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HEALTH RISKS FROM MARINE POLLUTION IN THE MEDITERRANEAN

PART II

REVIEW OF HAZARDS AND HEALTH RISKS

UNEP

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WATER QUALITY

By the year 2000, all people should have access to adequate supplies of safe drinking-water, and the pollution of groundwater sources, rivers, lakes and seas should no longer pose a threat to health.

Keywords

SEAWATER
WATER POLLUTION – prevention and control
WATER POLLUTANTS – adverse effects

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ABSTRACT

The risks to human health from marine pollution is acknowledged world-wide. Such risks normally arise from bathing in polluted seawater and from consumption of contaminated seafood, and are accentuated in those regions where climatic conditions result in relatively long bathing seasons and/or where seafood consumption is high. This document examines in depth the major microbiological and chemical pollutants in the Mediterranean, and provides an overall picture of the state of pollution of the Mediterranean Sea from the point of view of its impact on human health, taking into consideration the actual and potential health risks associated with bathing and consumption of certain species of seafood. In this framework, clarifying the situation for scientists, it would facilitate preventive and remedial action on the part of the various public health authorities throughout the region.

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PART 1

INTRODUCTION AND BACKGROUND

1.1 SOURCES OF MARINE POLLUTION

Pollution of the marine environment originates from various sources, the principal ones involved being the following:

1.1.1 Municipal sewage

This is discharged directly into the immediate coastal zone, either untreated or subjected to various treatment procedures, through outfall structures of variable length, or reaches the sea by seepage as a result of leaks in sewerage systems or other causes. Municipal sewage carries a heavy load of microorganisms, including bacterial and viral pathogens. In cities and large towns, it usually contains a variety of chemical wastes both from households and from industries discharging directly into the public sewerage system.

1.1.2 Industrial effluents and emissions

Industries located near the coastline discharge their wastes directly into the sea. Aerial emissions from industries located inland contain pollutants which can be transported out to sea through the atmosphere. Industrial effluents contain a large variety of chemical wastes, the type of pollutant depending on the particular industry in question.

1.1.3 Rivers

Polluted rivers carry a considerable amount of wastes to the sea. Apart from municipal and industrial wastes discharged upstream, rivers are also responsible for the transport of sometimes heavy loads of agricultural fertilizers and pesticides. Rivers contribute significantly to transboundary pollution, and effects can be felt at points comparatively distant from the origin. Run-off waters also contribute significantly not only to pollution of rivers but also to that of the sea.

1.1.4 Coastal waste disposal

Other forms of disposal of solid and liquid waste in or near the marine environment contribute to the direct or indirect pollution of the sea in various ways, depending on the type and amount of the material disposed of.

1.1.5 Maritime (offshore) sources

The pollutant discharged in greatest amounts is ballast oil from tankers. In many cases, beaches suffer a variable amount of damage. Ships also dispose of waste materials overboard.

1.2 GENERAL HEALTH RISKS FROM MARINE POLLUTION

In very general terms, the majority of the socio-economic consequences of marine pollution can be expressed as immediate or long-term effects on human health. In this context, the two main types of human exposure to pollutants in the marine environment are through direct contact with polluted seawater and/or beach sand, including ingestion of the former while swimming or bathing, and consumption of contaminated seafood. In some cases where the seawater, after being treated in desalinization plants, is used for drinking purposes, pollution may constitute a potential health risk.

A number of diseases have been tentatively associated with bathing in polluted waters. Such diseases have been reported to include bacterial diseases such as salmonellosis (typhoid and paratyphoid fever), shigellosis (bacillary dysentery), cholera, viral diseases such as hepatitis A and E, illnesses caused by a number of other enteroviruses, gastroenteritis caused by a number of bacteria and viruses, and diseases caused by a variety of animal parasites, such as amoebic dysentery. In addition, a number of diseases and disorders affecting the ear, eye, skin, and upper respiratory tract have been associated with bathing.

With the exception of those associated with pathogens having a relatively low infective dose, diseases affecting the gastrointestinal tract are much more easily contracted by humans through the consumption of raw or partially cooked food, particularly shellfish. A number of epidemics and outbreaks of various diseases attributed to the consumption of contaminated shellfish have occurred in various parts of the world, and the number of individual cases, particularly of the less insidious diseases, is likely to be very high. In those regions of the world where conditions favour the proliferation of certain species of algae (resulting in "red tides" or "algal blooms") which produce toxins, shellfish become contaminated by these algae and on ingestion by humans, the toxins cause a number of diseases, mainly Paralytic shellfish poisoning (PSP) and Diarrhoetic shellfish poisoning (DSP).

Pollution of the sea by persistent chemicals, such as mercury and other metals, DDT, PCB and a number of other organic substances, produces a completely different hazard. Following entry into the marine environment, these chemicals accumulate in plants and animals as they pass through the marine food-chain, reaching their highest levels in filter-feeders, such as bivalve molluscs, and in large predatory fish such as tuna and swordfish. Effects on humans through the consumption of chemically-contaminated seafood are essentially long-term, depending on the chemicals themselves, and the rate and amount of intake. In general, the principal risk is restricted to those individuals consuming seafood more than two to three times a week, although the risk varies with the type of seafood, the concentration of pollutant and the circumstances of the consumer. Clinically-recorded effects have occurred in population groups subsisting mainly on a seafood diet. It is probable, however, that relatively mild effects have gone unnoticed, or have not been associated with contaminated seafood because, in a number of cases, as with mercury, the symptoms are not specific, and the condition can easily be attributed to other causes.

Apart from the essential resources provided by the marine environment to human life, in the overall toxicological evaluation it should be taken into account that this environment is also an important source of protective factors, which in some cases may counteract hazardous agents (De Flora *et al.*, 1991).

1.3 CHARACTERISTICS OF THE MEDITERRANEAN SEA

The Mediterranean Sea is a relatively large body of water with an area of 3 million km^2 and a volume of over 4 million km^3. It is virtually enclosed, with narrow links with the Atlantic via the Straits of Gibraltar, the Black Sea via the Dardanelles and the Red Sea via the Suez Canal. The main water inflow and outflow are both through the Straits of Gibraltar, water entering through surface currents, and exiting via the intermediate layer (between 200 and 600m deep). The surface current system of the Mediterranean shows a migration of Atlantic water, with salinity slightly above 36, towards the east with numerous spin-off eddies along the way (Miller, 1983). The annual thermal changes of surface waters are very large and control the density of surface waters and the basic characteristics of the annual biocycle. There is no surface return system from the east to the west.

The return of Mediterranean water is by way of Levantine intermediate water and Mediterranean deep water flowing from east to west and spilling over the sill of Gibraltar into the deep Atlantic. Such intermediate and deep water is produced by very pronounced evaporation processes which gradually transform surface water with salinity slightly above 36 into denser water with salinity of 38.4 or more. Deep seawater in the Mediterranean has a temperature between 12.5° and 13.5° C in the west and between 13.5° and 15° C in the east with salinity between 38.4 and 39.

The estimated turnover time for Mediterranean waters is 80 years. The basic nature of the Mediterranean circulation system contains components of strong vertical convections which determine the distribution of salinity and produce vertical recycling of nutrients and other dissolved substances (Miller, 1983). When winter storms lower surface temperature in the western Mediterranean to 12° C, deep convection can take place; in the Algero-Provençal basin it was traced to the depth of 2,000 m. (State of the Mediterranean Marine Environment, M.A.P. Technical Reports Series, No. 28, UNEP, Athens, 1989).

The Mediterranean is relatively lacking in nutrients, though river outflows cause eutrophication phenomena in a number of coastal areas.

Health hazards from marine pollution in each region of the world can be accentuated as a result of several prevailing factors. In the Mediterranean, the factors responsible for accentuating health hazards include the following:

1.3.1 Oceanographic factors

The practically enclosed state of the Mediterranean Sea and the relatively long circulation time of its waters are conducive to pollutant retention. Substances entering from the Atlantic in its upper layers, as well as floating pollutants discharged into the Mediterranean itself, would remain there, as the outflow is via the intermediate layer. Similarly, heavy pollutants would sink to bottom sediments before they could be removed. At the same time, the comparatively low rate of interchange between coastal and open waters leads to concentration of pollutants in the former zone, where their effects are mainly felt.

1.3.2 Climatic factors

The relatively warm prevailing temperatures are responsible for long exposure periods to seawater and beach sand while bathing, in comparison to the situation in other, more temperate, zones. Furthermore, seafood preservation requires much closer attention.

1.3.3 Socio-economic factors

In many parts of the region, municipal sewage and industrial effluents are still being discharged untreated into the immediate coastal zone, or reach it in this stage through rivers. Exposed population groups are large as, apart from the 130 million inhabitants estimated to live permanently along the Mediterranean coastline, over 100 million tourists visit the area annually. In addition to heavy seafood consumption, the sea represents the main recreational amenity for both local and tourist populations during a long bathing season lasting from May to September in most areas; the heterogeneous nature of beach populations, together with the overcrowding problem in many beaches, facilitates the spread of infections. Seawater and seafood quality control measures vary from country to country and, in several cases, their effectiveness as a health protection tool can be considered doubtful.

During the past two decades, the general situation regarding Mediterranean pollution has registered a substantial improvement, both from the viewpoint of legal and administrative control measures enacted and enforced, and from that of infrastructural investments in the form of sewage treatment plants and submarine outfall structures in many coastal cities and towns. In 1975, the Mediterranean Action Plan, a comprehensive pollution prevention and control programme sponsored and organized by the major United Nations bodies and agencies, was formally adopted by Mediterranean states. This programme has been ongoing ever since, and has registered a considerable degree of success. However, in several of its areas, the Mediterranean still requires a comprehensive degree of improvement in order to minimize both actual and potential marine pollution hazards, including those affecting human health.

1.4 MARINE POLLUTION MONITORING AND RESEARCH IN THE MEDITERRANEAN REGION

From the scientific point of view, the seriousness of the pollution problem of the Mediterranean Sea is considered to have become particularly evident after the International Conference on Oil Pollution of the Sea, held in Rome in October 1968, and during the course of the International Conference on the Protection of the Sea, held in Milan in April 1969 (GFCM, 1972). During this period, studies on various aspects of the pollution of the Mediterranean Sea, previously relatively sparse, started to increase. These studies, however, were fragmentary in nature and generally limited to specific coastal areas, generally the most polluted ones. As a result, far from providing any form of accurate or realistic picture of the general situation, their main achievement was the resultant controversy between advocates of two extreme schools of thought, one describing the Mediterranean as a dying sea and the other considering the water body in question as large enough to assimilate the material being dumped and discharged into it.

The importance of starting a more organized approach towards studies on the pollution of the Mediterranean Sea was highlighted during the FAO Technical Conference on Marine Pollution and its Effects on Living Resources and Fisheries, held in Rome in December 1970. One year prior to this, the General Fisheries Council for the Mediterranean (GFCM) had decided to carry out a study on the state of pollution of the Mediterranean Sea, and had sent a questionnaire to all riparian countries in the area. The replies were reviewed by a group of consultants, who presented their preliminary report at the conference. The final report was published two years later (GFCM, 1972). Most of the conclusions had to be reached by indirect estimates, rather than by direct measurements, owing to the relative scarcity of available records.

The 1970 FAO Technical Conference and the 1972 GFCM report provided further impetus to marine pollution studies in various parts of the region, and led to several national and international activities aimed at providing more information on the situation. At national level, study programmes were accelerated in several universities and marine research institutions. At international level, practically all the major United Nations Specialized Agencies, as well as other international bodies, enlarged their marine pollution research programmes, or established new ones. By and large, the bulk of research was performed at individual level and, in the lack of an adequate mechanism, there was very little coordination between the various programmes.

This situation attracted greater attention on the part of the major United Nations Specialized Agencies, which immediately recognized the need for a properly-balance region-wide programme in order to reach a reasonably accurate estimate of the state of pollution of the Mediterranean Sea. This would enable actual and potential threats both to human health and to marine life to be more specifically identified, and data obtained would provide national authorities with the basis for the development and implementation of appropriate preventive and remedial measures.

By the end of 1974, such a programme - the Joint Coordinated Mediterranean Pollution Monitoring and Research Programme (MED POL Phase I) - had been finalized jointly by UNEP, FAO, WHO, UNESCO, IOC, WMO and IAEA. During the first meeting of Mediterranean States on the pollution of the Mediterranean Sea, held in Barcelona in January 1975, this programme was formally approved by the governments of the region within the overall framework of a more comprehensive undertaking - the Mediterranean Action Plan - which includes two other components: a set of legal instruments binding Mediterranean States to joint or individual action in various aspects of pollution control, and a socio-economic programme designed to link developmental processes with environmental preservation (UNEP, 1975).

During its initial phase, operational from 1975 to 1981, the MED POL programme consisted of a number of pilot projects each implemented by an *ad hoc* network of national institutions in Mediterranean countries, and coordinated jointly by the relevant UN Specialized Agency and the United Nations Environment Programme. During this phase, two projects were coordinated by the World Health Organization (WHO). The first was on coastal water quality control (WHO/UNEP, 1981), in which 30 laboratories from 14 Mediterranean countries regularly monitored the microbiological and related conditions of recreational and shellfish areas. In common with the other projects, the general aim was twofold. Firstly, to obtain a more accurate picture of the situation regarding the quality of coastal recreational and shellfish waters. Secondly, to lay the foundation, particularly in developing countries, of a flexible monitoring programme which such countries would eventually have to implement in order to ensure that water quality was in conformity with accepted criteria and standards.

From the information acquisition viewpoint, the pilot project's results provided data which were valuable insofar as the actual areas monitored, and their environs, were concerned. From the overall viewpoint, the geographical coverage attained was insufficient to allow the formulation of any assessment or evaluation on a regional scale. However, the pilot project provided extremely valuable experience on the Mediterranean situation as regards technical and related capabilities in the various countries to respond to the demands of the situation. Development of permanent monitoring programmes would have been difficult in the absence of such experience.

The second project coordinated by WHO during this period was a comprehensive survey on pollutants from land-based sources in the Mediterranean, in which practically every major UN Agency also participated. The project was implemented in 1976-1977 and provided a valuable input to the preparations, ongoing at the time, for finalization of the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources, eventually adopted and signed in Athens in 1980. Considerable information regarding the pollutant load entering the Mediterranean Sea was obtained, though most of this had to be so obtained by indirect estimates in the absence of available measurements. Furthermore, a wide divergence between individual countries was evident both in type of legislation, in monitoring procedures, and in waste management practices (UNEP *et al.*, 1984).

Other pilot projects related to health, carried out during the 1975-1981 period, concerned baseline studies and monitoring of heavy metals (particularly mercury and cadmium) and chlorinated hydrocarbons (particularly DDT and PCB) in selected marine organisms, including a number of edible fish and shellfish species. The scope of these projects, which were organized by FAO, was purely to obtain information on concentrations. Correlation with possible health effects was planned for a later stage.

As the matter of pollution of coastal recreational areas constitutes a major issue in the Mediterranean, particularly from the viewpoint of possible effects on tourism, WHO went beyond the strict scope of the pilot project on coastal water quality control in its implementation. Apart from laying down the foundation of sanitary monitoring programmes, the matter of recreational water quality standards was reviewed at length during WHO expert meetings. Results included an outline code of practice for waste management in coastal areas, preliminary guidelines for conducting of microbiological/epidemiological studies correlating coastal water quality with health effects (considered necessary for obtaining the sound epidemiological evidence on which quality standards and criteria would eventually have to be based), comprehensive guidelines for health-related monitoring, and even more comprehensive guidelines for waste discharge into the Mediterranean marine environment.

Based on the experience of the preliminary or pilot phase of MED POL, the second phase - the Long-term Programme of Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase II) - was developed and finalized in 1980, adopted successively at technical and formal levels in 1981, and became operational in 1982. Originally designed to cover the period 1981-1990, it was eventually extended up to the end of 1995. Basically, it consists of three elements: monitoring, research, and activities for the progressive implementation of the 1980 Athens Protocol on pollution from land-based sources.

The current phase of the MED POL programme contains an even stronger health-related component than was the case in the preliminary phase. Emphasis is given to the development and enhancement of national programmes for regular monitoring of pollution sources and recreational and shellfish waters. Research topics, in support of such monitoring, include studies on microbiological methodology with particular emphasis on seawater and shellfish analysis, and the development of common methodology for implementing quality control and for evaluating survival and adaptation of pathogens in the marine environment, pathogens/indicator relationships, epidemiological studies correlating recreational water quality with health effects on exposed population groups, carcinogenic and mutagenic marine pollutants, and biological monitoring of population groups exposed to pollutants via seafood consumption. Activities in connection with the progressive implementation of the land-based pollution Protocol include the preparation of guidelines on various aspects of waste management, with the aim of minimizing pollution at source. They also include the preparation of assessments on the state of pollution of the Mediterranean Sea by specific substances or groups of substances, based both on monitoring data and research results. These assessments incorporate an evaluation of the risks both to marine life and to human health, and include recommendations to governments on appropriate measures to be taken.

Currently, practically every Mediterranean country has a programme of recreational water quality monitoring in operation. Such programmes vary in extent and scope, particularly in developing countries. In this context, it should be borne in mind that in a number of these countries such programmes were virtually non-existent in the relatively recent past, and their progressive implementation has, in many instances, required the establishment of a new administrative and technical infrastructure not possible of realization overnight.

Within the framework of the MED POL programme, WHO has prepared recommended methods for determination of the principal traditional indicator bacteria, as well a number of selected bacterial pathogens. All methods are based on standard international ones, but adjusted, on the basis of local research, to meet the requirements demanded by conditions prevailing in the Mediterranean marine environment. All methods are updated periodically, depending on the feedback from institutions utilizing them. All these methods have recently been amalgamated into comprehensive five-part guidelines for health-related monitoring of coastal recreational and shellfish waters, each part respectively dealing with general issues, bacterial indicators, bacterial pathogens, statistical methods and laboratory quality control. (WHO/UNEP, 1994).

1.5 THE STATE OF MEDITERRANEAN RECREATIONAL AND SHELLFISH AREAS

There does not appear to be any doubt that improvements 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 European Union in terms of the 1975 bathing water quality Directive (E.C., 1988, 1989, 1993) 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 and 87% in 1992, with a corresponding reduction in lower water quality stations (23.5% in 1983, 16.7% in 1987 and 13% in 1992). In Italy, the number of stations conforming with Italian criteria (based on, but stricter than those in the Directive) showed a steady increase from 68% in 1984 to 89.3% in 1989 and 92% in 1992 (Ministry of Health, Rome, 1993). Again from information in E.C. reports, results from Greece show that in 1987, 77.7% of stations with at least five samplings per year were found to be in conformity with requirements, while this figure rose to 97% in 1992. In Spain, the number of stations with high quality water (A2) decreased from 65.2% in 1986 to 51.0% in 1987, but rose to 74% in 1992; in this last year, 93% of stations complied with the E.C. Directive's mandatory requirements.

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 former 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). Results obtained from the same countries, together with data from others (Albania, Egypt, Syria, Tunisia and Turkey) between 1988 and 1992 show that while the latter percentage has not been generally maintained an overall basis, there has been improvement in the situation in practically each individual country.

The information available with respect to the above sixteen countries has been considered (WHO, 1989) as sufficient to indicate a general trend of improvement during 1983-87 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.

PART 2

BIOLOGICAL POLLUTION OF RECREATIONAL - SHELLFISH AREAS IN THE MEDITERREANEAN

2.1 GENERAL

The main types of human exposure to pathogenic microorganisms 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.

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).

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 to be 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 provide a reasonable estimate of the degree of sewage pollution, and perhaps a relative 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 non-gastrointestinal pathogens and the biotoxins from algae (P.S.P., D.S.P.). In general, there is very little control over the quality of beach sand, which has only recently commenced to be recognized as a factor to be considered in the transmission of a number of skin and other contact infections, including fungal ones.

The situation outlined in (e) above is global in character, rather than specific to the Mediterranean. It does however constitute an accentuated risk factor in the region when considered in combination with the other specifically-Mediterranean factors. The same holds true 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 limited particularly in the interpretation of results, by a number of confounding factors over which satisfactory control has been found difficult to achieve.

2.2 PATHOGENIC MICROORGANISMS

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 in 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, and results obtained so far show a considerable increase in sewage treatment plants and submarine outfall structures, particularly around the northwestern seaboard. Considering the increase in coastal development over the past two decades, one would expect an increase in the total amount of wastewater discharged, but also a concomitant increase in the proportion of treated wastewater with respect to that found in the previous survey.

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 possible source 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 contaminated by the bathers themselves may at times serve as an effective route of transmission of some viral diseases. This could also apply to other bacterial and fungal infections (Papadakis *et al.*, 1992). 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.

Untreated or inadequately-treated sewage remains the main factor of concern in a substantial part of the Mediterranean. 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 achieved in the receiving seawater and the consequent adverse effects produced in the areas near the points of discharge (UNEP/WHO, 1985).

Apart from pathogenic microorganisms (bacteria, viruses, fungi and parasites) discharged into the marine environment through sewage effluents or other terrestrial sources, other groups of naturally-occurring marine microorganisms, which can be considered to be pathogenic through either by causing infections or their ability to produce various toxins and to which human 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 known as algal bloom or red tide when their concentration in sea water reach levels of 10^4 to 10^6 cells/litre. Although 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 has led to consider that land drainage plays 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.

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 E.C. 1975 Directive on bathing water Quality (E.C., 1976) which has a zero tolerance for *Salmonella* and enteroviruses in 10 litre volumes, 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 pathogen 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 pathogenic organisms in question, expected concentrations of pathogens in seawater or in shellfish would be much lower than those of indicators. Routine examination of wastewater or seawater for pathogenic microorganisms is rendered impracticable because: (a) no single procedure is available for isolation and identification of all pathogens, (b) negative findings for specific pathogens can only be considered provisional because state-of-the-art 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 the 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.

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 faecal indicators commonly used to evaluate assess marine pollution 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, references where such links were conclusively established, particularly in the case of outbreaks of diseases such as Hepatitis A, cholera.

A brief summary of available data on individual pathogens in the Mediterranean marine environment is given in the next 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.

2.2.1 Bacteria

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 former 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 direct infection as a result of bathing or other recreational activities is not very likely to occur due to the relatively high infective dose required in the case of most serotypes. On the other hand, the infective dose for *S. typhi* and *S. paratyphi* A and B is considerably lower. Consumption of seafood is a different problem, as the bacteria are concentrated either by filter-feeding shellfish or on fish gills. In shellfish, their bacterial concentration may be 50 times that in water (UNEP/WHO/IAEA, 1988).

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 survival 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.*

Cholera is one of the major diseases associated with the consumption of sewage-contaminated shellfish, and the causative agent, *Vibrio cholerae*, was discovered by Koch in Egypt during the 1883-84 epidemic. The 7th pandemic wave of cholera, due to *Vibrio El Tor*, which was originated in 1961 in Indonesia and is still active, spread to Mediterranean countries in the early '70's. 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 carriers, occurred in Italy in 1973 where it caused 277 cases and 24 deaths (Baine *et al.*, 1974). Brisou *et al.* (1962) isolated 44 strains of vibrios from the Algerian coast. Although only some of them are considered pathogenic, there is clear evidence that some strains of naturally-resident aquatic bacteria are capable of causing gastroenteritis, systemic infections and intoxications in humans (Shumway and Hurst, 1991). NAG (non-agglutinable) vibrios, which cause gastroenteritis, are also frequently found in shellfish in the region. As in the case of the *Vibrio cholera*, vibrios are also 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 *Vibrio* population in the effluent was the most diverse, including several species of sanitary interest such as *V. fluvialis*, *V. cholerae* (non-01) and *V. metschnikovii*; those three species, however, were not found in seawater or mussels.

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 humans 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 1979-80, 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. Recent studies indicate that the old concept considering *V. cholerae* as pathogenic organism found mainly in countries with no sanitary facilities and that survival of the type of *Vibrio* outside the human intestine is limited, has to be radically revised. *V. cholerae* and a number of other *Vibrio* species are commonly found as natural residents of aquatic environment in cholera-free areas and their presence is not necessarily associated with faecal contamination (West, 1989).

In the last 20 years increasing number of epidemiological and ecological observations (in U.S.A. and other countries) indicate that many environmental strains of *Vibrio* sp. are human pathogens. From 11 *Vibrio* species considered as causing illness in man (West, 1989), *V. cholerae* 01, *V. cholerae* non 01, *V. parahaemolyticus, V. vulnificus, V. mimicae* (previously as *V. cholerae*) and *V. alginolyticus* have been found more often associated with human illness, either through seafood consumption or water contact and abrasion. More attention has received *V. vulnificus*, which can cause fatal septicemia and gastroenteritis or wound infections. However, the presence of *Vibrionaceae* organisms, especially of *V. vulnificus* in seawater and shellfish depends on temperature and salinity, and it appears as seasonal findings (Kelly and Dan Stroh 1988, O'Neil *et al.* 1992, Kaspar and Tamplin 1993, Cook 1994).

Staphylococcus aureus and related species, particularly *S. epidermidis*, are potential pathogens associated with skin, skin glands and mucous membranes of warm-blooded animals including humans. 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 ater (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, 61% 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 seawater and sand of heavily-populated and slightly-populated beaches in Greece (Papadakis *et al.*, 1992) 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. Although *Staphylococcus aureus* is linked with food-poisoning in general, records of transmission through shellfish are relatively sparse. In Egypt, shellfish taken from highly-polluted water have been considered as of unacceptable quality due to the presence of pathogenic microorganisms, which include *S. aureus* (El-Sharkawi *et al.*, 1982).

Pseudomonas aeruginosa causes ear and eye infections as well as wound, burn and urinary tract infections and enteritis. Like *Staphylococcus aureus*, it is endemic in the Mediterranean as in other parts of the world. 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 vehicle of transmission.

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 of faecal pollution (*E. coli*) (faecal coliforms) was either low in concentration or absent (Yoshpe-Purer, 1987).

High concentrations of *Pseudomonas* species have also been recorded in shellfish. Volterra (1989) reported concentrations as high as 110,000/100 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.

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 (Helicobacter)* 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 humans (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 and 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 C) have been recorded from bathing waters (WHO, 1982), and their transmission to humans by this route is suspected.

Other non-enteric or not wholly enteric bacterial pathogens in the Mediterranean include *Aeromonas hydrophila*, which causes septicaemia in immunosuppressed hosts, diarrhoea, pneumonia, abscesses 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 beach waters 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 per 100 mL in *Donax trunculus* and 22,000 per 100 mL in *Ensis siliqua* (Volterra, 1989).

2.2.2. Viruses

More than 140 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 families, including enteroviruses (polioviruses, coxsackieviruses, and echoviruses), the new genus Hepatovirus family Picornaviridae (hepatitis A virus), reoviruses, adenoviruses, parvoviruses (adeno- associated viruses) and Calciviridae (Norwalk virus). These groups, with the number of types and the diseases carried, are outlined in Table 2.2.1.

The frequency of isolation and the quantity of virus recovered from sewage depend not only on the infections caused by naturally-occurring viruses and those induced by oral poliovirus vaccine, but also on the efficiency of the recovery procedures. According to Sellwood *et al.* (1981), the serotypes that can be detected at any specific time in sewage, except for polioviruses, reflect to a greater or lesser extent those viruses circulating 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

Table 2.2.1

Human enteric viruses that may be present
in polluted water (Schwartzbrod and Deloince, 1995)

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

present in urban sewage. In more than one Mediterranean country, polioviruses are detected in every sewage sample tested (Krikelis *et al.*, 1985).

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. Viruses so far identified in the Mediterranean marine environment are listed in Table 2.2.2.

As even a few plaque-forming units (PFU) or (as alternatively termed) cytopathogenic unit (CPU) of virus may lead to infection when swallowed, provided they reach target cells in the organism, the presence of human viruses in seawater has to be taken seriously, and the danger of infection as a result of bathing in polluted waters is therefore not imaginary (Katzenelson, 1977). Although epidemiological studies have not, so far, shown any clear correlation between swimming in polluted 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). In the Tyrrhenian Sea the concentration of enteroviruses in coastal waters ranged between 2 and 160 TCD_{50} per litre (Crovari *et al.*, 1974). In nearshore shallow waters (0.5 to 12 m deep) enteroviruses were shown to accumulate in clastic sediments, with concentrations ranging between 0.8 and 40.2 TCD_{50}/100 mL sediment eluate. Viruses were easily released into water by means of mechanical shaking simulating wave motions and bottom currents (De Flora *et al.*, 1975). Among other 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). In sewage effluents (after a biological treatment) in the central Mediterranean (French coast) the quantity of virus ranged during the years 1990-1991 from <1 to 250 MPNCU/L. (Hugues *et al.*, 1993).

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, and E, which however is not endemic in the Mediterranean, 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 an unidentifiable viral agent, rather than a bacterial pathogen (Geldreich, 1985).

A number of epidemics of Hepatitis A have occurred in Europe and worldwide over the last 30 years, in addition to the endemic background of sporadic cases that may total several hundred per year (Shuval, 1986). It has been indicated (Stille *et al.*, 1972) that consumption of contaminated molluscs accounted for an estimated 19% of the cases of

Table 2.2.2

Viruses isolated in the Mediterranean marine environment (WHO, 1991)

Virus	Type	Location	Matrix
Enteroviruses:			
Poliovirus	2 (vaccine-like)	Italy(a)	sediments
"	1,2,3 (vaccine-like)	Italy(b)	seawater
"	3	Italy	mussels
"	1,2,3	Greece	wastewater, seawater
Echovirus	3,5,6,8,9,12,13	Italy, France	mussels
"	9,11	Italy(b) France	seawater
"	1,3,7,15,19,21,23,30	Greece	wastewater
"	3,7,23	Greece	seawater
"	6, unspecified	France	seawater
"	11,5,20	France(c)	seawater
"	4,6,11,12,19	France(d)	wastewater
Coxsackie virus A	18	Italy	mussels
"	18	France	mussels
"	7	France	seawater
"	7,16,21	France	wastewater
Coxsackie virus B	1,2,3,5	Italy(b), France	seawater
"	1,2,3,4,5,6	Greece	wastewater
"	1,5,6	France	wastewater
"	4,5	France	seawater
"	2,4,5,6	Greece	seawater
"	1,6	France(c)	seawater
Hepatitis A virus		Greece	mussels
"		France	mussels
"		Spain	wastewater
Unspecified, non-polio		France	seawater
"		Italy(a)	sediments
Unspecified		Greece	wastewater, seawater
		Italy	wastewater, seawater
Other viruses:		France	wastewater
Adenovirus	1,2,3,4,5,7,15	Greece	wastewater
"	1, unspecified	France	wastewater
"	unspecified	France	seawater
"	unspecified	Italy	seawater
Rotavirus	unspecified	Spain	wastewater

(a) from De Flora *et al.* (1975)
(b) from Crovari *et al.* (1974)
(c) from Hugues (1994)
(d) from Hugues *et al.* (1993)

Hepatitis A in Frankfurt, and that 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).

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

2.2.3 Fungi

A number of fungal species are pathogenic to humans, 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.

Candida albicans, together with other *Candida* species, have 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). About 80% of the total isolations consisted of *Penicillium*, *Aspergillus* and *Cladosporium*. The latter two 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). A study on the microbiological content of beach sand and seawater in Israel has revealed a considerable fungal flora, which still awaits identification (Ghinsberg, 1994). 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.

2.2.4 Other microparasites

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*, *Heminolepis nanna, Oxyuris* and *Trichurus* are able to survive for months in the marine environment, and ingestion of a single egg is theoretically 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).

Protozoan parasites of either worldwide distribution or present in the Mediterranean region include *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli*, *Naegleria* and *Cryptosporidium pavum* 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.

2.2.5 Toxic algae

Blooms of toxic algal species are common occurrences in shellfish-growing areas worldwide. The algal species involved, which produce potent toxins, mainly belong to the dinoflagellate group. 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. The resulting 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).

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 Shumway'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. Saxitoxin was not detectable both in these samples and in shellfish collected from the market by the available methods (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.

Also, *G. breve* is a particularly fragile species which is broken up by the pounding action of surf. Aerosolised particles can be released that have, at least elsewhere in the USA and New Zealand, and associated with red algal tides, caused a respiratory irritation syndrome with dry cough and eye irritation (Steidinger and Joyce, 1973; Bates *et al.*, 1993).