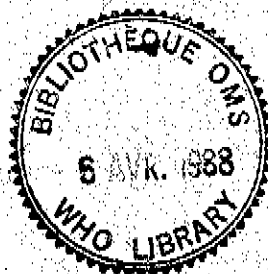




Long-term Programme for Pollution Monitoring and Research  
in the Mediterranean Sea  
(MED POL Phase II)



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MICROBIAL POLLUTION OF MEDITERRANEAN COASTAL AREAS  
AND ASSOCIATED HEALTH EFFECTS

Report on a Joint WHO/UNEP Consultation

Athens  
22-26 September 1987

1988

EUR/HFA target 20

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## TARGET 20

### Water pollution

By 1990, all people of the Region should have adequate supplies of safe drinking-water, and by the year 1995 pollution of rivers, lakes and seas should no longer pose a threat to human health.

#### Index:

WATER POLLUTION %AE%  
WATER QUALITY  
ENVIRONMENTAL MONITORING  
RESEARCH  
MEDITERRANEAN SEA

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## FOREWORD

A considerable proportion of municipal sewage originating from Mediterranean coastal cities is still discharged into the sea in its raw state. As a result, coastal waters, including many recreational beaches and shellfish-growing areas, are subject to varying degrees of microbiological pollution, and in many areas there is a significant incidence of diseases and other health disorders caused by, or known to be associated with, bathing in polluted seawater or consumption of contaminated seafood.

At their Fourth Ordinary Meeting in Genoa in September 1985, Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its Related Protocols adopted a formal declaration that included ten targets to be achieved during the period 1986-1995 (the second operational decade of the Mediterranean Action Plan). One of these targets consists in 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.

In the meantime, coastal water quality monitoring programmes are now operational in practically all Mediterranean countries. Some of these are long-standing. Most of the others have originated during the last few years within the framework of the long-term programme of pollution monitoring and research in the Mediterranean Sea (MED POL Phase II). In the case of recreational and shellfish waters, monitoring is still basically restricted to the orthodox microbiological parameters (i.e. bacterial indicator organisms), and studies carried out within the framework of the research component of MED POL Phase II over the past four years, particularly on the relationship between concentrations of the more commonly used indicator organisms and the presence of pathogens and on the correlation of seawater quality with health effects on bathers, have indicated the need to review these parameters so as to further ensure safety from adverse health effects. In addition, experience has shown that some of the standard methods used for determination of the concentrations of microbiological parameters in seawater require updating and modification, and the necessary research work in this regard has been in progress for the last few years, again within the research component of MED POL Phase II.

Epidemiological studies to correlate the microbiological quality of coastal recreational waters with health effects have commenced in a number of Mediterranean countries. The performance of such studies is necessary so as to ensure that environmental quality standards and criteria for such waters are in keeping with public health requirements, taking specifically Mediterranean conditions into account.

The present Consultation on Microbial Pollution of Mediterranean Coastal Areas and Associated Health Effects was jointly convened by WHO and UNEP within the framework of MED POL Phase II. Its objectives included the following:

- to review coastal recreational and shellfish water quality monitoring programmes in the region and to determine problem areas;

- to review the draft revised versions of the reference methods for determination of faecal coliforms and faecal streptococci in seawater;
- to review progress in research projects operational under MED POL Phase II in the areas of survival of pathogens, indicator/pathogen relationships, viral contamination of seawater, neurotoxins, and microbiological methodology, as well as in epidemiological and related studies on the correlation of coastal water quality and health effects;
- to formulate a joint research programme for 1988-1989 for studies on coastal recreational and shellfish waters, taking into account the requirements of the programme;
- to formulate a preliminary list of pathogenic microorganisms, which would have to be considered in terms of Annex II to the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources.

A number of experts from Mediterranean institutions that were either already participating in the microbiological or epidemiological components of the appropriate research activities of MED POL Phase II, or had shown a positive interest in participating, were invited to attend the Consultation. In addition, the following international organizations were invited to send representatives: the Food and Agriculture Organization of the United Nations (FAO), the Intergovernmental Oceanographic Commission (IOC), the United Nations Educational, Scientific and Cultural Organization, the World Meteorological Organization, the International Atomic Energy Agency and the Commission of European Communities.

### 1. Opening of the meeting (agenda item 1)

The meeting took place in the offices of the Coordinating Unit for the Mediterranean Action Plan, Athens, from 22 to 26 September 1987. It was attended by 17 temporary advisers from eight Mediterranean countries, one representative each from FAO, IOC and UNEP, and one staff member of the WHO Regional Office for Europe. A list of participants is given in Annex 1.

Dr L.J. Saliba, Senior Scientist, Mediterranean Action Plan, WHO Regional Office for Europe, opened the meeting and welcomed the participants on behalf of the Regional Director, Dr J.E. Asvall. He briefly outlined the events leading to the meeting and explained its context within the general framework of the Long-term Programme of Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase II).

Mr A. Manos, UNEP Coordinator of the Mediterranean Action Plan, welcomed the participants on behalf of Dr Mostafa K. Tolba, Executive Director of UNEP, as well as on behalf of the Coordinating Unit for the Mediterranean Action Plan. He stressed the importance of the subject to be discussed. During their Fourth Ordinary Meeting in Genoa in September 1985, Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its Related Protocols had approved, on an interim basis, environmental quality criteria for bathing waters. It was important that firm criteria consonant with the requirements of the region be eventually developed, and the MED POL Phase II programme had, as one of its major objectives, the performance of studies on the results of which these and other criteria could be so developed. He augured all participants a fruitful meeting.

### 2. Scope and purpose of the meeting (agenda item 2)

Dr L.J. Saliba outlined the scope and purpose of the meeting. The MED POL monitoring programme was going well in the field of coastal recreational waters, but, to date, no monitoring of shellfish waters had been performed. In this regard, it was important for the meeting to review the *Guidelines for monitoring the quality of coastal recreational and shellfish-growing areas* before issue of the first substantive version of the publication. This would ensure a further degree of harmonization between participating laboratories. It was similarly essential to review current research projects of a microbiological nature and ensure that future work would be geared more intimately to the actual needs of the programme. Furthermore, by 1989, it was planned to prepare the first assessment on the state of pollution of the Mediterranean Sea by pathogenic microorganisms, and one of the tasks of the present meeting was to formulate a preliminary list that could also eventually assist Mediterranean governments in their compliance with the appropriate obligations under Article 6 and Annex II of the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources.

### 3. Election of officers (agenda item 3)

Professor F.M. El Sharkawy was elected Chairman, Dr A. Vassallo Vice-Chairman and Ms Susana Sotirakopoulos Rapporteur. Dr L.J. Saliba acted as Secretary to the meeting.

4. Adoption of the agenda (agenda item 4)

The provisional agenda was unanimously adopted.

5. Organization of the meeting (agenda item 5)

The meeting agreed on the general organization of its work, including session hours and other arrangements. It was agreed that all work would be held in plenary apart from specific topics, such as detailed review of reference methods, for which small working groups would be established.

6. Review of results obtained in coastal recreational and shellfish water quality monitoring programmes within the framework of MED POL Phase II (agenda item 6)

Participation in the MED POL Phase II monitoring programme was organized on a country basis through an agreement signed between the national MED POL Coordinator (on behalf of the national authorities concerned) and the United Nations Environment Programme. Agreements were comprehensive and covered pollution sources, coastal waters and reference areas. The microbiological component was mainly contained in the second of these three sections. The agreement, renewable annually, specified the monitoring areas and stations, matrices and parameters to be monitored in each, together with monitoring frequency, the institutions responsible for each aspect of the monitoring, and the assistance to be provided. To date, national MED POL monitoring programmes had been signed with ten Mediterranean States (Algeria, Cyprus, Egypt, Israel, Lebanon, Libya, Malta, Morocco, Syria and Yugoslavia). All these agreements incorporated a microbiological component, related mainly to the monitoring of coastal recreational waters, but in certain cases also covering shellfish waters and/or municipal effluents. Apart from these ten countries, another six (France, Greece, Italy, Monaco, Spain and Turkey) had either long-standing monitoring programmes organized at national or regional level or carried out monitoring of coastal recreational and shellfish waters on a regular basis. These programmes were, however, independent of MED POL and organized on the basis of national requirements. Evaluation of results was also carried out under the terms of current national legislation and/or administrative procedures.

Within the framework of agreements signed as part of the MED POL Phase II monitoring programme, in the ten participating countries a total of 45 institutions were engaged in microbiological monitoring (involving mainly coastal recreational waters). Between them, a total of 402 stations (347 in coastal waters and 55 in effluents) were being monitored regularly. All institutions were determining faecal coliforms. In addition, some were also determining total coliforms and faecal streptococci. A number of institutions were also determining concentrations of other microbiological parameters, either regularly or occasionally. This, however, was outside the framework of the MED POL agreements, and normally results were not reported. Details of country participation (institutions, sampling stations and parameters monitored) are given in Table 1.

Guidelines for monitoring the quality of coastal recreational and shellfish waters had been developed for the MED POL programme, and the first substantive version of this document, incorporating comments and suggestions by a number of expert group meetings, had been finalized for issue in late 1987. For coastal recreational waters, microbiological parameters to be

Table 1. Country participation in the microbiological component of the MED POL Phase II monitoring programme

Country	Number of institutions	Number of stations		Parameters monitored <sup>a</sup>
		Coastal waters	Effluents	
Algeria	21	42	-	Total coliforms, faecal coliforms, faecal streptococci
Cyprus	1	88	-	Faecal coliforms
Egypt	1	14	5	Faecal coliforms
Israel	3	64	-	Faecal coliforms
Lebanon	1	9	-	Faecal coliforms
Libya	1	8	-	Total coliforms faecal coliforms, faecal streptococci
Malta	3	7	4	Total coliforms, faecal coliforms faecal streptococci
Morocco	4	21	10	Total coliforms, faecal coliforms faecal streptococci
Syria	1	5	9	Faecal coliforms
Yugoslavia	9	89	27	Faecal coliforms

<sup>a</sup> In seawater. In effluents, only faecal coliforms are being monitored.

determined in "minimum" monitoring programmes were faecal coliforms and faecal streptococci, and at least one pathogen causing infection through contact, the species to be determined according to local circumstances. In "extended" monitoring programmes, apart from the minimum parameters, a number of additional ones (including both indicator organisms and pathogens) were indicated, the selection to depend on national or local requirements. It was agreed that these additional parameters would be reviewed by the meeting under the appropriate agenda item. Apart from serving to update the guidelines before the issue of the first substantive version, the list could also serve as a preliminary indication of what pathogens countries should consider in terms of Annex II to the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources.

The meeting reviewed the draft revised version of the *Guidelines for monitoring the quality of coastal recreational and shellfish-growing areas* (Reference Methods for Marine Pollution Studies, No. 1). Various modifications were agreed on, concerning mainly the pathogens and indicator organisms considered as important in extended monitoring programmes. The meeting recommended that such modifications should be incorporated in the first substantive version of the document to be issued towards the end of 1987 or early in 1988.

To facilitate comparability of results obtained by different laboratories in the various countries, which would be of extreme importance when carrying out assessments of the state of microbiological pollution of the Mediterranean as a whole, a series of standard reference methods had been developed for determination of the main parameters, including the orthodox bacterial indicator organisms by both techniques in current use in the region: the membrane filtration culture (MF) method, and the multiple test-tube, or most probable number (MPN), method, as well as supporting parameters (BOD, COD, nitrogen and phosphorus). A number of methods for determination of the more common pathogens had also been prepared. This list, as well as each individual method, was constantly being updated. It was agreed that the list should continue to be extended as far as possible, and should accord more importance to pathogenic fungi as well as bacteria. Also, the methods should continue to be based on existing ones, geared to the capabilities of Mediterranean laboratories.

The monitoring component of MED POL Phase II included intercalibration and quality control. In the case of microbiological parameters, the possible distribution of standard samples had been considered but discarded because of the time factor involved in such distribution (i.e. the necessary unavoidable delay between preparation or collection of the samples at the central reference point and their arrival in participating laboratories). A series of intercalibration exercises in microbiological methods for coastal water quality monitoring had been organized between 1982 and 1985. These had involved the bringing together of selected scientists from different participating institutions to "central" laboratories, where the same samples were analytical by each participant, using the same methodology and the same standard equipment. The microbiological parameters involved in this series of exercises were mainly faecal coliforms and faecal streptococci in seawater (using both the MF and MPN methods) and faecal coliforms in shellfish (using the MPN method). Results obtained had shown differing degrees of variation between individual readings, due partly to the disparity in experience between the participants, especially in the interpretation of results. As a result, it had been concluded that the whole series, while proving extremely useful from the viewpoint of training, had a less successful outcome in terms of assurance of comparability and intercalibration, particularly since there was no guarantee that each "home" laboratory would have the same facilities and environmental conditions as those prevailing in the institutions where the group exercises had been carried out. It had therefore been proposed to include two short training courses in microbiological methods (one in English and one in French) in the MED POL work plan and budget for the 1988-1989 biennium. The 1988 course had been approved by the Fourth Ordinary Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its Related Protocols in Athens in September 1987 (two weeks before the present meeting). The detailed activities for 1989 would be considered by the Contracting Parties within the framework of the approved global budget at the "expanded bureau" meeting in September 1988. In accordance with the recommendations of the Working Group on Scientific and Technical Cooperation for MED POL, the planned exercises would be designed primarily for young scientists involved in microbiological monitoring within the framework of MED POL, and would be restricted to determination of faecal coliforms and faecal streptococci in seawater (by the MF and MPN methods) and faecal coliforms in shellfish (by the MPN method). Emphasis would be given to basic methodology, quality control and interpretation of readings.

The meeting noted that the environmental quality criteria adopted by Contracting Parties for recreational waters and shellfish waters in 1985 and 1987 respectively were confined to only one microbiological parameter: i.e. faecal coliforms. It was accepted that this possibly constituted the only practicable common measure that Mediterranean States could adopt. However, taking all circumstances into consideration, the participants agreed that in the formulation and implementation of national monitoring programmes, other parameters, depending on local conditions, should also be monitored.

The meeting also noted the steps that WHO intended to take with a view to improving the microbiological component of the MED POL monitoring programme, as already agreed to by the WHO Consultation on Health-related Aspects of Marine Pollution Control in the Mediterranean, held in Copenhagen from 29 June to 4 July 1987. These lines of action included extension of direct contacts with health laboratories in the region already performing microbiological monitoring of coastal recreational and shellfish areas, with the aim of eventually drawing them into full participation in the MED POL programme, the provision of all relevant information about the Mediterranean Action Plan to the health authorities of the region to assist in the avoidance of duplication with other programmes and, from the technical viewpoint, the promotion of training programmes and increased contacts between different laboratories.

7. Review of revised draft versions of reference methods for determination of (a) faecal coliforms and (b) faecal streptococci in seawater (agenda item 7)

7.1 Determination of faecal coliforms in seawater by the MF method

The standard reference method for determination of faecal coliforms in seawater by the MF method, developed for use by Mediterranean laboratories participating in the MED POL II programme, defined faecal coliforms as aerobic and facultatively anaerobic, gram-negative non-spore-forming rods that fermented lactose while producing acid and gas, at both 36°C and 44°C, in less than 24 hours, and produced indole in tryptone water containing tryptophan at 44°C. The method also recommended that suspect and doubtful colonies could be tested for acid and gas development by means of a confirmatory test using MacConkey broth or brilliant green bile broth and that, in areas where industries discharged polysaccharides (paper mills, sugar beet industries, etc.), further confirmation by the indole test might be necessary.

During an intercalibration exercise and consultation meeting on microbiological methods for coastal water quality monitoring convened jointly by WHO and UNEP in Athens from 25 to 29 June 1984, it had been considered that, since it was being recommended that for faecal coliform determination Mediterranean laboratories should be free to use either the MF or MPN method, the ability to use lactose and grow at 44°C should be the only metabolic process on which to base identification, and the formation of indole should therefore be omitted. Furthermore, it had also been considered by a number of the participants at the above-mentioned meeting that indole production alone would exclude the *Escherichia coli* type. However, lactose use at 44.5°C as the only criterion would introduce confounding factors when testing seawater near industrial areas, as the presence of *Klebsiella* could result in high counts when determining faecal coliforms.

The value of *E. coli* as an indicator organism had also been discussed. Some epidemiological approaches to the selection of the best indicator organism for establishing a correlation between coastal water quality and health effects had demonstrated that enterococci and *E. coli* provided a better correlation with the occurrence of gastrointestinal disease than did *Klebsiella*, *Enterobacter* and *Citrobacter*. During a consultation on environmental quality criteria for shellfish-growing waters and shellfish in the Mediterranean, convened jointly by WHO and UNEP in Athens from 26 to 27 March 1987, it had been noted that differences in the bacterial flora of the waters in various parts of the Mediterranean could lead to noncomparable determinations of faecal coliforms, and, in future, recourse to *E. coli* as a more specific indicator organism could be considered as an alternative. In this regard, however, the meeting had considered that comparability studies in the different countries were necessary and had recommended that such studies, both on the comparability of faecal coliforms and *E. coli* and on the usefulness of other indicators should be undertaken within the framework of the research component of MED POL Phase II.

The meeting discussed the recently completed study on the quantitative determination of *E. coli* from faecal coliforms in seawater, which had been performed within the framework of the research component of MED POL Phase II by Dr A. Mates of the District Public Health Laboratory, Haifa, Israel. In this study, a simple rapid method was developed for counting *E. coli* in seawater using the MF method. Following filtration, the membrane filters were incubated on mFC medium for  $24 \pm 2$  hours at  $44.5 \pm 0.2^\circ\text{C}$  for the determination of faecal coliforms. An *in situ* test for determination of *E. coli* was performed by transferring the membrane filters to nutrient agar containing 4-methylumbelliferyl-B-D-glucuronide (MUG) and incubating for 3 hours at  $35^\circ\text{C}$ , followed by detection of *E. coli* colonies by their fluorescence under long-wave UV light. Extensive biochemical confirmation tests on the isolates showed that all the fluorescent colonies (either lactose-positive or lactose-negative) were *E. coli*. False negative reactions amounted to 8.4%. According to the author, the MUG test was simple, found to be specific for *E. coli* that were either lactose-positive or negative, and had no connection with indole production. As a result of the short incubation period and the release of the end-product of MUG that diffused into the surrounding medium, membrane filters containing up to 35 colonies per filter could easily be counted. The results obtained by this method gave an indication of both faecal coliforms and *E. coli*, thus providing a clue as to the source of contamination.

The study had provided the basis for possible modifications to the current version of the reference method for determination of faecal coliforms in seawater by the MF method, by the proposed addition of a test for *E. coli*. While still leaving the determination of faecal coliforms as such as a self-contained (and the main) component of the reference method, it would provide for the necessary comparability studies through the monitoring component of MED POL itself, thus enabling the acquisition of the relevant data on which future action at the appropriate time could be based.

Following comprehensive discussion, the meeting agreed that the reference method in question should be expanded to include the determination of *E. coli*. However, it was noted that the MUG test was comparatively new, and few laboratories in the Mediterranean had experience in its use. In this regard, it was agreed that the MUG method should be compared with other methods under Mediterranean conditions, preferably by more than one laboratory.

## 7.2 Determination of faecal streptococci in seawater by the MF method

The standard reference method for determination of faecal streptococci in seawater by the MF method, developed for use by Mediterranean laboratories participating in the MED POL Phase II programme, laid down KF-streptococcus agar as the culture medium to be used. The same medium was recommended in the American Public Health Association's *Standard methods for examination of water and wastewater*.

Following reports from a number of Mediterranean laboratories to the effect that they were getting relatively high counts of faecal streptococci as compared to those obtained for other bacterial indicators in the same samples, it was considered that the designated medium might be responsible. Originally developed for determination of faecal streptococci in potable water, its value for seawater appeared dubious as many naturally occurring marine bacteria were able to grow on it and produce colonies difficult or impossible to distinguish from faecal streptococci, thus producing abnormally high counts, the majority of which were false positives.

A study on the determination of the most suitable medium for enumeration of faecal streptococci in seawater, performed by Dr Yona Yoshpe-Purer of the "A. Felix" Public Health Laboratory, Tel-Aviv, Israel, within the framework of the research component of MED POL Phase II, had recently been completed. The meeting discussed the results of this study, as well as the conclusions drawn by the author.

The study consisted primarily of a comparison of KF-streptococcus agar, m-enterococcus agar and bile-esculin azide agar at 35°C and 42°C. All these media were recommended in standard literature. Results showed that, while none of these media could be described as really specific for faecal streptococci, m-enterococcus agar was superior to KF-streptococcus agar in the monitoring of seawater as, although the former could be termed as only slightly more selective for faecal streptococci, it was much more specific through inhibiting the growth of many marine bacteria that gave false positive results on the latter medium. At first sight, therefore, there appeared to be a case for amending the relative reference method by substituting KF-streptococcus agar by m-enterococcus agar.

The meeting agreed that the results of the study carried out indicated that, until a medium specific for faecal streptococci in seawater was developed, m-enterococcus agar appeared to be better indicated as a culture medium than KF-streptococcus agar. However, in view of the fact that the study was carried out in only one locality and the naturally occurring bacterial flora was known to differ in the various parts of the Mediterranean, it was considered that further comparison between the two media should be made in a number of other localities before a decision as to whether the currently recommended medium (KF-streptococcus agar) was taken.

## 8. Review of research projects operational within the framework of MED POL Phase II (agenda item 8)

Microbiological and related projects being undertaken within the framework of the research component of MED POL Phase II could be described as falling under the following broad categories:

- development of sampling and analytical techniques for determination of pathogenic and indicator microorganisms;

- formulation of the scientific rationale for microbiological quality criteria;
- epidemiological studies related to the confirmation, or eventual revision, of existing microbiological quality criteria for bathing waters, shellfish-growing waters and edible marine organisms;
- development of proposals for guidelines and criteria related to the design of submarine outfall structures for municipal waste discharge, including pretreatment;
- survival of pathogens in the Mediterranean Sea.

In the first activity, seven projects had been completed since 1982 and four were currently ongoing. All these projects were concerned with the development of microbiological methodology for the determination of bacterial indicator organisms, including comparisons between different methodologies. From the purely technical point of view, the only projects considered and accepted under this particular activity were those pertaining to situations where the development of a method or the modification of an existing one required actual field or laboratory research to solve one or more problems specifically linked to prevailing Mediterranean conditions. Results obtained so far from completed projects, apart from the suggested recommendations for revision of the two reference methods on determination of faecal coliforms and faecal streptococci respectively (considered by the meeting under agenda item 7), included (a) a preliminary indication on the prevalence of pathogenic fungi in bathing beaches (mainly in sand), together with a draft reference method for determination of *Candida albicans*; (b) data on the comparability of the two principal methods (the MF and MPN methods) used in the Mediterranean for determination of the three main bacterial indicator organisms in seawater (total coliforms, faecal coliforms and faecal streptococci), which had enabled the recommendation of both methods to MED POL participating laboratories as recognized alternatives; and (c) data on fluctuations in the population density of a number of bacteria (including the three above-mentioned indicators) as a result of various environmental factors. Ongoing projects dealt with the feasibility of using bacteriophages as indicators of viral pollution of coastal seawater, the comparative distribution of microbial and fungal populations in seawater and sand, and the development of a simple rapid method for determination of *E. coli* in seawater.

In the second activity, microbiological research projects had so far been restricted to comparability studies on the various methodologies used in Mediterranean countries to determine the microbiological quality of coastal recreational and shellfish-growing waters, including evaluation of results, with the aim of developing common criteria which, while ensuring that the necessary safety standards would be met, would also ensure, in so far as possible, the minimum amount of modification to existing national criteria and statutory methodological procedures. So far, these studies had resulted in the development, and adoption by Contracting Parties, of interim environmental (microbiological) quality criteria for both recreational and shellfish waters. Ongoing projects now also included the comparative value of bacterial and chemical indicators in the assessment of coastal water pollution, and comparative studies on the relationship between bacterial indicator organisms and eutrophication, including plankton blooms.

The third activity formed one of the major research and study fields. One major component constituted microbiological-epidemiological studies on the correlation between microbiological water quality and health effects. One project recently completed in this field had confirmed a definite relationship in the Mediterranean between bacterial indicator and pathogen levels in coastal recreational beaches and gastrointestinal (and, to a lesser extent, non-gastrointestinal) disease incidence among bathers. Another study correlating microbiological water quality with the incidence of virus infections was in progress. Other studies performed within the framework of the same activity included the relationship between gastrointestinal pathogens and bacterial indicator organisms in polluted seawater, the incidence of enteric viruses in marine coastal waters, and the incidence of fungi in polluted beaches, together with an assessment of their impact on human health. Ongoing projects also included an assessment of dermal diseases in bathers and a study on the relationship between tourist activity and coastal pollution.

The fourth activity contained a relatively small microbiological component, included in studies leading to the development of guidelines for the design of submarine outfall structures for municipal effluents. This was currently being covered by one research project aimed at the development of a computer model for outfall design and by an *ad hoc* multilaboratory project on the monitoring of selected outfall structures in the Mediterranean, including bacterial determinations both at the source itself and in affected areas in the immediate vicinity.

Research projects in the last activity had nearly all been concerned with the various environmental factors affecting survival time of bacteria in the Mediterranean marine environment, including relationship with the point of discharge. Completed projects had dealt with bacterial indicator densities along outfall plumes, accumulation in sand and sediments, and survival of pathogenic and indicator organisms under the influence of physicochemical factors. Ongoing projects dealt with similar themes and also included a study on the adaptation of pathogenic enterobacteria to seawater.

Altogether, 19 projects, either totally or partially of a microbiological nature, had been completed and another 18 were ongoing. Completed projects would now be further reviewed and results evaluated.

The meeting recognized that there was a varying degree of overlap between the various overall activities as originally approved by the Contracting Parties at their Second Ordinary Meeting in Cannes in March 1981 (when the MEDÚPOL Phase II programme was adopted). However, it was equally recognized that any recommendations on possible restructuring were outside the scope of the meeting, and a matter of more immediate importance was the listing of research and related work necessary for the further prevention and control of microbiological pollution in the Mediterranean region. It was also understood that, in keeping with general policy, research performed within the framework of MED POL would necessarily have to take into account similar work in progress under the aegis of other programmes, so as to prevent undue duplication and/or overlap and to ensure maximum cost-effectiveness. In this regard, the meeting was informed that, in accordance with a recent overall decision regarding the reorientation of the whole research component of MEDÚPOL Phase II, priority would now be accorded to research projects of purely Mediterranean dimensions as opposed to those reflecting overall global interest. This would therefore involve the application of existing basic data

to Mediterranean conditions, with such modifications as available local experience, or where necessary local research, dictated.

9. Formulation of a joint research programme for 1988-1989 for coastal recreational and shellfish waters, including epidemiological studies on health effects (agenda item 9)

The meeting was informed of the current line of approach to reorientation of those components of MED POL research under the responsibility of WHO. Instead of relying mainly on the current practice of passive consideration of proposals originating from Mediterranean laboratories, a renewed emphasis on preselection of, and agreement on, proposals before formal submission through recognized MED POL channels would be pursued. This line of approach had already paid considerable dividends during the last three years, and a number of gaps in essential action-oriented microbiological research had been identified, and the necessary data obtained through appropriate research projects. Because of the limited financial resources available to the MEDÜPOL programme, every effort would have to continue to be made to identify, to the maximum extent possible, those institutions in the region that were already performing identical, or at least similar, research to that required as part of their own national or institutional programmes, and which could be drawn into or at least linked with the relevant (in this case microbiological and epidemiological) components of MED POL with minimal financial repercussions.

The availability of extra sources of funds was particularly important with regard to those project areas where the work involved was, by its very nature, extensive and resource consuming. This was the case with epidemiological studies involving correlation of coastal water quality with health effects where, apart from regular monitoring of the microbiological quality of the water in specific areas, a considerable amount of field survey work in connection with the occurrence of disease was necessary. In this context, the data obtained would be of particular benefit to the national or local authority carrying out or commissioning the studies, as they would provide the basis for remedial or preventive measures adapted to their own specific circumstances, thereby justifying the expenditure involved.

Another point, however, would also have to be taken into account. Mediterranean coastal resorts were used, for a significant portion of the year, by a large number of tourists from outside the region, particularly from northern Europe. For this reason, WHO was making all possible efforts to obtain at least partial financing of appropriate projects linking coastal water quality with health effects from funding sources in those non-Mediterranean countries regularly sending tourists to the Mediterranean region, in the form of voluntary donations. So far, regrettably, no concrete results had been achieved although efforts were continuing.

The meeting agreed on the broad lines of microbiological research required to meet the objectives of the Mediterranean Action Plan in general and the MED POL programme in particular, and that the 1988-1989 research component should emphasize such aspects. One necessity was the updating of existing information on diseases present in the Mediterranean region caused by pathogenic microorganisms and contractable through exposure to contaminated seawater and/or sand in marine coastal recreational areas or through consumption of contaminated marine seafood. In this regard, it was pointed out that, so far, insufficient attention appeared to have been accorded to pathogenic fungi, and this would have to be rectified. Another need was for

the identification of indicator organisms for non-gastrointestinal pathogens and also for viruses. In the latter case, the current and medium-term capabilities of most Mediterranean countries precluded the isolation of the pathogen itself. Moreover, the accumulating evidence of the gradual adaptation of various indicator organisms and pathogens to the marine environment and of accompanying changes in their characteristics (including their virulence) necessitated research in this field under conditions specific to the Mediterranean region.

Apart from work of a general regional nature, it was also essential to acquire the necessary data on the relative importance of the various individual diseases attributable to marine pollution in the different geographical subregion of the Mediterranean coastline. At local level, it was necessary to determine the relationships between specific pollution sources and affected areas, with a view to minimization of problems at source wherever possible.

Regarding specific lines of action, the need for more research on the occurrence of diseases associated with contact with contaminated water or sand was expressed. These included dermatophyte diseases (caused by fungi), otitis (*Pseudomonas aeruginosa*), mucosal wounds and infections (staphylococci, *Aeromonas*), ocular infections (staphylococci, *Pseudomonas*), skin infections (*Bacteroides*) and diseases caused by ectoparasites. Regarding diseases caused by seafood consumption or ingestion of seawater, more data was required on hepatitis virus A, rotavirus, enteroviruses, salmonellosis, enterotoxic diseases (toxins of *Clostridium*, *E. coli*, staphylococci, vibrios and dinoflagellates), and kidney infections (*Leptospira*). Work was also necessary on other gastroenteric diseases (*Lactobacillus*, *Bifidobacterium*, enteropathogenic *E. coli*, adenovirus, Norwalk virus, *Aeromonas*, *Campylobacter* and *Yersinia*). Parasite infections were also considered as an important field of investigation.

In particular, attention was drawn to the need for further adequately conducted epidemiological studies, and to the importance of the creation of a sense of awareness and understanding of the considerable amount of organization and expenditure necessitated by their obvious complexity. In this regard, the meeting reviewed the protocol for microbiological-epidemiological studies to correlate the quality of coastal recreational waters in the Mediterranean with health effects. Reservations were expressed about the feasibility of successfully implementing such a protocol in most Mediterranean countries. For this reason, and because of the financial implications of such studies, it was considered that immediate action should take the form of relatively simpler pilot studies. Apart from having a common basis to enable the necessary comparability of results, such studies would also have to take into account the conditions prevailing in the different countries. A simpler protocol to cover the interim period therefore had to be developed.

Most participants indicated that their institutions could possibly participate in a microbiological-epidemiological study on the basis of a simplified protocol. The meeting agreed that such a study would require an interdisciplinary approach, involving microbiologists, sanitary engineers, epidemiologists, statisticians and clinicians, and that locally available resources should be drawn on to the fullest possible extent. The study should involve both local populations and tourists. It was pointed out, however, that because of the relatively high cost of such studies, even on the basis of

a simplified protocol, apart from funds obtained from national or local sources, WHO and UNEP should allocate more funds to such projects from the Mediterranean Trust Fund, and also that WHO should continue to seek other sources of funding, possibly through voluntary donations.

10. Formulation of preliminary list of pathogenic microorganisms to be recommended for consideration in terms of Annex II to the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources (agenda item 10)

As a basis for discussion, the meeting considered the list of pathogenic microorganisms contained in the *Guidelines for monitoring the quality of coastal recreational and shellfish-growing areas* (Reference Method for Marine Pollution Studies, No. 1), reproduced in document ICP/CEH 052/10.

It was agreed that pathogenic microorganisms should be broadly categorized into those that could cause infection through (a) ingestion and (b) contact.

It was considered that the more important pathogens causing infection through ingestion were the following.

Salmonellae. These do not survive long in seawater, and infection directly as a result of bathing or recreational activities is not very likely owing to the relatively high infective dose required. On the other hand, for *Salmonella typhi* and *S. paratyphi* A and B, the infective dose is considerably lower. Consumption of seafood is a different problem because of concentration of the bacteria either by filter-feeding shellfish or on fish gills. In shellfish, the concentration may be fifty times that in water. This route of infection assumes importance when associated with consumption of raw seafood such as oysters and mussels.

Pathogenic forms of *E. coli*. Enteropathogenic *E. coli* causes gastroenteritis. Infection is not very likely to occur through ingestion of seawater as a result of bathing, but contaminated shellfish could be an important route of transmission.

Vibrios. *Vibrio cholerae*, NAC vibrio and *V. parahaemolyticus* are all important pathogens, the most likely route of infection being through consumption of raw shellfish rather than recreational activity. It should be noted that no correlation exists between *V. parahaemolyticus* and sewage pollution. Other vibrios, such as *V. alginolyticus*, could also be important.

Enteric viruses. There is still relatively sparse information on waterborne transmission of viral disease, and research required include the development of appropriate techniques to concentrate the very dilute virus suspensions. Unlike bacteria, viruses are not effectively inactivated by conventional chlorination of sewage, and there is evidence that they survive longer in seawater than many bacterial indicators and pathogens. From the evidence available, human health hazards from viruses in the marine environment are concerned mainly with seafood consumption.

Campylobacter. *Campylobacter jejuni* and *C. coli* cause both diarrhoea and fever and can be transmitted through ingestion of water and seafood consumption. Considerable basic study on these organisms is, however, still required.

Shigellae. These are causative agents of bacillary dysentery, and cases reported from the Mediterranean call for a more comprehensive assessment of the situation than has hitherto been undertaken.

Yersinia. This has been recognized as an important type of bacteria in terms of epidemiology and zoonoses, and its presence has been demonstrated in seawater.

Aeromonas hydrophila. This 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.

Parasites. While little information is available on the human health aspects of contamination of the marine environment by parasites such as nematodes (e.g. *Ascaris*, *Trichurus*, *Toxoplasma*, *Oxyuris*, etc.), it appears that there is little danger of infection through seawater ingestion. On the other hand, nematode eggs as well as protozoans such as *Entamoeba*, *Giardia* and *Naegleria* can constitute a health hazard from consumption of contaminated seafood, particularly when mussels and oysters are collected from areas in the vicinity of sewage outfalls.

As regards contact pathogens, the following were considered as the most important.

Staphylococci. Coagulase-positive strains are potential pathogens, causing a wide range of infections. As they are salt-tolerant, they are able to survive in the marine environment, constituting a potential hazard to bathers on crowded beaches.

Pseudomonas aeruginosa. This is a potential pathogen that has been increasingly implicated in ear, throat and skin infections through bathing in contaminated waters.

Pathogenic fungi. These are very important causative agents of contact diseases. *Candida albicans* is present in both seawater and sand. The presence of *Pityrosporum furfur* and of other dermatophytes in sand play a considerable role in the occurrence of superficial dermatomycoses. *Microsporum canis*, resulting from the presence of healthy carrier dogs on beaches, has also been reported as an agent of *Taenia capitis* in children.

It was agreed by the meeting that the relative importance of a number of pathogens varied from country to country. It was therefore important that the final list of pathogenic microorganisms developed should be issued as a general guideline, but should also indicate as much geographical distribution as possible on the basis of records collected from the various countries.

## 11. Recommendations

The meeting made the following recommendations:

- (1) The proposed amendments to the document *Determination of faecal coliforms in seawater by the membrane filtration culture method* (Reference Methods for Marine Pollution Studies, No. 3) should be incorporated into a revised draft version to be submitted to Mediterranean laboratories for comments. Before this, however, the methylumbelliferyl glucuronide medium

should be tested by a number of Mediterranean laboratories. In this context, a standardized supply of this medium should be distributed to such laboratories.

(2) The proposed amendments to the document *Determination of faecal streptococci in seawater by the membrane filtration culture method* (Reference Methods for Marine Pollution Studies, No. 4) should be deferred until more information is available on the comparability of the current and proposed media for use in monitoring marine water in various parts of the Mediterranean. Arrangements should be made for such comparability tests.

(3) The conclusions of the Consultation on modifications to the *Guidelines for monitoring the quality of coastal recreational and shellfish-growing areas* (Reference Methods for Marine Pollution Studies, No. 1) should be distributed to Mediterranean laboratories for comments, so that a suitably revised version can be issued as soon as possible.

(4) The microbiological and related monitoring of coastal water used for recreation and shellfish cultivation should be linked, as far as possible, with monitoring of pollution sources. This will allow more comprehensive evaluation and indicate the remedial action required.

(5) As soon as possible, WHO should develop a simplified version of the current protocol for epidemiological and microbiological studies to correlate the quality of coastal recreational water with effects on health.

(6) Microbiological and related research within the framework of MED POL Phase II should be redirected towards action to fulfil the objectives and needs of the programme. Such action includes:

- the determination of the correlation between the main bacterial indicator organisms, to facilitate the interpretation of results among institutions using different indicators;
- the determination of indicators for viruses and potential pathogens, particularly agents causing non-gastrointestinal diseases associated with contaminated coastal water;
- the study of the environmental factors affecting the fate of viruses;
- the study of the somatic and genetic changes of pathogenic and indicator microorganisms in the marine environment, with special reference to their virulence;
- comparative studies on pathogens of recognized importance, such as *Campylobacter* spp., by networks of Mediterranean institutions;
- epidemiological studies correlating the quality of coastal water with effects on health;
- studies on the prevalence of fungal pathogens and of parasites on beaches, along with their possible impact on health.

(7) In the allocation of funds to MED POL research projects, particular note should be taken of the fact that epidemiological and microbiological studies require a larger degree of assistance owing to their relatively high costs.

(8) All possible avenues of obtaining complementary funding for epidemiological and microbiological studies should continue to be explored. In particular, voluntary donations could be solicited from funding institutions in European countries from which tourists regularly come to the Mediterranean region.

Annex 1

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