7 (1) of the EC Directive) is adopted for the Mediterranean. The main method of identifying threats to public health is, after all, through monitoring of the appropriate media. Perhaps the same procedure as for enteroviruses, *i.e.* monitoring at the discretion of national or local health authorities when the presence of *Salmonella* is suspected, could be initially adopted.

Data quality assurance

7-25. Since 1994, training courses and intercalibration exercises in microbiological methods are being organized at national level, with financial and related assistance from MED POL. While these courses should continue, arrangements will have to be made to cater for those countries where the number of laboratories and personnel is so small as to preclude the organization of national courses at a sufficiently high level of participation. This could be achieved by combining participants from two to three countries in one course. The traditional "international" exercises should not be stopped altogether. These exercises were the only events which not only enabled comparison between different laboratories, but also brought participants from different countries together in a working environment particularly favourable for the discussion of common problems.

7-26. The financial resources available to MED POL only allow relatively short-term training at both individual and group level. The only training possible is therefore of the familiarization type, i.e. designed for participants already having a knowledge of the relevant procedures and requiring mainly updating. The necessary long-term training in microbiological methods will have to continue to be borne by countries out of their own resources. The cost of such training, as well as the costs of equipping and maintaining laboratories, would be very much reduced if, wherever possible, the relevant work is allocated to already-existing microbiological laboratories within the countries, such as those engaged in the analysis of drinking water, even if such laboratories belong to Ministries or other national or local bodies different from those responsible for bathing and shellfish water quality.

Bathing areas and beach quality

7-27. The 1985 Mediterranean resolution incorporating the interim environmental quality criteria for bathing waters does not include a definition of bathing areas. This should be done, and the definition in Article 1 of the EC Directive could possibly be adopted. Mediterranean States should similarly be asked to identify bathing areas within their territories in line with such definition, and provide lists of such areas to the Mediterranean Action Plan Secretariat.

7-28. As has been described, there are a number of non-gastrointestinal diseases associated with bathing which appear to be independent of the degree of sewage pollution of recreational waters. The evidence available from a number of studies shows that incidence of such cases may be due to already-infected individuals contaminating the water, coupled with the over-crowded nature of a number of beaches. There is obviously no measure regarding the former, but the feasibility of controlling overcrowding on beaches, possibly by extension of beach area availability, could be studied in the various countries having this problem. The matter of general beach hygiene, as described in paragraphs 7-10 and 7-11, should be studied with a view to possible remedial action.

Criteria and standards for shellfish waters

7-29. The 1987 criteria and standards for shellfish waters are, as already stated, based on the monitoring of one bacterial indicator (faecal coliforms) at quarterly intervals. On the one hand, it could be argued that this represents only a preliminary screening phase solely aimed at acceptability of the particular water body as a growing medium, and that there is a much stricter control procedure, including both depuration as necessary and further analysis on a

wider range of microbiological parameters, during the period between harvest and sale for consumption. On the other hand, as has already been stated earlier in this document, reliance on one bacterial parameter at infrequent intervals cannot be viewed as sufficient, even for area acceptability, and there is at least one Mediterranean country in which compliance with these standards also constitutes acceptability of the shellfish for consumption without further control procedures. Moreover, while the bulk of commercially produced shellfish pass through sanitary control procedures after harvesting, very little information is available as to what quantity of shellfish are harvested from naturally-growing areas, and what proportion of this by-passes the normal control procedure between gathering and consumption.

7-30. It is not considered that any specific immediate action in the form of revised standards for shellfish waters is indicated at this particular stage in time, but there is the immediate need for this matter to be studied further to determine whether the monitoring parameters should be increased and, if so, what additional parameters should be introduced, and to look into the matter of sampling frequency. A specific resolution by the Contracting Parties on such a study should be developed and adopted at the earliest opportunity. In the same resolution, Contracting Parties should, as interim measures pending the results of such a study, pledge (a) to monitor for any additional parameters whenever this is considered necessary on public health grounds, (b) to identify natural areas in which shellfish grow and are harvested, and to ensure that such areas are monitored in accordance with the provisions of the currently-existing quality criteria, and (c) as is already the practice in some countries, to prohibit the sale of shellfish from non-approved areas, and to warn the public of the risks involved in the consumption of raw shellfish collected at individual, as opposed to commercial, level from such areas.

Microbiological and epidemiological research

7-31. Countries should encourage the following lines of research, both within the framework of MED POL and otherwise:

Microbiological surveys to satisfy the requirement for a more comprehensive catalogue of the presence and (where feasible) density of pathogenic microorganisms in sewage effluents and in those marine areas (recreational and shellfish-growing) known to be affected by such effluents. This would provide essential data for the design of new sewage treatment facilities and outfall structures in such localities, and for any possible modifications required in the case of existing ones.

Surveys on the aesthetic and hygienic quality of beaches to determine what improvements are required on a case-by-case basis. Within this framework, analysis of sand samples from major beaches for bacteria and fungi should be performed as a baseline study. The results of such a study would determine whether health risks from polluted sand and sediments are such as to require criteria and standards for these media at a future date.

Epidemiological studies on the correlation between recreational water quality and observed health effects on exposed population groups, covering the main water sport activity areas (including bathing). This will provide a useful index as to the effectiveness or otherwise of prevailing quality standards.

Epidemiological studies correlating the incidence of specified diseases and disorders with beach overcrowding.

Epidemiological studies and surveys aimed at identifying the extent to which contaminated seafood (as opposed to other sources) is responsible for the incidence of gastrointestinal diseases and disorders in both local populations and tourists.

Microbiological studies correlating the density of bacterial indicator organisms with the presence and density of pathogens such as *Salmonella*. As this correlation will vary in each locality according to the proportion of diseased individuals and carriers in the community, studies will have to be performed in a number of localities. The results will provide a useful indication as to when the determination of additional parameters in any particular locality would be necessary for a more accurate determination of health risks.

Microbiological studies that will try to interpret the real pathogenic significance of pathogens such as *Salmonella* and enteroviruses in seawater samples.

Investigations on the presence and density of pathogenic microorganisms, particularly viruses, in specimens collected from shellfish waters considered as acceptable in terms of current criteria and standards, and in samples of shellfish following depuration, with the objectives of reviewing the efficacy of depuration techniques in use, and the eventual establishment of criteria for viruses.

Studies aimed at enhancing current microbiological methods for the determination of concentrations of pathogens and indicator organisms under Mediterranean conditions, including the development of less expensive techniques for determination of viruses.

Studies on the survival and adaptation of pathogenic and indicator microorganisms released into the Mediterranean marine environment, including the mechanisms responsible for change.

Studies to extend the present data base on toxic algal blooms in those areas in the Mediterranean subject to regular or sporadic eutrophication.

Control of sewage pollution at source

7-32. In view of the fact that, in several parts of the Mediterranean, municipal sewage is still being discharged to sea in a raw or partially-treated state, countries should accelerate, to the fullest degree possible, the establishment of sewage treatment plants and the construction of submarine outfall structures in accordance with the terms of the 1985 Genoa Declaration. This is essential for the rehabilitation of bathing and shellfish areas currently affected by sewage pollution.

Internal country organization and provision of information

7-33. Mediterranean countries should enhance, as necessary, their relevant marine pollution prevention and control mechanisms (including the improvement of internal organization and inter-ministerial liaison where there is divided responsibility), in order to prevent duplication

of effort, reconcile divergent interests, and enable enforcement of standards adopted as effectively as possible. Countries should also ensure that all relevant details of their legislation, programmes, and measures taken in accordance with the provisions of the Barcelona Convention and Protocols, as well as any Resolution adopted in terms of such Convention and Protocols, are submitted to the Mediterranean Action Plan Secretariat, as provided by the terms of the said Convention and Protocols.

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