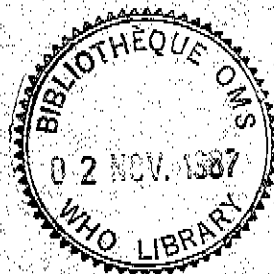


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Long-term Programme for Pollution Monitoring
and Research in the Mediterranean Sea
(MED POL Phase II)

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ENVIRONMENTAL QUALITY CRITERIA FOR SHELLFISH-GROWING WATERS AND SHELLFISH IN THE MEDITERRANEAN

Report on a joint WHO/UNEP meeting

Athens
26-27 March 1987



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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:

SEAWATER %ST%
SHELLFISH %ST%
MICROBIOLOGICAL TECHNICS
MEDITERRANEAN SEA

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FOREWORD

The consumption of shellfish raised in the Mediterranean is estimated at approximately 12,000 metric tons per year. This constitutes one of the principal kinds of human exposure to microbial pollutants, especially in coastal areas where shellfish are consumed most, principally raw, by both local populations and tourists, in view of the fact that shellfish-growing waters in the region, especially if located in the vicinity of inhabited urban coastal areas, are subject to varying degrees of pollution by sewage. In this regard, in the Mediterranean region, approximately 90% of municipal sewage is still currently discharged in the raw state.

A pilot project on coastal water quality control (MED VII), jointly co-ordinated by WHO and the United National Environment Programme (UNEP) within the framework of the Joint Co-ordinated Mediterranean Pollution Monitoring and Research Programme (MED POL Phase I), and implemented by thirty Mediterranean Laboratories between 1976 and 1981, included the regular monitoring of a number of shellfish-growing waters and shellfish for the presence of microbiological indicators of sewage pollution. In several of these areas, both the water and the shellfish themselves were found not to comply with accepted microbiological quality standards. The pilot project also produced interim recommendations for common environmental quality criteria for shellfish waters and shellfish in the Mediterranean region.

While the results of the pilot project could not be interpreted as representative of the situation in the Mediterranean in general, principally because of the relatively small number of stations from which sampling was performed, as well as the geographical imbalance of such stations with respect to the region as a whole, it was clear that such results did provide an approximate indication of the situation prevailing, and general improvement was necessary. In this context, common criteria were considered essential in terms of Article 7 of the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources (signed in Athens in May 1980) which stipulates that Contracting Parties shall progressively formulate and adopt, in co-operation with the competent international organisations, common guidelines and, as appropriate, standards or criteria, dealing in particular with, *inter alia*, the quality of seawater used for specific purposes that is necessary for the protection of human health, living resources and ecosystems.

As the first step towards this objective, a preliminary assessment of the state of microbial pollution in the Mediterranean sea, along with proposed control measures, was prepared by WHO and UNEP in 1983. The scope of this document, which covered both coastal recreational waters and shellfish waters (including shellfish) was to assess the current state of microbial pollution in the Mediterranean sea, mainly on the basis of analysis of the results of the MED VII pilot project, to review the existing national provisions, as well as the international arrangements and agreements within or relevant to the region in this field, to describe the scientific rationale on the basis of which environmental quality criteria for the Mediterranean could be formulated, and to recommend interim measures for adoption by Mediterranean Governments, pending the acquisition of the more comprehensive data required for the formulation of more permanent measures.

In the case of shellfish waters and shellfish, in view of the number of standards and criteria already in force in various Mediterranean countries, WHO and UNEP were requested to review the comparability of the proposed interim criteria with those already existing in the region, on the basis of laboratory studies, with a view to finding a common solution which, while not prejudicing the necessary quality requirements, would also involve the least possible modification to current national statutory provisions. In accordance with this request, a number of Mediterranean laboratories performed the necessary studies within the framework of the research component of the Long-term Programme of Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase II).

The objectives of the present consultation meeting, which was convened jointly by WHO and UNEP within the framework of the MED POL Phase II Programme, included the following:

- to review the current statutory provisions relating to microbiological quality standards and criteria for shellfish waters and shellfish in/or applicable to the Mediterranean;
- to review the comparability of the various methodologies utilised in the enforcement of such standards and criteria, based on the data obtained from the recent laboratory studies;
- to formulate revised interim environmental quality criteria, to be considered by Mediterranean Governments for adoption on a common basis.

A number of microbiologists responsible for the analysis and evaluation of the microbiological quality of shellfish waters and shellfish in their respective countries were invited to participate in the consultation, as well as other scientists involved in the decision-making process on relevant national legislation. In addition, the Food and Agriculture Organization of the United Nations (FAO) and the Commission of the European Communities (EEC) were also invited to send representatives.

1. Opening of the meeting (agenda item 1)

The meeting took place in the premises of the Co-ordinating Unit for the Mediterranean Action Plan, Athens, Greece, from 26 to 27 March 1987. It was attended by eight temporary advisers from seven Mediterranean countries, one representative each from EEC, FAO and UNEP, and one Staff Member from WHO/EURO. A list of participants is given in Annex 1.

Dr L.J. Saliba, Senior Scientist, Mediterranean Action Plan, World Health Organization, Regional Office for Europe, opened the meeting on behalf of the Regional Director, Dr J.E. Asvall. He welcomed the participants and gave a brief outline of the events leading to the present meeting. He emphasised the importance of the Mediterranean region having rational and effective environmental quality criteria for shellfish waters and shellfish on a common basis, on the understanding that such criteria would apply to the quality of culture areas (including the shellfish in them), and would not impinge on post-harvest assessments of shellfish quality as a food product, which would be performed by national authorities as part of their food-safety procedures and were beyond the scope of the proposed criteria.

Dr L. Jeftic, Senior Marine Scientist, United Nations Environment Programme, welcomed participants on behalf of the Co-ordinating Unit for the Mediterranean Action Plan, and also stressed the importance of the subject matter of the present meeting, which should lead to proposed regional environmental quality criteria for shellfish waters and shellfish capable of being adopted by Mediterranean States on a joint basis for eventual translation into their respective national statutory control measures.

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

Dr L.J. Saliba explained the scope and purpose of the meeting. Participants had to study the survey of the national and other legislation with regard to quality standards and criteria for shellfish waters and shellfish in the Mediterranean, including the interim criteria proposed by WHO and UNEP. The results of the laboratory exercises on the comparability of the various parameters measured and the particular methodologies utilised also had to be studied and discussed. On the basis of these discussions, the meeting was to formulate revised interim criteria for consideration by Mediterranean States later in the year.

3. Election of Officers (agenda item 3)

Professor J.A. Papadakis was elected Chairman. Dr Laura Volterra Vice-Chairman and Dr S. Sobot 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. Organisation of the meeting (agenda item 5)

It was agreed that the sessions of the meeting would all be held in plenary, with the provision of establishing small working groups for any particular topic, should the necessity arise.

6. Review of existing environmental criteria for shellfish-growing waters and shellfish in the Mediterranean, and their comparability vis-a-vis the proposed WHO/UNEP interim criteria (agenda item 6)

6.1 Environmental quality criteria and standards in the Mediterranean region

Information available prior to the meeting was from four Mediterranean countries - France, Greece, Italy and Spain, and from the Commission of the European Communities (EEC) whose relevant Directive is applicable to the same four countries. Information from Egypt, Morocco and Yugoslavia was submitted during the meeting.

6.1.1 Egypt

There are no specific statutory standards or criteria under Egyptian law regarding the microbiological quality of shellfish-growing waters or shellfish. These are however examined at regular intervals, and their quality evaluated according to prevailing international (global) standards. Enforcement is through internal administrative procedures.

6.1.2 France

French law prescribes that shellfish waters must conform with a microbiological quality criterion of less than 300 faecal coliforms per 100 ml of flesh of shellfish in the waters in question. In addition to this, an internal administrative standard (which is not often used and in any case appears to have no statutory standing) classifies shellfish-waters into four categories as follows:

A. Satisfactory	0 <u>E. coli</u> /100 ml seawater
B. Acceptable	1-60 <u>E. coli</u> /100 ml seawater
C. Doubtful	61-120 <u>E. coli</u> /100 ml seawater
D. Unsatisfactory	Above 120 <u>E. coli</u> /100 ml seawater

Shellfish for consumption are subject to conformity with the following criteria:

Aerobic micro-organisms, 30°C	up to 100,000/gram flesh
Faecal coliforms	up to 300/100 ml flesh
Faecal streptococci	up to 2,500/100 ml flesh
<u>Staphylococcus aureus</u>	up to 10/gram flesh
Anaerobic sulphur-reducing bacteria, 46°C	up to 10/gram flesh
<u>Salmonella</u>	absent in 25 g flesh

There is a detailed procedure for evaluating results and deciding on acceptability whenever any particular sample exceeds the above limits at any time during the stipulated sampling period (26 samplings during 12 consecutive months).

The method utilised in France for determination of the main microbiological indicators of sewage pollution, (E. coli, faecal coliforms and faecal streptococci) in both seawater and shellfish is the Most Probable Number (MPN) technique. In the case of shellfish, although results are expressed in terms of the flesh, which is separated from the intervalvular fluid prior to weighing, the fluid is incorporated into the diluent in making up the stock solution. Any microorganisms in the fluid therefore represent an addition to the concentrations already present in the flesh itself when these are determined.

6.1.3 Greece

Up to recently, Greek Law classified shellfish-growing waters on the following basis:

A. Suitable	0-70 total coliforms/100 ml seawater
B. Moderately infected	71-700 total coliforms/100 ml seawater
C. Unsuitable (heavily infected)	Above 700 total coliforms/100 ml seawater

Shellfish for consumption were subject to classification into the following three categories:

A. First Class	up to 500 <u>E. coli</u> /100 ml flesh
B. Second Class	500-1500 <u>E. coli</u> /100 ml flesh
C. Third Class	above 1500 <u>E. coli</u> /100 ml flesh

For both seawater and shellfish, the method utilised was the MPN technique. In the case of shellfish, the flesh only was analysed, not the intervalvular fluid.

Under new legislation, enacted in 1986, waters are considered suitable for shellfish if they do not contain more than 70 Total coliforms per 100 ml seawater in 75% of the samples over a period of one year, based on a minimum sampling frequency of once every 3 months. Waters in which samples contain concentrations of total coliforms between 71 and 700 per 100 ml seawater are only considered suitable if depuration stations are in operation. The acceptable concentration for shellfish is up to (and including) 300 faecal coliforms per 100 ml flesh plus intervalvular fluid in at least 75% of the samples over a period of one year, based on a minimum sampling frequency of once every 3 months. For both seawater and shellfish, the prescribed method is the MPN technique.

6.1.4 Italy

The Italian Law is probably the most comprehensive. Under its provisions, shellfish waters are subject to the following classification and standards:

A. Approved zones

Seawater should not contain more than 2 E. coli per 100 ml. Up to 7 E. coli per 100 ml seawater is tolerated in not more than 10% of the samples, provided the shellfish themselves come up to the required standards. Shellfish should not contain more than 4 E. coli per ml of flesh plus intervalvular fluid, and Salmonella must be absent in 25 ml flesh plus intervalvular fluid.

B. Conditioned zones

Seawater should not contain more than 34 E. coli per 100 ml. Up to 49 E. coli per 100 ml is tolerated in not more than 10% of the samples. Shellfish should not contain more than 39 E. coli per ml of flesh plus intervalvular fluid.

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

6.1.5 Morocco

Microbiological quality standards and criteria for shellfish waters are generally based on French Law. For acceptability of shellfish waters, the concentration of faecal coliforms in the flesh of shellfish therein, must not exceed 300 per 100 ml. The MPN method is used for determinations.

6.1.6 Spain

Spanish Law fixes the following limits regarding the acceptability of shellfish waters from the point of view of their microbiological quality:

1. Concentration of E. coli must not exceed 15 per 100 ml of seawater in more than 50% of the samples;
2. Concentrations of E. coli must not exceed 50 per 100 ml of seawater in more than 10% of the samples.

Depurated shellfish destined for consumption must conform to the following quality standards:

Aerobic micro-organisms	up to 100,000 per gram
<u>E. coli</u>	up to 500 per litre
<u>Salmonella</u>	absent in 25 ml
Streptococci (Group D)	up to 100 per gram
<u>Vibrio parahaemolyticus</u>	up to 100 per gram

The Law does not specify whether determinations should be made on the flesh alone or on the flesh plus intervalvular fluid. Determinations, however, are normally made on the flesh alone. Methodology used is the MPN technique.

6.1.7 Yugoslavia

According to Yugoslav Law, shellfish waters are considered acceptable if the concentration of total coliforms does not exceed 10 per 100 ml. There is no specific legislation regarding shellfish, but market quality standards demand the absence of:

<u>Salmonella</u> in 25 grams or ml
Coagulase-positive Staphylococci in 0.01 gram or ml
Sulphite-reducing clostridia in 0.01 gram or ml
<u>Proteus</u> species in 0.001 gram or ml
<u>E. coli</u> in 0.001 gram or ml

The Membrane Filtration (MF) method is utilised for determination of total coliforms in seawater, and the Most Probable Number (MPN) for determination of E. coli in shellfish.

6.1.8 Commission of the European Communities

The 1979 EEC Directive on the quality of shellfish waters is based on microbiological limits in the shellfish therein, and does not impose any similar limit on the waters themselves, except indirectly by limiting the content of suspended solids in waters affected by discharges to 30% above that of unaffected waters. The microbiological limit for shellfish in the growing area (on which the suitability of the area itself is assessed) is not more than 300 faecal coliforms in 100 ml of flesh plus intervalvular fluid, in at least 75% of the samples, based on a minimum sampling frequency of once every three months over a period of twelve months. In this regard, however, the Directive adds that, pending the

adoption of a Directive on the protection of consumers of shellfish products, it is essential that this value be observed in waters in which live shellfish are directly edible by man.

6.2 WHO/UNEP proposed interim criteria

The following interim microbiological criteria for shellfish-growing waters and shellfish were proposed for joint adoption by Mediterranean States in 1983:

Shellfish-growing waters:

- Faecal coliforms : - not more than 10 per 100 ml seawater in 80% of the samples;
- not more than 100 per 100 ml seawater in the remaining 20% of the samples.
- Shellfish : - up to 2 per gram - sale permitted;
- 3-10 per gram - temporary prohibition of sale;
- over 10 per gram - sale prohibited.

Recommended methodology for seawater was the Membrane Filtration (MF) or the Most Probably Number (MPN) method. The latter was recommended for shellfish, utilising the flesh alone, and not including the intervalvular fluid.

6.3 Comparability studies

Comparability studies on the various methodologies, including the evaluation of data obtained according to the different criteria and standards, were performed by the following institutions either within the framework of, or as a direct contribution to, the MED POL Phase II Programme:

- Athens School of Hygiene, Athens, Greece (bacteriological quality of seawater and harvested mussels);
- High Institute of Public Health, Rome, Italy (development of analytical techniques for monitoring the hygienic quality of shellfish);
- General Directorate of the Environment, Madrid, Spain (accumulation of pathogenic micro-organisms by marine molluscs);
- Institute of Oceanography and Fisheries, Split, Yugoslavia (comparison of faecal coliform levels in mussel flesh and flesh/intervalvular fluid).

The Greek study involved the daily collection of mussels from the growing area of Loutropyrgos at a depth of 3.80 metres. Overlying seawater samples were collected from the same area at a depth of approximately 30 cm. Both seawater and shellfish were analysed for total coliforms, faecal coliforms and

enterococci. In the case of shellfish, two separate samples were utilised. In one, the flesh only was analysed, in the other the flesh and intervalvular fluid together.

No definite correlation was established between concentrations of any of the above three microbiological parameters in seawater and in shellfish. With regard to comparability between bacterial concentrations in the flesh alone and in flesh plus intervalvular fluid, the best correlation was obtained in the case of faecal coliforms. Most of the samples were, however, of high quality from the microbiological viewpoint, with none of the tubes giving positive reactions, leading to an MPN index of less than 2 per 100 ml. No obvious correlation was found between the three parameters in any of the matrices investigated. This, however, was outside the scope of the meeting's objective.

The Italian study utilised shellfish collected from natural banks along the Tyrrhenian and Ionic coasts of Italy. These were analysed for faecal coliforms by (a) the MPN technique, using three different media, (b) the Pour Plate method, in each case analysing different sub-samples for concentrations in flesh alone, and in flesh plus intervalvular fluid. Samples were also analysed for faecal streptococci and anti-*E. coli* phages, in both cases determining the concentrations in flesh plus intervalvular fluid by the MPN technique.

On calculation of analysis of variance, data obtained on flesh alone and on flesh plus intervalvular fluid showed a certain correlation ($r_{20} = 0.6631$ for MPN in MacConkey broth, $r_{20} = 0.7903$ for MPN in A1 medium, and $r_{20} = 0.7728$ for direct counts on MacConkey Agar. The general observation was made that, as was already noted in previous experiments, the intervalvular liquid appeared sometimes to increase the colorimetric titre of the flesh, and sometimes to decrease it. This may be due to factors such as the particular environmental conditions at the time of sampling, the pumping efficiency of the molluscs as a function of seasonal variations, and the accumulation or depuration stage of the shellfish in relation to the pollutant concentration in the water and sediment. As in the Greek study, no apparent correlation existed between the three microbiological parameters.

The Spanish study was a comprehensive exercise involving collection of mussels and seawater samples from three locations along the Mediterranean coast of Spain, and determining the concentrations of three bacterial parameters - total coliforms, faecal coliforms and faecal streptococci - in sub-samples of (a) flesh alone, (b) flesh plus intervalvular fluid, and (c) intervalvular liquid alone, utilising the MPN method. Seawater quality was determined on the basis of the same three parameters, utilising the Membrane Filtration (MF) method. In a number of cases, the coliform content of phytoplankton and zooplankton was determined concurrently.

From the comparability point of view, the conclusions reached were that a definite correlation could be established between concentrations of total coliforms and faecal coliforms in seawater and those in shellfish. Analysis of variance showed $r = 0.52$ ($p < 0.05$) and $r = 0.41$ ($p < 0.05$) respectively. This correlation was not found in the case of faecal streptococci. The same was observed when comparing concentrations of the three parameters in shellfish flesh alone and in flesh plus intervalvular fluid.

The Spanish study also included a review of existing legislation in the various Mediterranean countries, and a comparative account and evaluation of the different criteria utilised for acceptability of shellfish waters and shellfish.

The Yugoslav study consisted in comparative studies of faecal coliform concentrations in mussel flesh and in flesh plus intervalvular fluid, using samples from three localities in the area of Split. The MPN method was used. By the time of the meeting, a total of twelve analyses had been performed. On statistical analysis of these preliminary results, a correlation coefficient of 0.48 was obtained. At this stage, neither a regular pattern nor a statistically significant difference between the two methods was recorded, and concentrations recorded in the flesh alone were less than those in flesh plus intervalvular fluid in 50% of the samples, equal in 25% and more in the remaining 25%.

The meeting considered these results, as well as results obtained from previous studies of a similar nature and concluded that, insofar as the comparability of results from determinations of faecal coliform concentrations in shellfish flesh alone and in flesh plus intervalvular fluid was concerned, although no firm correlation could be established, data obtained showed that, in general, the final results were similar. In spite of variation in individual samples, overall concentrations of the indicator organism in the two sub-matrices (flesh and flesh + fluid) tended to agree when calculated over a long period. The meeting considered that, on the basis of evidence and data to date, it was not possible to arrive at any definite conclusion regarding the correlation between the different indicator organisms themselves, and the comparative density of these in seawater and in shellfish.

The meeting also noted that differences in the bacterial flora in the water of various parts of the Mediterranean could lead to non-comparable results when determining faecal coliform concentrations and, in future, recourse to E. coli as a more specific indicator organism could be considered as an alternative. In this regard, however, comparability studies in the different countries would be necessary.

7. Formulation of proposed environmental quality criteria for shellfish-growing waters and shellfish in the Mediterranean

The meeting recognised the fact that, in view of the considerable variation existing between the systems practised in the different countries, it would not be possible to formulate common environmental quality criteria for shellfish-growing waters and shellfish for the Mediterranean region as a whole which, apart from satisfying public health requirements, would also enable all countries to adopt them without modification to their own national legislation. Furthermore, any common numerical criteria (i.e. upper limits of tolerance on bacterial concentrations) would have to be based on harmonised or at least comparable methodologies.

In spite of this variation in national legislation, however, the meeting felt that acceptable interim criteria could be formulated on shellfish water quality, to determine whether specific coastal areas were suitable for cultivating or collecting shellfish, through the proposed use of a single minimum matrix for analysis, a common indicator, and a common methodology, together with comparative alternatives where feasible. In this regard, the

meeting agreed that recommendations, in line with the provisions and limitations of Article 7.1(c) of the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources, should be limited to shellfish water quality.

The two main matrices (seawater and the shellfish therein) used to determine the quality of shellfish waters were considered, and the meeting agreed that the microbiological quality of the shellfish in the area in question should be the compulsory analytical matrix, although this could, wherever appropriate, be supplemented by analysis of the water.

The meeting also agreed that criteria for water quality would in no way determine the acceptability of shellfish for direct human consumption, which would continue to be determined by appropriate sanitary procedures in each country.

8. Recommendations

The meeting made the following recommendations and requested WHO and UNEP to submit revised interim environmental quality criteria for shellfish waters on such basis, to be considered by Mediterranean Governments for adoption:

- (a) The term "shellfish waters" should mean the coastal and brackish waters in which shellfish (bivalve and gastropod molluscs) live;
- (b) For the assessment of the microbiological quality of these waters, the shellfish should be taken into account;
- (c) For the determination of microbiological parameters, preference should be given to analysis of shellfish flesh and intervalvular fluid, rather than the flesh alone;
- (d) The results of analysis of microbiological quality should be expressed by the number of faecal coliforms recorded in 100 ml of shellfish flesh and intervalvular fluid or of flesh alone, which should not exceed 300/100 ml;
- (e) The method of analysis used should be incubation at 37 ± 0.5 °C with fermentation on a liquid substrate for a period of 24 or 48 hours, followed by a confirmation test at 44 ± 0.2 °C for 24 hours. The number of faecal coliforms should be estimated according to the Most Probable Number (MPN) method;
- (f) Water should be sampled and analysed at least once every three months. This frequency should be increased as much as possible, in accordance with specific local conditions;
- (g) Regarding microbiological parameters, research should be undertaken within the framework of MED POL on the comparability of faecal coliforms and E. coli, as well as on the usefulness of other indicators;

Annex 1

LIST OF PARTICIPANTS

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