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ASSESSMENT OF THE STATE OF POLLUTION OF THE MEDITERRANEAN SEA BY PATHOGENIC MICROORGANISMS

In cooperation with

WORLD HEALTH ORGANIZATION

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CONTENTS

BACKGROUND

1. Article 8 of the Convention for the Protection of the Mediterranean Sea against Pollution, adopted by the coastal states of the region in Barcelona on 16 February 1976, stipulates that Contracting Parties shall take all appropriate measures to prevent, abate and combat pollution of the Mediterranean Sea Area caused by discharges from rivers, coastal establishments or outfalls, or emanating from any other land-based sources within their territories (UNEP, 1982).

2. In conformity with the provisions of this article and others of a more general nature contained in the Convention, Mediterranean Coastal states adopted the Protocol for the Protection of the Mediterranean Sea against Pollution from land-based sources in Athens on 17 May 1980.

3. Article 6 of the Protocol stipulates that Contracting Parties shall strictly limit pollution from land-based sources in the Protocol area by substances or sources listed in Annex II to the Protocol and, to this end, shall elaborate and implement, jointly or individually as appropriate, suitable programmes and measures. The same article also stipulates that discharges shall be strictly subject to the issue, by the competent national authorities, of an authorization taking due account of the provisions of Annex III to the Protocol. Pathogenic micro-organisms constitute one of the items listed in Annex II to the Protocol.

4. Article 7 of the Protocol stipulates that Contracting Parties shall progressively formulate and adopt, in cooperation with the competent international organizations, common guidelines and, as appropriate, standards or criteria dealing in particular with, *inter alia*, the quality of seawater used for specific purposes that is necessary for the protection of human health, living resources and ecosystems.

5. The 1976 Barcelona Convention entered into force on 12 February 1978 and has been ratified by all eighteen Mediterranean coastal states and by the European Economic community, the last ratification being on 29 June 1990. The 1980 Athens Protocol entered into force on 17 June 1983 and, as of 31 December 1990, has been ratified by fifteen Mediterranean Coastal states and by the European Economic Community.

6. At their Fourth Ordinary Meeting, held in Genoa from 9 to 13 September 1985, Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its related Protocols agreed that, with regard to the technical implementation of the Protocol for the Protection of the Mediterranean Sea against pollution from land-based sources, the Secretariat to the Convention and Protocols would propose an order of priority and a realistic time-table for the development of programmes and measures for at least two substances annually, including common emission standards and standards of use, required for the implementation of the Protocol, and that in preparing such a proposal, substances listed in Annex I to the Protocol should be accorded priority. Contracting Parties also agreed, however, that an exception should be made in the case of pathogenic micro-organisms, which should be accorded the same degree of priority as Annex I substances. At the same meeting, Contracting Parties adopted joint interim criteria for coastal recreational waters, based on concentrations of one bacterial indicator organism (faecal coliforms). At the same time, they agreed that studies should be undertaken on the comparison of these interim criteria with those already in force in Mediterranean countries, and also that epidemiological and other studies within the framework of the Long-term Programme on Pollution Monitoring and Research in the Mediterranean Sea should continue (UNEP, 1985a).

7. A meeting of experts on the technical implementation of the Protocol for the Protection of the Mediterranean Sea against Pollution from land-based sources was convened by UNEP in Athens from 9 to 13 December 1985. The meeting approved a workplan and time-table for the progressive implementation of the Protocol (UNEP, 1985b), which included the phased preparation of assessments of the state of pollution of the Mediterranean Sea by the substances listed in Annexes I and II to the Protocol, together with proposed control measures on the basis of such assessments. It was agreed that assessment documents should include, *inter alia*, chapters on :

- sources, points of entry and amounts of pollution from industrial, municipal and other discharges into the Mediterranean Sea;
- levels of pollution;
- effects of pollution;
- present legal, administrative and technical measures at national and international level.

8. At their Fifth Ordinary meeting in Athens in September 1987, Contracting Parties adopted environmental quality criteria for shellfish waters. These criteria, which are identical to the relevant EEC Directive on the subject, are also based on faecal coliform concentrations (UNEP, 1987). In this case, it was understood that the scope of the resolution in question was only designed to cater for acceptability of marine areas for shellfish growing and/or harvesting, and did not in any way intrude on acceptability of the shellfish themselves for human consumption, which aspect would continue to be handled by *ad hoc* public health or related legislation in the various countries (WHO, 1989).

9. The present document, which has been prepared by the World Health Organization within the framework of the workplan and time-table detailed above, attempts to provide an assessment of the state of pollution of the Mediterranean Sea by pathogenic micro-organisms on the basis of information available to date, outlines prevention and control measures in force at the present time, and proposes short-term and long-term action which could be taken by Mediterranean states within the framework of the Protocol for the Protection of the Mediterranean Sea against Pollution from land-based sources to further alleviate the situation.

INTRODUCTION

10. The main types of human exposure to pathogenic micro-organisms in the marine environment are through direct contact with polluted seawater and/or sand, including ingestion of the former while swimming or bathing, and through consumption of contaminated seafood.

11. Concern about actual and potential adverse health effects arising out of such exposure has been expressed worldwide, particularly during the last two decades. In the case of shellfish, this has led to the progressive development of various quality criteria and standards not only for shellfish themselves within the framework of public health and food quality legislation, but also for the growing and harvesting waters themselves. Apart from the variation between statutory requirements in the different countries, doubts have also been raised 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 epidemiological studies aimed at establishing 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).

12. Health hazards arising from the presence of pathogenic micro-organisms in the Mediterranean marine environment can be considered as particularly significant as a result of a heterogeneous variety of factors, including the following (WHO, 1989):

(a) 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 currently being discharged untreated into the immediate marine coastal zone, in many instances in the vicinity of recreational and/or shellfish areas.

(b) 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 week-ends. The heterogeneous nature of beach populations further facilitates the spread of infections.

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

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

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

13. The situation outlined in 11(e) above is global, rather than Mediterranean, in character. It does however constitute an accentuated risk factor in the region when considered in combination with the other specifically-Mediterranean factors. The same holds good regarding epidemiological studies correlating recreational water quality with health effects, which have been conducted in various countries of the region over the past two decades. These studies were essentially based on internationally-recognized protocols utilized for studies outside the region, but were relatively small-scale. To a variable extent, all were handicapped, particularly in the interpretation of results, by a number of confounding factors over which satisfactory control has been found difficult to achieve.

14. Practically all Mediterranean states have now established programmes to monitor the microbiological and related quality of their bathing beaches. These vary to a significant extent in scope and infrastructural support and, moreover, some countries having relatively long-standing and comprehensive programmes, others with such programmes still in the early stages of development or implementation. Considerable attempts have been made, and are still being made, by WHO and UNEP, within the framework of the Long-term Programme of Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase II), to provide as much assistance as possible in the form of equipment and training to laboratories in the region, particularly those in developing countries, to bring them up to adequate levels of capability in the performance of routine microbiological monitoring. Although the degree of such assistance can only be described as catalytic, it has contributed in no small way to the remarkable progress effected during the last decade.

15. In line with the activities approved by Mediterranean States for the research component of the MED POL Phase II Programme at the Second Ordinary Meeting of Contracting Parties in Cannes in March 1981 (UNEP, 1983), and with subsequent resolutions of the Contracting Parties concerning recreational and shellfish waters, WHO has been organizing studies on the development of methodology suitable for determination of concentrations of selected pathogenic and indicator microorganisms (including pathogen/indicator relationships) in various marine and related matrices under specific Mediterranean conditions, on factors affecting the survival and adaptation of pathogens, and, in particular, microbiological/epidemiological studies on the correlation between coastal water and sand quality and health effects (WHO/UNEP, 1990). An outline of the results of these and other relevant studies to date, together with their contribution to

an assessment of the state of pollution of the Mediterranean Sea by pathogenic microorganisms, is provided in the appropriate sections of this document.

ENTRY OF PATHOGENIC MICROORGANISMS INTO THE MARINE ENVIRONMENT

Sources and inputs

16. Pathogenic micro-organisms enter the marine environment mainly through municipal wastewater discharges. The results of a pilot project on pollutants from land-based sources in the Mediterranean (UNEP/ECE/UNIDO/FAO/UNESCO/WHO/IAEA, 1983), carried out in 1977 within the framework of the Joint Coordinated Mediterranean Pollution Monitoring and Research Programme (MED POL Phase I), provided an estimate of approximately $2x10^9$ 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 420 x 10^9 m^3 /year of fresh water 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 by microorganisms (pathogenic and otherwise) has not been assessed, and it has been assumed that the high concentration of microorganisms in wastewater discharges directly ink coastal waters makes such discharges the major source of microbiological pollution reaching the Mediterranean Sea (UNEP/WHO, 1985). An updated survey is currently under way, but results are not expected to be available before 1992. Considering the increase in coastal development over the past decade, one would expect an increase in the total amount of wastewater discharged since the last survey.

17. The atmosphere may serve as a pathway for the entry of pathogenic microorganisms into the coastal marine environment. Brisou (1976) states 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 source indicated is bathers themselves. Shuval (1986) states that recreational waters not receiving sewage effluent 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. Thus, bathing waters so 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. Results of epidemiological and related studies directed at this specific point are outlined in the appropriate section of this document. 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 micro-organisms is a subject which calls for serious consideration.

18. Untreated or inadequately-treated sewage remains the main factor of concern, and disposal of this into the immediate coastal marine environment through relatively short outfalls, in many cases at the coastline itself, explains the low dilution and dispersion in the receiving seawater and the consequent adverse effects produced in the areas near the points of discharge (UNEP/WHO, 1985).

19. Apart from pathogenic microorganisms (bacteria, viruses and fungi) discharged into the marine environment through sewage effluents or other terrestrial sources, another group of naturally-occuring marine microorganisms, which can be considered to be pathogenic through their ability to produce various toxins, to which man is exposed mainly through shellfish consumption, can pose a similar threat to health when present in large numbers. These microorganisms, mainly dinoflagellate algae, constitute a phenomenon termed an algal bloom or red tide when their concentration in sea water reach levels of 10^4 to 10^6 cells/litre. While the environmental conditions under which these microorganisms are able to reproduce asexually at high rates does not appear to have been fully elucidated, the fact that red tides are essentially a coastal phenomena, land drainage has been considered as playing a role in their initiation (WHO, 1984). It has also been demonstrated that the size of algal blooms is in proportion to the magnitude of incoming nutrient masses through rivers (WHO, 1991). In a recent review (Shumway, 1990), 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. The same author states that it has also 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.

20. During the Fourth Ordinary meeting of the Contracting Parties in Genoa in 1985, Mediterranean States adopted a Declaration (subsequently known as the Genoa Declaration) wherein they committed themselves to a number of environmental targets to be achieve within the next decade of operation (1986-1995) of the Mediterranean Action Plan. These targets include 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.

21. Since the adoption of the Genoa declaration in September 1985, the number of municipal sewage treatment plants and submarine outfall structures conveying the effluents out to sea has increased, and more are in the planning stage. The Mediterranean as a whole, however, is still far from a satisfactory overall remedy to the situation, particularly as regards those specific areas where algal blooms are known to occur, and for which preventive or remedial measures at source are more complex.

Survival and fate

22. Over the past century, many studies have been carried out to estimate the fate of enteric or non-enteric pathogenic microorganisms (viruses, bacteria, fungi and protozoa) in estuaries and seawater through both field and laboratory observations and experiments. 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 stated that it could be expected that concentrations of the three major groups of indicator bacteria normally utilized for determining the state of seawater pollution by sewage (total coliforms, faecal coliforms and faecal streptococci) would not remain unaltered in the receiving seawaters but would rather 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 a subject of considerable debate and of continuing research.

23. Results from field studies carried out during the pilot project on Coastal Water Quality Control (MED VII) between 1976 and 1980, within the framework of the Joint Coordinated Mediterranean Pollution Monitoring and Research Programme (WHO/UNEP, 1981), and elsewhere, pointed out the different survival patterns of the three bacterial indicator organisms mentioned in the preceding paragraph. 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 percentual reduction.

24. Microorganisms discharged into seawater are rapidly absorbed 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 organisms per unit of volume (Brisou, 1976). these adsorbants are diluted, dispersed, flocculated, sedimented or carried back towards the coasts. The physicochemical process of flocculation of microbial cells and their subsequent sedimentation to the sea bottom are considered as the mechanism responsible for the microbiological enrichment of sediments in the areas surrounding wastewater discharges (Mitchell, 1975). Natural turbulence and sea currents can be considered as a plausible mechanism by which contaminated sediments can be resuspended, with the consequent impairment of the microbiological quality of the overlying waters (Volterra, 1980; Velescu, 1982).

25. Most research on the survival of microorganisms discharged into the marine environment

in sewage effluent has concentrated on bacterial indicator organisms. Information on pathogens is comparatively sparse. The general situation regarding the fate of pathogenic microorganisms in the marine environment has been reviewed by a WHO Working Group on the Health impact of human exposure to fresh and saline recreational waters, held in Rimini in February/March, 1990. The information provided in the next ten paragraphs is essentially based on the relevant parts of the meeting report (WHO, 1991), except where other references are specifically cited.

26. The ability of allochtonous microorganisms to survive in marine natural environments largely varies, according to the compartment colonized (i.e. water, sediment or animal intestinal organs and body tissues). In seawater, both field and laboratory studies have shown that light, salinity, temperature and nutrient availability are the most important parameters that affect viability, and in particular survival, of microbial pathogens of human origin. Light appears to have the highest motivating potential. Its lethal effect increases with intensity: the T90 values (time taken for a 90% reduction of bacterial counts) for bacteria are generally 50 to 100-fold lower in the light than in the dark. Furthermore, light acts synergistically with salinity and, to some extent, temperature. Maximal lethal action is observed in water with high solar irradiation, high salinity and high temperature. Large variations in T90 values for enteric pathogens are observed in different marine areas. These differences are attributable to very different environmental conditions. The effect of temperature depends more 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 temperature. *Vibrio* species *(V. cholerae, V. alginolyticus, V. parahaemolyticus)* are very sensitive to low temperatures; they generally become undetectable in marine environments at temperatures below 15-18 ° C.

27. Organic substances from sewage are also important factors affecting the survival of pathogens since they can obviously favour their growth. This is, of course, not the case for strict parasites such as viruses.

28. However, these physicochemical 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 (microorganisms, plants or animals) is possible. However, 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.

29. Furthermore, both *in situ* experiments and laboratory studies have shown the antagonistic activity of marine organisms on microbial pathogens. Many marine animals use the latter, together with the autochtonous microflora, as a source of nutrients, and a large number of marine organisms (bacteria, fungi, algae, invertebrates) produce antibiotic or lytic substances that can inhibit or destroy telluric bacteria and viruses. Once can assume that such antagonistic activity through allelopathic products could be higher in eutrophic waters or in sediments.

30. Whatever the source of the antagonistic processes, both pathogenic viruses (enteric viruses, polio virus, hepatitis A virus) and fungi *(candida albicans)* survive for longer periods of time in seawater than bacteria.

31. The mechanisms responsible for the evolution and disappearance of allochtonous 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 telluric 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.

32. This regulatory activity can be considered an adaptative 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 allochtonous 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 (Gauthier, 1990) 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.

33. 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 telluric bacteria.

34. Besides these "classical" considerations, account should also be taken of some recent findings that have followed the development of direct microscopic methods that allow the enumeration of viable bacterial cells. Colwell and co-workers at the University of Maryland have stated that bacterial pathogens can evolve toward a *viable but not culturable* state in seawater under which they could retain infectivity and virulence. This could be considered as an evolution to dormancy. Although this would have to be confirmed with several bacterial pathogens, one can assume that such viable non-culturable cells remain potentially virulent to humans. However, this evolution to dormancy appears to be mainly tied to nutrient starvation; it could be precluded in the case of eutrophicated waters.

35. 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 autochtonous pathogens may be present in seawater and may overgrow under such abnormal conditions.

36. 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. Uptake of viruses by shellfish has been clearly demonstrated and, like bacteria, the majority of viruses are concentrated in the digestive system of the host. Once inside a shellfish, the survival of viruses appears to be further prolonged (Metcalf and Stiles, 1965).

37. 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). 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 *E.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 *E. 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.

PATHOGENIC MICROORGANISMS IN THE MEDITERRANEAN MARINE ENVIRONMENT

General considerations

38. With very few exceptions, estimation of the presence of pathogenic microorganisms in marine recreational and shellfish water pollution monitoring programmes continue to rely largely on concentrations of one or more bacterial indicator organisms as an index of acceptability or otherwise. Among the exceptions is the EEC 1975 Directive on bathing water Quality (EEC, 1976) which 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. Since bacterial indicator concentrations provide a measure of the degree of total faecal pollution, while the concentration of pathogens in wastewaters is a function of the number of ill or subclinical cases excreting the specific pathogen organisms in questions, 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 by the facts that (a) no single procedure is available that can be used to isolate and identify all pathogens, (b) negative findings for specific pathogens could only be considered provisional because state-of-the-act methodology is not sufficiently sensitive to detect a level of 1 pathogen in the volumes of seawater (100 ml) normally used for indicators, and (c) even in the case of what is probably the most ubiquitous organism, *Salmonella,* isolation techniques involve relatively complicated procedures that exceed the capabilities of many routine-type laboratories. In this case of viruses, monitoring can only be carried out in relatively well-equipped laboratories and, more often than not, as part of special research projects and other related studies.

39. 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. Overall morbidity statistics in themselves are insufficient, as practically all diseases caused by pathogens are capable of being contracted through pathways other than the marine environment. There have been, however, cases where such links were conclusively established, particularly in the case of shellfish.

40. On a very general level, pathogenic microorganisms 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 dyssentery), cholera and gastroenteritis caused by enteropathogenic *E. 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 dyssentery, giardiasis, ascariasis, etc.

41. Insofar as the actual ingestion of water during swimming or bathing is necessarily limited, with the exception of pathogens with a relatively low infective dose, the diseases mentioned above can be contracted more easily through the consumption of raw or partially-cooked fish or shellfish. Other pathogens reported (Wood, 1976) as causing human infections through this 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.

42. 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.

43. Apart from diseases affecting the gastrointestinal tract, a number of diseases or disorders affecting the eye, ear, skin, 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 may cause infection as a result of 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, 1985). 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 four species mentioned may also be found in polluted water, 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 organism beforehand, and the disease may be largely determined by the individual's susceptibility, rather than by exposure to the organisms in the marine environment.

44. A brief resumé of available data on individual pathogens in the Mediterranean marine environment, is given in the succeeding paragraphs of this section. These data are designed to provide a general overview with specific examples, rather than an attempt to compile all the individual records in the research literature.

Bacterial pathogens

45. *Salmonella* species, which are the agents of typhoid and paratyphoid fevers, food poisoning and gastroenteritis, have a worldwide distribution and are abundantly represented in the Mediterranean. 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 *S. typhi, S. paratyphi* A and *S. 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 Yugoslavia (Brisou, 1976). In a 3-year survey on *Salmonella* pollution of coastal sea waters 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 *Salmonella* strains have been isolated from sewage effluents in Alexandria (El-Sharkawi *et al,* 1982). Salmonellas do not survive long in seawater, and infection directly as a result of bathing or other recreational activities is not very likely 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 concentration may be 50 times that in water (UNEP/WHO/IAEA, 1988).

46. *Shigella* species, which are the agents of bacillary dyssentery, are also, like *Salmonella,* widely distributed throughout the world. *Shigella* is endemic on the Eastern and Southern shores

of the Mediterranean (Brisou, 1976). Cases reported from the region call for a more comprehensive assessment of the situation than has hitherto been undertaken, and statistics on the occurrence of the species in sewage should be the first step in correlating this with cases of dyssentery (UNEP/WHO/IAEA, 1987). These microorganisms are reported by a number of authors as having a relatively short time in the marine environment. However, it has been reported (El-Sharkawi, 1986) that no major differences in survival time in seawater was found for *Shigella flexneri* as compared to *Salmonella typhi, S. wien* and *Escherichia coli.*

47. Cholera is one of the major diseases associated with the consumption of sewage-contaminated shellfish, and the causative agent, *Vibrio cholera*, was discovered by Koch in Egypt during the 1883-84 epidemic. Cases have been reported in various parts of the Mediterranean, including France, Spain, Algeria and Morocco (Brisou, 1976) and a major outbreak, in which mussels were identified as the aetiological agents, occurred in Italy in 1973 (Baine *et al,* 1974). *Vibrio cholerae* non -01 was isolated from two samples of seawaters in bathing areas in the North Adriatic during a 1989 survey (Maini *et al,* 1990). Brisou *et al* (1962) isolated 44 strains of vibrios from the Algerian coast, only some of which are considered pathogenic, but there is clear evidence that some strains of naturally-resident aquatic bacteria are capable of causing gastroenteritis, systemic infections and intoxications in man (Shumway and Hurst, 1991). NAG (non-agglutinable) vibrios, which cause gastroenteritis, are also frequently found in shellfish in the region. As in the case of the Cholera vibrio, they are discharged through sewage-effluents and infection is most likely to occur through consumption of polluted shellfish. A total of 214 *Vibrio* serotypes were analyzed and identified during a recent study in Toulon (Martin and Bonnefont, 1990), comprising effluent, seawater and mussel samples. The effluent population was the most diversified, including several species of sanitary interest such as *V. fluvialis, V. cholerae* (non-01) and *V. metschnikovii,* which three, however,
were not found in seawater or mussels.

48. 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 *V. parahaemolyticus,* the main cause of infection is again through shellfish (UNEP/WHO/IAEA, 1988), though wound infection by contact with seawater is another route of transmission (WHO, 1982). *Vibrio alginolyticus* causes otitis, sore throat, and wound infections. It occurs in coastal marine areas and its main route of transmission to man is through contact with seawater and sediments. Isolations of *Vibrio parahaemolyticus* and, to a lesser extent, *V. 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. Volterra (1989) reports relative concentration of *V. parahaemolyticus* of 10^3 in mussels *(Mytilus galloprovincialis),* 10^4 in *Ensis siliqua minor* and 10^5 in *Chamela gallina,* as compared to 10^1 in seawater. The study was conducted in various mollusc beds located along the Tyrrhenian coast. In a total of 165 samples collected in the neighbourhood of Alexandria in 1979/1980, average counts of *V. parahaemolyticus* per 100 ml or 100 grams were 36 for seawater, 349 for *Echinus,* 436 for sediments, 534 for *Tapes* and 1872 for *Donax trunculus.* Samples collected during summer contained higher levels than those collected during winter (El-Sahn *et al,* 1982). *V. parahaemolyticus* and *V. alginolyticus* were also isolated from samples of seawater in North Adriatic bathing areas in 1989 (Maine *et al,* 1990), and the latter species was found to be the prevalent one in seawater and mussels in the Toulon survey (Martin and Bonnefont, 1990). Levels of *Vibrio* species recorded in seawater during an epidemiological study on two spanish beaches gave counts of 100-2800 per 100 ml for the less polluted beach, and 250-12000 for the more polluted one (Borrego *et al,* 1988). No distinction between species was made.

49. *Staphylococcus aureus* and related species (particularly *S. 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 coagulate-positive strains case 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 *S. aureus,* as well as others affecting the skin and naso-pharyngeal tract, are suspected of being transmitted by bathing water (WHO, 1982). The

origin of the pathogen in seawater is attributed to human activity, as all strains were found to be shed by bathers under all conditions of swimming (Robinson and Mood, 1966). In 628 samples of coastal water monitored in Israel, 60.7% contained *S. aureus.* A break-up revealed a range of 49.5% in the less populated beaches load (Yoshpe-Purer, 1987). A comparison between concentrations of *S. aureus* in the seawater and sand of heavily-populated and slightly-populated beaches in Greece (Papadakis *et al*, 1989) showed positive readings in all samples. While concentrations in sea water between beaches were not significantly different, considerably higher concentrations were found in the sand of highly-populated beaches. *S. aureus* was not isolated from seawater and sand on beaches in Spain during a study conducted in 1988 (Borrego *et al*, 1988), but the authors confirm its presence in previous surveys.

50. Although *Staphylococcus aureus* is linked with food-poisoning in general, mention of transmission of this through shellfish is relatively sparse. In Egypt, shellfish taken from highly-polluted water have been considered as of unacceptable quality due to the presence of pathogenic microorganisms, which includes *S. aureus* (El-Sharkawi *et al*, 1982). In France, the microbiological standards relevant to shellfish for human consumption include a maximum acceptable limit of 100 *S. aureus* per gram (UNEP/WHO, 1987).

51. *Pseudomonas aeruginosa* causes ear and eye infections as well as wound, burn and urinary tract infections and enteritis. Like *Staphylococcus aureus*, it is prevalent in the Mediterranean. The route of transmission was originally considered to be mainly infected swimming pools (WHO, 1982), but the organism is now becoming increasingly implicated in ear, throat and skin infection through bathing in contaminated seawater (UNEP/WHO/IAEA, 1988). Numerous cases of folliculitis, dermatitis, ear and urinary tract infections due to *P. aeruginosa* that were acquired by bathing in contaminated water have been reported (Yoshpe-Purer, 1987), through the author does not provide any differentiation between swimming pools and seawater as the medium of transmission.

52. *P. 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 1600/100 ml have been recorded in Spain in a polluted river near its outlet to the sea (Alonso Molina *et al*, 1984) and lower counts (0-210) in seawater (Borrego *et al*, 1988). In Israel, out of 652 samples of seawater from various beaches collected between 1983 and 1984, nearly 50% contained *P. aeruginosa.* In a small number of these samples, the normal indicator (faecal coliforms) was either low in concentration or absent (Yoshpe-Purer, 1987).

53. High concentrations of *Pseudomonas* species have also been recorded in shellfish. Volterra (1989) reports concentrations as high as 110,000 per 100 ml in *Mytilus galloprovincialis,* 460,000/100 ml in *Donax trunculus,* 42,000/100 ml in *Ensis siliqua* and 34,000/100 ml in *Chamelea gallina.* Concentrations varied from one to four orders of magnitude greater than that in the surrounding waters.

54. Other bacterial pathogens or potential pathogens recorded in the Mediterranean (UNEP/WHO/IAEA), 1988) and capable of causing, varying degrees of gastroenteritis through consumption of contaminated shellfish or, to a lesser degree, ingestion of polluted seawater while bathing include *Clostridium perfringens, Campylobacter* species, and enteropathogenic *Escherichia coli. C. perfringens* is discharged in considerable amounts in sewage, where it is mainly of human origin. It has a relatively high rate of survival in the marine environment, but does not reproduce in sediments. High counts of total clostridia in sediments, as compared with total coliforms, faecal coliforms and faecal streptococci in the same samples, have been recorded (Volterra *et al*, 1985). *Campylobacter* has only recently been recognized as an important bacterial pathogen of man (Geldreich, 1985), *C. jejuni* and *C. coli* causing diarrhoea and fever. *Campylobacter jejuni* and, to a lesser extent, *C. faecalis*, have been isolated from sewage outfalls and polluted seawater in Greece fairly regularly, particularly during July/August (Papadakis, 1987). It is considered that basic studies on these organisms is still required (UNEP/WHO/IAEA, 1988). Members of Lancefield's Group D of Streptococci *(S. faecalis, S. faecium, S. bovis* and *S. equinum)* have been incriminated in outbreaks of food-borne diseases associated with mainly non-marine sources.

Cases involving shellfish are less documented. Haemolytic streptococci (Lancefield's Group A and streptococci (Lancefield's Group A and C) have been recorded from bathing waters (WHO, 1982), and their transmission to man by this route is suspected.

55. Other non-enteric or not wholly enteric bacterial pathogens in the Mediterranean include *Aeromonas hydrophila,* which causes septicaemia in immunosuppressed hosts, diarrhoea, pneumonia, abcesses and wound infections. It can be transmitted through contact or ingestion of water, or through consumption of contaminated seafood. Levels recorded in seawater from Spain have varied from 0-50/100 ml in non-polluted beaches to 80-11800/100 ml in polluted ones (Borrego *et al,* 1988). Levels recorded in shellfish include 36,000 per 100 ml in *Mytilus galloprovincialis,* 740,000/100 ml in *Donax trunculus* and 22,000/100 ml in *Ensis siliqua* (Volterra, 1989).

Viruses

56. More than 120 different virus types 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 groups, including enteroviruses (polioviruses, coxsackieviruses, echoviruses and hepatitis A virus), reoviruses, adenoviruses, and parvoviruses (adeno-associated viruses). These groups, with the number of types and the diseases carried, are outlined in Table 1. The frequency of isolation and quantity of virus recovered from sewage depends not only on the infections caused by normally-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 in 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 will be present in urban sewage, and it has been suggested that they may constitute an adequate indicator of the virological quality of water (Payment *et al,* 1979). In more than one Mediterranean country, polioviruses are detected in every sewage sample tested (Krikelis *et al,* 1985).

Table 1

HUMAN ENTERIC VIRUSES THAT MAY BE PRESENT IN POLLUTED WATER*

Virus group	No. of types	Disease caused
Enteroviruses:		
Poliovirus	3	Paralysis, meningitis, fever
Echovirus	31	Meningitis, respiratory disease, rash, diarrhoea, fever
Coxsackievirus A	23	Herpangina, respiratory disease, meningitis, fever
Coxsackievirus B	6	Myocarditis, congenital heart anomalies, rash, fever, meningitis, respiratory disease, pleurodynia
New enteroviruses:		
Types 68-71	>4	Meningitis, encephalitis, respiratory disease, acute haemorrhagic conjunctivitis, fever
Enterovirus type 72 (hepatitis A virus)	1	Viral hepatitis A
Gastroenteritis (Norwalk) virus	2	Epidemic vomiting and diarrhoea, fever
Rotavirus	4	Epidemic vomiting and diarrhoea, chiefly of children
Reovirus	3	Not clearly established
Adenovirus	37	Respiratory disease, eye infections, gastroenteritis
Parvovirus (adeno-associated virus)	3	Fever, rash, aplastic anaemia

* After Rao *et al,* 1986.

57. Enteroviruses have been recorded in sewage effluents and/or in seawater in many parts of the Mediterranean. According to available literature, isolates include all three serotypes of polioviruses, serotypes 1-5 of Coxsackie B virus and serotypes 1,7 and 30 of Echovirus. (Krikelis *et al*, 1985a, 1985b, 1986), Hepatitis A virus (Papaevangelou *et al*, 1990). and a large number of either untyped or unspecified serotypes (Krikelis, 1987; Petrilli *et al*, 1980; Volterra, 1989; Maini *et al*, 1990). Seven serotypes of Adenoviruses (Nos. 1, 2, 3, 4, 5, 7 and 15) have also been isolated (Krikelis *et al*, 1985a), together with a number of untyped isolates.

58. As even a single plaque-forming unit (PFU) or (as alternatively termed) cytopathogenic unit (CPU) of virus may lead to infection when swallowed, 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 waters and viral epidemics, sporadic cases of infection cannot be ruled out. Most research work performed in the Mediterranean on viruses in sewage effluents or seawater has 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 CPU per litre and adenovirus concentrations from 70 to 3200 CPU per litre. In coastal waters, a total virus range of 5 to 145 CPU per litre has been recorded (Krikelis *et al*, 1985a, 1987). Among records from the Western Mediterranean, mean values for total enteroviruses include 258 CPU per 10 litres in raw sewage, and 1.35 to 2.1 CPU per kg in marine sediments. (Jofre, 1987). In another series of experiments, mean values for recovery of enteric viruses from 24 sediment samples varied between 200 CPU enteroviruses and 57 FF rotaviruses per kg, and 130 CPU enteroviruses and 140 FF rotaviruses per kg, depending on the specific elution procedure used (Jofre *et al*, 1989).

59. The role of shellfish as vectors in human enteric virus disease is well documented. Viruses which have been shown epidemiologically to be transmitted by shellfish are Hepatitis A, non-A, non-B hepatitis, Norwalk, Snow. Mount agent, astroviruses, coxsackie virus 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 and unidentifiable viral agent, rather than a bacterial pathogen (Geldreich, 1985).

60. A number of epidemics of Infectious Hepatitis A have occurred in Europe and the United States 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 type A Hepatitis in Frankfurt, and the German cases were mainly attributable to eating oysters and mussels on the Mediterranean littoral. Poliovirus, Coxsackie virus A18, and Echovirus 3, 5, 6, 8, 9, 12 and 13 in mussels have been variously reported from France and Italy, and in Greece, Hepatitis A virus and/or Hepatitis A Antigen have been recorded in shellfish from polluted waters (Papaevangelou *et al*, 1990). The records for virus contaminants of mussels recorded in the Mediterranean are outlined in Table 2.

Table 2

VIRUS CONTAMINANTS OF MUSSELS RECORDED IN THE MEDITERRANEAN

Contaminant	Location	Reference
Echovirus 5, 6, 8, 12 Coxsackie virus A18	Italy	Bendinelli & Ruschi, 1969
Poliovirus 3	Italy	Petrilli & Crovari, 1965
Echovirus 3, 9, 13	Italy	Bellelli & Leogrande, 1967
Coxsackie virus A18	France	Denis, 1973
Hepatitis virus A Hepatitis virus antigen	Greece	Papaevangelou *et al*, 1990

61. It should be noted that clinical diagnosis of virus diseases depends on isolation of the virus and a positive seroreaction. The relative unavailability of the necessary specialized diagnostic facilities on a routine basis in many Mediterranean countries indicates that the extent of virus disease, particularly on an individual case basis, is still largely unknown.

Miscellaneous animal parasites

62. 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).

63. 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.

Fungal pathogens

64. 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.

65. *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, 1988) and Israel (Ghinsberg, 1990). In Greece, its presence in seawater has been associated with sewage pollution, as evidenced by bacterial indicator counts (Papadakis, 1987). Its presence in seawater is currently under investigation in a number of Mediterranean areas. Work 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). An ongoing 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, 1990). Recent 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.

Toxic algal blooms

66. 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 include paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP) and diarrhoeic shellfish poisoning (DSP). PSP toxins constitute a well-characterized group of tetrahydropurines, saxitoxin being the first component identified, and are produced by a well-defined dinoflagellate group, mainly *Gonyaulax* and *Gymnodinium* species, occurring in both tropical and temperate seas, NSP is caused by *Gymnodinium breve*, with symptoms similar to, but milder than, PSP. DSP 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* and related species. A further form - Amnesic Shellfish Poisoning (ASP), produced by toxins causing abdominal cramps and neurologic responses involving memory loss and disorientation has also been described. (WHO, 1984, Shumway, 1990).

67. A summary of toxic and noxious algal blooms and their effects on shellfish has been recently compiled (Shumway, 1990). *Dinophysis sacculus* in shellfish was responsible for a ban on marketing affected seafood in France between 1987 and 1989. The same species has also been recorded as causing DSP in Portugal from shellfish. According to the same author, DSP is also widespread in the Adriatic. PSP toxins caused by *Gonyaulax tamarensis* in mussels have been reported from Spain, as were also similar toxins caused by *Gymnodinium catenatum* in the marine bivalves *Venus verrucosa* and *Cytherea*, first recorded in Spain's Mediterranean coast in 1990. *Alexandrium minutum* in mussels is also reported as causing the first recorded case of PSP in France in 1989. The same author's distribution list also shows PSP as occurring in the Tyrrhenian Sea. Other records afford further information. 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). 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, 1989). In a recent review (Berland and Bellan, 1990), blooms of *Gymnodinium breve* (responsible for NSP) are also mentioned as having been recorded in the North of Spain and in the Eastern Mediterranean.

CORRELATION BETWEEN SEAWATER AND SEAFOOD QUALITY AND HEALTH EFFECTS

General considerations

68. In line with global practice, recreational 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 four countries (France, Greece, Italy and Spain) which are member states of the European Economic Community are bound by the relevant EEC Directive (EEC, 1976), which also includes an assortment of physicochemical parameters apart from the microbiological. Standards and criteria exist in all countries of the region, but differ to a large extent both as to the particular microorganism(s) monitored, and the "acceptable" levels of each (WHO, 1989). The situation has not changed to any appreciable extent since the adoption of common interim criteria for bathing waters by Mediterranean States in 1985, 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.

69. 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 arguments still run high 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. The natural marine bacterial flora often interfere with negative readings, and as natural flora vary with the area within the Mediterranean itself, it has been found difficult to produce standard methodology guaranteed to produce the same results wherever applied. Comparison of the state of microbiological pollution in coastal recreational (or, for that matter, shellfish-growing) waters in different areas of the region can therefore be quite meaningless if restricted solely to counts obtained, without taking sampling and analytical methodology, not to mention quality control, into account (Saliba and Helmer, 1990).

70. The use of faecal indicators for indexing the health hazards in recreational (and drinking) waters 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, 1977) 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.

71. 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.

72. Bacterial and other indicators (often defined as health effects water quality indicators) are expected to meet certain requirements, which have been outlined by the 1977 WHO/UNEP Group of Experts. Indicators must:

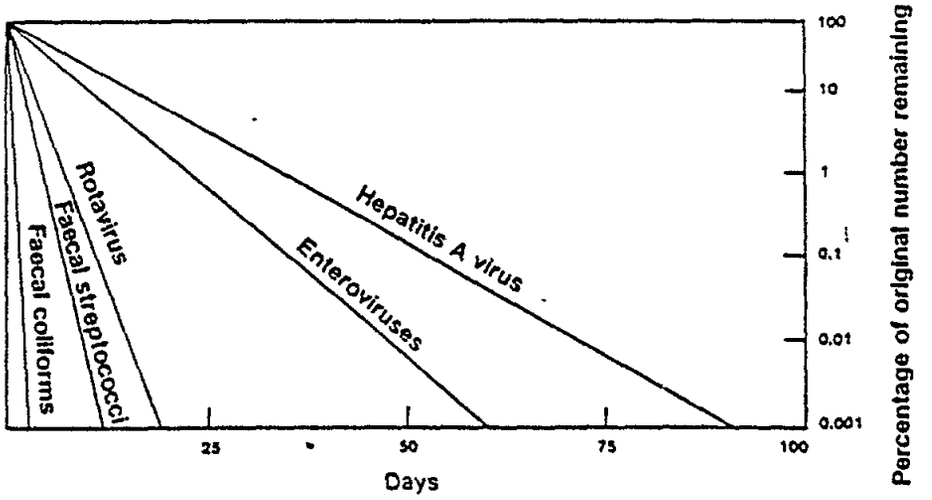
- be consistently and exclusively associated with the sources of pathogens or noxious substances;
- be present in sufficient numbers or quantities to provide an "accurate" density estimate whenever the level of each of the pathogens is such that the risk of illness is unacceptable;
- approach the resistance 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.

73. It has always been acknowledged that no individual indicator is ideal and no indicator system perfect. 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.

74. The situation as regards viruses is perhaps more serious. Apart from the fact that they may survive for considerable periods of time, particularly in association with sediments, it has been shown (Vasl *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 recently been summarized (Wheeler, 1990) at the request of the United Kingdom House of Commons Environment Committee Inquiry into Pollution of Beaches (Figure 1). 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 such a conclusion, based primarily on conditions prevailing in Northern European waters would have to be looked into on the basis of Mediterranean conditions (both climatic and otherwise), it accentuates the inadequacy of current bacterial indicators as a monitoring tool for pollution by viruses.

Figure 1

TYPICAL SURVIVAL CHARACTERISTICS OF FAECAL BACTERIA AND HUMAN ENTERIC VIRUSES IN SEAWATER*



* After Wheeler, 1990.

Coastal recreational waters

75. 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 list of the summary results of the main studies of this nature, which include four in the Mediterranean, is provided in Table 3. This list does not include one study recently completed in Israel and one study nearing completion in Spain.

Table 3

SUMMARY RESULTS OF THE MAIN CABELLI-STYLE PROSPECTIVE EPIDEMIOLOGICAL STUDIES[a]

Author	Date	Country/area	Fresh/sea	Indicator	R^2[b]	Symptoms[c]
Stevenson	1953	United States of America	Both	Total coliforms	NR	ENT/GI/R
Cabelli	1982	United States of America	Both	Enterococci	0.56	GI
Seyfried	1985	Canada	Fresh	Total staphylococci Faecal coliforms Faecal streptococci	0.19 0.08 0.03	R/GI
Cheung	1988	Hong Kong	Sea	*E. coli* Staphylococci	0.53	S/GI
El Sharkawi	1983	Egypt	Sea	Enterococci *E. coli*	0.79 0.77	GI
Fattal	1986	Israel	Sea	Enterococci *E. coli*	NR NR	GI GI
Mujeriego	1982	Spain	Sea	Faecal streptococci	NR	S/E/ENT/GI
Foulon	1983	France	Sea	Faecal streptococci Total coliforms Faecal coliforms	NR	E/S/GI

[a] After Jones, F. & Kay, D. *Recreational water quality: the relationship between epidemiological studies and recreational activities in water* Report of a biological standards seminar, Middlesex Polytechnic, 9 February 1990.

[b] R^2 = Coefficient of determination; NR = not reported.

[c] E = eye infections, S = skin complaints; GI = gastrointestinal symptoms, ENT = ear nose and throat infections, R = respiratory illness

76. 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) and Wheeler (1990). 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, *E. 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.

77. The Alexandria study 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, enterococcus and *E. coli* densities reaching 10^4/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 *E. coli* densities. A comparison with previous studies of a similar nature in New York showed that at equivalent enterococcus 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*, 1982) 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.

78. The 1983 Israeli study, carried out at bathing beaches in the Tel-Aviv area, was carried out at three beaches 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, *E. 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 *E. 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).

79. Of the two studies conducted in Spain, the former (Mujeriego *et al*, 1982) 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 latter study (Marino *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. A positive correlation, but in this case, 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.

80. 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.

81. A later study (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 100ml) 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.

82. A study currently in progress in Spain is being 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 first year of the study (Borrego *et al* 1988), 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 seawaters; (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. The study is continuing with a larger population group.

83. A prospective epidemiological study was conducted in Israel 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).

84. 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: (i) difficulties in defining exposed and non-exposed population groups; (ii) uncertainty in the degree and duration of bathing exposure; (iii) availability of only limited information on pollutants present in the bathing water; (iv) inadequate identification of illnesses and their relationship to bathing; and (v) difficulties in the interpretation of any associations between illnesses and exposure to polluted water, and in ascribing a cause-effect relationship. He also points 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.

85. It has recently 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 recently 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 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.

Shellfish waters

86. 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).

87. Apart from the two studies on Typhoid fever in Egypt (El-Sharkawi *et al*, 1982) 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. 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.

88. 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. 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.

89. 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.

CONCLUSIONS

Analysis of the current situation

90. 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. It is equally clear that the situation is currently resulting in adverse health effects on both local and tourist populations. Regarding the latter, apart from the German attribution of Infectious Hepatitis to the region mentioned earlier in this document, participants at a fairly recent WHO/UNEP consultation on the microbiological pollution of the Mediterranean Sea reported that (a) 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, (b) 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 dyssentery were imported cases and (c) 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 (WHO/UNEP, 1990).

91. 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.

92. While such records provide evidence of occurrence and indications of magnitude, the extent of damage to health on a Mediterranean-wide basis still has 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. Furthermore, there are still large stretches of the Mediterranean coastal zone mainly in the Southern and Eastern parts, from which records are sparse.

93. There does not appear to be any doubt that ameliorative changes of a general nature have occurred over the last decade, one factor being the increase in sewage treatment and disposal facilities in various parts of the region. One other indication of a positive trend is provided by the results of coastal water monitoring programmes. From information supplied by member states to the EEC in terms of the 1975 bathing water quality Directive (EEC, 1988, 1989) 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). 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 (Ministry of Health, Rome, 1990). Again from information in the two EEC reports available, results from Greece 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. The only negative trend recorded is in Spain, where the number of stations with high quality water (A2) decreased from 65.2% in 1986 to 51.0% in 1987.

94. An evaluation of monitoring data for recreational waters submitted to the Coordinating Unit for the Mediterranean Action Plan by seven other Mediterranean countries (Algeria, Cyprus, Israel, Lebanon, Malta, Morocco and Yugoslavia) within the framework of national MED POL monitoring agreements for the period 1983-1987 was performed in 1989. According to this evaluation, the conformity of sampling stations with the interim environmental quality criteria adopted in 1985 rose from 78% in 1983 to 96% in 1987 in the case of stations with at least six samplings a year (UNEP, 1989).

95. The information available with respect to the above eleven countries has been considered (WHO, 1989) as sufficient to indicate a general trend of improvement during the period covered over a stretch of the Mediterranean coastline covering practically the whole of the northern seaboard, the western part of the southern, and part of the central and eastern areas. Apart from this general trend, no other conclusions could be reached, and no comparisons between the different areas attempted, as (a) the larger portion of the southern and eastern seaboards had to be considered as still unmapped in terms of water quality, (b) the criteria and standards adopted by the different countries for acceptability showed a wide range of divergence both in the number of parameters measured and in the actual limit values applied for each, and (c) the analytical methods employed differed.

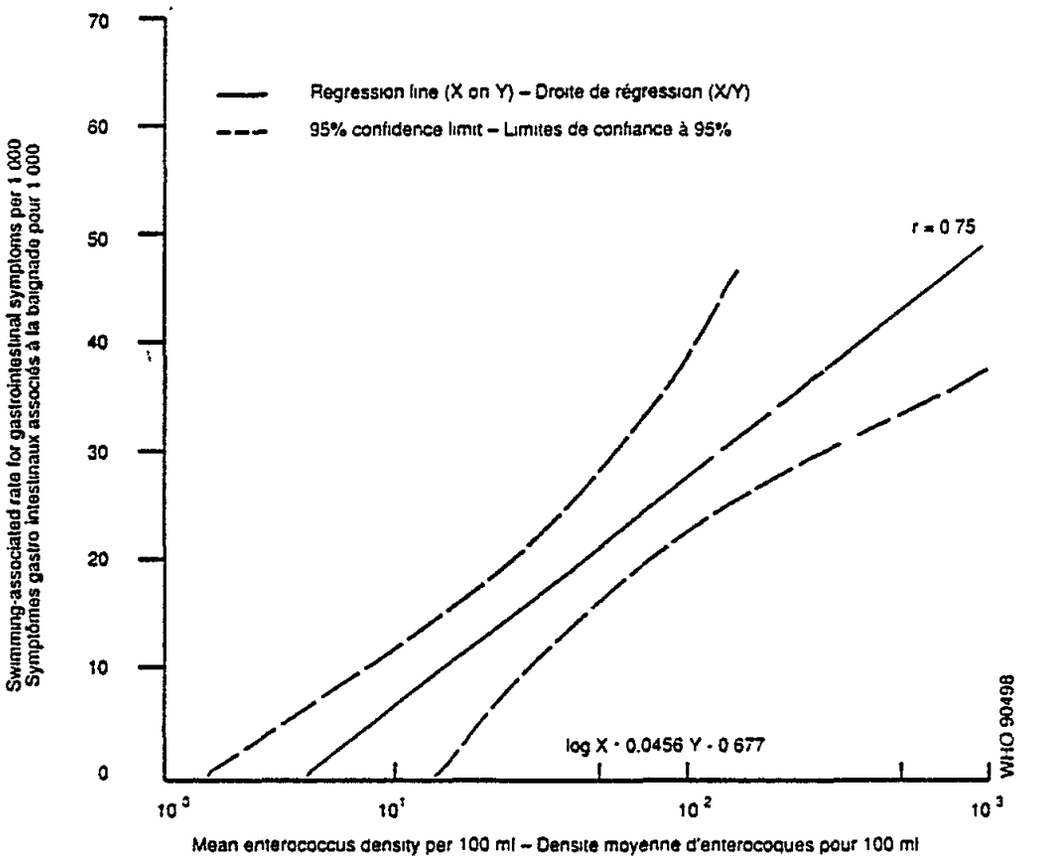
96. Considerable caution should be exercised in any hypothetical extrapolation of the situation-trend outlined above to the incidence and density of pathogenic microorganisms in affected coastal areas or to human health hazards arising from bathing activities. On the one hand, it is clear that any increase in the ratio of treated to untreated sewage and/or in the number of submarine outfall structures deviating effluent discharge from the immediate coastline to points out at sea should, both in theory and in practice, reduce the overall incidence and density of pathogenic microorganisms in previously-affected areas, with a consequent reduction in health risks. On the other hand, the positive trend in water quality is evidenced primarily by the reduction in density of indicator organisms whose capability both to survive in the marine environment and to withstand sewage treatment is generally less than that of pathogens, most pronouncedly so in the case of viruses. One point of concern is also that the main (in many cases the sole) criterion of acceptability is based on concentrations of one particular indicator (faecal coliforms) which most epidemiological studies, irrespective of other variations between their results, have indicated as an unsatisfactory basis for correlation with morbidity symptoms in exposed human population groups.

97. One specific example of the potential risk to health from bathing in sewage-polluted coastal waters is provided by the 1972-1979 USEPA study (Cabelli, 1983), which produced what is perhaps the best attempt at quantification of bathing-associated health risks by developing data for regression lines for both total and highly-credible gastrointestinal symptoms (i.e. nausea, vomiting, diarrhoea and stomach-ache). The latter data were used by the USEPA in developing a regression line in a recommended health effects criterion for marine recreational waters, as it was considered that there was greater credibility for those types of severe symptoms, and that the consequences of illness were of greater public health importance and thus more amenable to economic analysis (Cabelli *et al*, 1984).

98. The USEPA recommended health effects criterion is reproduced in figure 2. As a result, the proposed USEPA standard for marine bathing waters, based on an acceptable swimming-associated gastroenteritic rate of 19 per 1000 swimmers was a limit of 33 enterococci 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. In a hypothetical population of one million, this would mean 25,000 to 40,000 cases, and further extrapolation to normal tourist and local populations in various regions of the world, particularly in tropical and subtropical climates, would accentuate the concern one has to accord to such a situation (Saliba and Helmer, 1990).

Figure 2

USEPA RECOMMENDED HEALTH EFFECTS CRITERION
FOR MARINE RECREATIONAL WATERS*



*After Cabelli *et al*, 1984

99. The development of a similar health effects criterion for the Mediterranean would go a long way towards a fairly accurate estimate of the hypothetical risks to health expected from waters of varying quality, and the eventual arrival at reasonably safe water quality criteria and standards for the bathing waters of the region. As no parallel exercise on the same scale has been performed to date in the Mediterranean, this is not currently possible. Direct application of the USEPA criterion towards even an approximate estimate of what degree of health risks would be expected in the Mediterranean is similarly not possible as the indicator organism currently used in routine monitoring is different, as are also exposure conditions of Mediterranean bathing populations as compared to those studied in the United States. In addition, it has recently been stated that re-analysis of the data on which the 1986 USEPA standards are based suggest that the mathematical relationship between bather morbidity and enterococcus concentration is of dubious quality, in addition to significant bias due to measurement error in bacterial enumerations not accounted for in the original analysis (Fleisher, 1990, 1991; Godfree *et al*, 1990). In very general terms, however, taking into account the particular climatic and socio-economic considerations prevalent in the Mediterranean region, one would expect the health effects in terms of hypothetical morbidity figures in the region to be associated with lower bacterial concentrations in seawater than US equivalents.

100. The situation regarding shellfish varies throughout the Mediterranean region, and health-risks 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 practised 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.

101. 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).

102. 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, for shellfish themselves, consists in faecal coliforms. This accentuates the contention which has, in fact, been expressed in the relevant Mediterranean fora 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.

103. 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. A comprehensive document on marine biotoxins is currently in preparation under WHO auspices, but this will not obviate the need for comprehensive field studies in the appropriate areas.

Recommended action

104. The practical end-point of microbiological and epidemiological investigations on health hazards associated with the use of the marine environment is the acquisitions of data utilizable for the development and implementation of practical preventive and control measures by national and local authorities.

105. The primary requirement is 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.

106. From the general viewpoint of controlling pollution at source, acceleration of implementation of that part of the 1985 Genoa Declaration concerning sewage treatment plants and outfall structures for cities and towns is necessary. One extra measure of a more immediate nature could be to provide separate treatment facilities for hospital wastes (where such is not already being done) prior to their entry into the municipal sewage system.

107. The current interim criterion for coastal recreational waters jointly adopted by Contracting Parties in 1985 should be clearly recognized as what it actually constitutes - a temporary minimum palliative measure based on the immediate capabilities of the lowest common denominator. It is important that long-term planning, particularly in the design of treatment and disposal facilities, should not be performed solely on the basis of this criterion.

108. The eventual requirement will be the development of modified criteria and standards, based on more reliable indicators. However, at this particular stage in time, it is not recommended that any formal action be taken to amend the current criterion, even on a further interim basis, as the global situation regarding the validity of the several bacterial indicators currently in use is in a state of flux. However, 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 can be done without major legislative modifications.

109. The question of the aesthetic and hygienic quality of beaches deserves immediate attention. Within this framework, analysis of sand samples from major beaches for bacteria and fungi should be performed as a baseline study. The question of beach overcrowding is a more complex problem which will have to be solved on a case-by-case basis.

110. In the case of shellfish waters, it is similarly recommended that the criteria jointly adopted by Contracting Parties in 1987 should not be modified at the present time. Investigations should however be undertaken to the extent possible on the presence and density of pathogenic microorganisms, particularly viruses in specimens collected from acceptable waters, with the objectives of reviewing the efficacy of depuration techniques in use, and the eventual establishment of criteria for viruses.

111. The question of epidemiological studies correlating coastal recreational waters with health effects deserves particular attention. Up to the present time, Mediterranean studies have been essentially directed at the adaptation of globally-recognized techniques to conditions prevailing in the region. Given the present state of global uncertainty regarding all past studies, and taking into account recent emphasis in Northern European countries on the overall problem, work in the Mediterranean on this subject should be closely coordinated with existing and planned European initiatives, and closer links between the competent international organizations (particularly WHO and CEC) on a combined European/Mediterranean dimension, would be of great help towards the eventual solution of this problem.

112. Within the field of health-related microbiological research in the Mediterranean, a more pronounced shift in the relevant MED POL component from individual studies to coordinated multi-country projects on defined priority topics within already-approved research areas is indicated. Apart from epidemiological studies (which require coordination both within and outside the region, and substantial external funding in addition to the 'catalytic' MED POL contribution), target topics of a priority nature would appear to be (a) comparative densities of pathogens (including viruses) and indicator organisms in coastal recreational waters and shellfish in representative areas around the Mediterranean, (b) survival of viruses in seawater and shellfish and their resistance to sewage treatment and depuration techniques, and (c) adverse health effects of algal and related blooms, particularly concerning the presence and concentration of algal biotoxins under Mediterranean conditions.

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