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ASSESSMENT OF THE STATE OF MICROBIAL POLLUTION
OF SHELLFISH WATERS IN THE MEDITERRANEAN SEA AND PROPOSED MEASURES

In collaboration with



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INTRODUCTION

1. One of the principal kinds of human exposure to microbial pollutants in the Mediterranean Sea is the consumption of seafood contaminated by such pollutants. The magnitude of the potential danger from this exposure can be illustrated by mentioning that about 100 million people live permanently on the shores of the Mediterranean and about the same number visit the coast each year during which time they consume locally-caught shellfish. The consumption of shellfish raised in the Mediterranean is estimated as about 12000 metric tons per year.

2. Microbial pollution is one of the direct results of discharge of untreated sewage into the marine environment. In the Mediterranean region, over 90% of municipal waste is discharged in the raw state (UNEP/ECE/UNIDO/FAO/UNESCO/WHO/IAEA, 1983). The importance of ensuring adequate sea water quality is rendered more acute by the fact shellfish are abundantly grown and consumed throughout the region, thus making it equally important to apply quality criteria for shellfish-growing waters as well as for shellfish themselves.

3. This problem was specifically recognized by Mediterranean Governments when adopting the Joint Coordinated Mediterranean Pollution Monitoring and Research Programme (MED POL Phase I) as the scientific component of the Mediterranean Action Plan in February 1975 (UNEP, 1975). One particular pilot project, forming part of this component, Coastal Water Quality Control (MED POL VII), included the regular monitoring of shellfish-growing waters and shellfish flesh by a network of national laboratories throughout the region, the main parameters being microbial (FAO/UNESCO/IOC/WHO/WMO/IAEA/UNEP, 1983). During the course of this pilot project, coordinated by the World Health Organization (WHO), desirable environmental quality criteria for both shellfish-growing waters and shellfish were elaborated by participants in the pilot project and proposed for adoption as interim criteria. These (WHO/UNEP) interim criteria have been used to assess the microbiological quality of the Mediterranean shellfish and shellfish-growing waters surveyed during MED POL VII. The results of this part of the pilot project (which also included coastal recreational waters) are described in the appropriate sections of this document.

4. The Intergovernmental Review Meeting of Mediterranean Coastal States and the First Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its related Protocols (Geneva, 5-10 February 1979) recommended that:

"Work should be continued on the development of the scientific rationale for the criteria applicable to the quality of recreational waters, shellfish-growing areas, waters used for aquaculture, and seafood. Based on this rationale and taking into account existing national provisions and international arrangements and agreements, the criteria should be formulated on a scientific basis and submitted to the Governments and the EEC for their consideration" (UNEP, 1979).

5. In addition, Article 7 of the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-Based Sources (adopted in Athens on 17 May 1980), stipulates that:

"1. The Parties shall progressively formulate and adopt, in cooperation with the competent international organizations, common guidelines and, as appropriate, standards or criteria dealing in particular with:

.....
(c) the quality of sea water used for specific purposes that is necessary for the protection of human health, living resources and ecosystems;
.....

2. Without prejudice to the provisions of article 5 of this Protocol, such common guidelines, standards or criteria shall take into account local ecological, geographical and physical characteristics, the economic capacity of the Parties and their need for development, the level of existing pollution and the real absorptive capacity of the marine environment" (UNEP, 1982).

6. The Second Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its related Protocols and Intergovernmental Review Meeting of Mediterranean Coastal States on the Action Plan (Cannes, 2-7 March 1981) approved the Long-term Programme for Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase II) (UNEP, 1983). Activities in the research component include the performance of "epidemiological studies related to the confirmation (or possible revision) of the proposed environmental quality criteria (standards of use) for bathing waters, shellfish-growing waters and marine organisms". These studies were initiated in 1983, but so far have been mainly concerned with correlating recreational water quality with health effects in the exposed sectors of Mediterranean populations, rather than with effects associated with consumption of contaminated shellfish.

7. In conformity with the above recommendations, a document entitled "Assessment of the present state of microbial pollution in the Mediterranean Sea and proposed control measures" (UNEP/WG.91/6) was prepared by UNEP and WHO in 1983 and submitted to the Second Meeting of the Working Group for Scientific and Technical Co-operation (WGSTC) for MED POL in November 1983. The scope of this document was to assess the current state of microbial pollution in the Mediterranean based on analysis of the results of the pilot project on Coastal Water Quality Control (MED POL VII), to review the existing national provisions, and international arrangements and agreements relevant to the Mediterranean Sea in this field, to describe the scientific rationale on the basis of which environmental quality criteria for recreational waters and shellfish-growing waters in the Mediterranean Sea can be formulated, and to recommend measures to be taken by the Contracting Parties at their next meeting.

8. The meeting, as well as the Extraordinary Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its related Protocols (Athens, 10-13 April 1984) (UNEP/IG.49/5) did not agree with the proposed measures, particularly those relating to shellfish-growing waters and shellfish, and requested the Secretariat to prepare a revised version of the document on the basis of written comments received. As a result, a slightly modified version (UNEP/WG.118/6) of the document was prepared by UNEP and WHO in 1985, and submitted to the Third Meeting of the Working Group on Scientific and Technical Co-operation for MED POL (Athens, 27-31 May 1985).

9. The Meeting took note of the section of document UNEP/WG.118/6 relevant to the environmental quality of shellfish-growing waters and shellfish, including the recommendations belonging to that section of the document, but the Working Group felt that it was not prepared to discuss the subject in depth and work on recommendations which could be transmitted to the Contracting Parties. Consequently, the Meeting recommended that the consideration of the subject be proposed for the Fourth Meeting of the Working Group and agreed to provide the secretariat, by 31 December 1985, with written comments relevant to the environmental quality of shellfish-growing waters and shellfish, including additional information on national legislation, administrative measures, policies and practices. The secretariat was requested to prepare a new document on the assessment of the present state of microbial pollution in the mediterranean Sea relevant to shellfish and shellfish-growing waters, including recommendations which could be considered for adoption by the Contracting Parties. The new document should be based on the relevant parts of document UNEP/WG.118/6, on the information expected to be received from the MED POL National Co-ordinators by 31 December 1985 and on any other information at the disposal of the secretariat (UNEP/WG.118/9 para 45).

10. It was also agreed that a number of countries would organize, in the form of country contributions, a pilot study on the measures proposed in paragraph 175 of UNEP/WG.118/6:

"The coastal waters of the Mediterranean will be considered as satisfactory and safe for shellfish-growing if the concentration of faecal coliforms (FC) of representative water samples, sampled monthly (in winter) and fortnightly (in summer), does not exceed 10 FC per 100 ml in 80% of the samples and 100 FC per 100 ml in any of the samples. Shellfish will be considered as suitable and safe for direct sale to the general public if the concentration of FC in representative shellfish flesh, sampled monthly (in winter) and fortnightly (in summer), does not exceed 2 FC per gram (wet weight) of flesh. Shellfish with concentrations of 3 - 10 FC per gram of flesh should be temporarily prohibited for direct sale until the concentration of FC is lowered to at least 2 FC per gram of flesh by adequate purification methods. Shellfish with more than 10 FC per gram of flesh should be prohibited for sale. The concentration of FC should be determined by agreed reference methods or by methods yielding comparable results, proved by intercalibration with relevant reference methods."

as well as the alternative measures which could be considered as suitable in the Mediterranean region. The results of that pilot study would be available in time for consideration by the Fourth Meeting of the Working Group (UNEP/WG.118/9, para 46).

11. The Fourth Ordinary Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its related Protocols (Genoa, 9-13 September 1985), after exhaustive discussions on the proposed interim environmental quality criteria for shellfish and shellfish-growing waters, requested that these subjects should be taken into account at the Meeting of the Working Group on Scientific and Technical Co-operation with a view to their application and enforcement, as far as possible, by appropriate measures (UNEP/IG.56/5, I, para 110, III, F, para 7).

12. The Fourth Meeting of the Working Group on Scientific and Technical Cooperation for MED POL (Athens, 16-20 June 1986) reviewed the position in the light of updated information provided by the Secretariat (document UNEP/WG.144/10). In view of the fact that the necessary studies on the

comparability of the various criteria and their respective methodologies had not been completed, a time-table was agreed on to enable a revised version of the above-quoted working document, complete with proposed measures, to be submitted to the Working Group at its next session (UNEP/WG.144/13, para 57).

13. Following receipt by the Secretariat of the necessary reports on the results of the comparability studies, a Consultation on Environmental Quality Criteria for Shellfish-growing waters and Shellfish in the Mediterranean was jointly convened by WHO and UNEP in Athens on 26-27 March 1987 (WHO, 1987). The meeting reviewed both the results of studies carried out and the various criteria already in force in the Mediterranean, and agreed on a set of recommended proposed measures regarding environmental quality criteria for shellfish waters.

14. The present document includes most of the basic material contained in earlier documentation to ensure self-containment and avoid undue cross-reference. The review of legal and related measures currently in force has been updated to include newly-available material. The recommendations of the March 1987 Consultation Meeting have been reproduced in their entirety, and on the basis of these recommendations, measures are proposed for adoption by the Contracting Parties.

ASSESSMENT OF MICROBIAL POLLUTION OF SHELLFISH AND SHELLFISH-GROWING WATERS IN THE MEDITERRANEAN SEA

Introduction

15. Part of the objectives of the pilot project Coastal Water Quality Control (MED POL VII) were to obtain statistically significant data, scientific information, and technical principles necessary to assess the public health implications derived from unsatisfactory quality conditions in the shellfish-growing areas of the Mediterranean. The results obtained by the collaborating laboratories during the execution of the pilot project have provided a sound basis for the first regional assessment of the microbiological quality of such areas.

16. Furthermore, results from the MED POL X pilot project (UNEP/ECE/UNIDO/FAO/UNESCO/WHO/IAEA, 1983), carried out in the framework of MED POL Phase I, to study the type and quantity of the pollution loads reaching the Mediterranean from land-based sources, have provided additional insight into the sources of the microbial pollution in the Mediterranean waters.

17. As a result of the studies carried out during the execution of MED POL VII and MED POL X, a series of national and international programmes have been established, and are being carried out, to effectively manage the disposal at sea of wastewater effluents, one of the main objectives being the amelioration of unsatisfactory sanitary conditions in the coastal waters of the Mediterranean Sea.

Sources and inputs of domestic sewage

18. The volume of wastewater discharges from coastal communities, estimated according to MED POL X, approximately as 2×10^9 m³/year is marginal compared to the 420×10^9 m³/year of fresh water discharged by rivers. Although rivers may also add a considerable amount of microbial pollution, mainly from upstream wastewater discharges, their actual relative contribution to the microbial pollution of the Mediterranean has not been assessed. It is

assumed that the high concentration of micro-organisms in coastal wastewater discharges makes them the major source of the microbial pollution reaching the Mediterranean Sea.

19. Disposal of inadequately treated or untreated sewage through short outfalls, having in most cases a small number of diffuser ports, explains the low dilution and dispersion achieved in the receiving seawater, and the consequent adverse effects produced in the coastal areas near the discharge point.

20. The considerable reduction of the natural transparency of seawater, the increased presence of extraneous floating materials such as plastics and oils, and the development of undesirable sanitary conditions in the nearby coastal bathing areas, are the more apparent and immediate effects of inadequate disposal of sewage in coastal waters.

21. The public health implications both for the local population and the numerous visitors reaching the Mediterranean coastal areas, are becoming a source of concern for many national and international authorities, as well as for public opinion at large.

Fate of microbial pollution in the Mediterranean

22. Assessment of microbial pollution in the Mediterranean Sea has been essentially based on three groups of indicators of faecal pollution: total coliforms, faecal coliforms and faecal streptococci. Although they do not fulfil all the requirements for an "ideal indicator", they are generally considered and used as acceptable indicators of the sanitary quality of recreational waters (WHO/UNEP, 1976, 1977a).

23. Sea water is not the natural environment for most of the micro-organisms discharged in wastewater effluents, particularly those originating in the intestinal tract of human and other warm-blooded animals. Consequently, it can be expected that the three microbial indicators referred to in the preceding paragraph will not remain unaltered in the receiving seawaters, but rather will disappear progressively (WHO/UNEP, 1979a).

24. Salinity, natural light, temperature, dissolved substances, and natural predators are among the factors known to affect the survival of these micro-organisms in seawaters. Specifically, solar radiation has been pointed out as the single most important factor responsible for microbial inactivation (Gameson, 1975).

25. Whether or not all the micro-organisms discharged in wastewater effluents are permanently inactivated during the hours following their mixing with receiving seawaters is a subject of considerable debate and of continuing research.

26. Results available (Kapusinski, 1981) indicate that the sublethal injuries induced by solar radiation in the catalase enzymatic system of Escherichia coli render the cells sensitive to otherwise innocuous peroxide concentrations. Although standard cultivation techniques do not allow recovery of all the stressed microbial cells, the addition of peroxide scavengers, and particularly the catalase enzyme itself, have proved capable of recovering a considerable portion of the affected E. coli cells. Nonetheless, considering the high level of supplemental enzyme required in laboratory cultures to recover injured cells, it can be expected that this enrichment process will not take place under natural conditions, and

consequently most of the sunlight injured organisms will not be able to survive, thus being permanently lost after a certain time.

27. Results from field studies carried out during MED POL VII (WHO/UNEP, 1981) and elsewhere, have pointed out the different survival patterns of the three microbial indicators referred to in paragraph 16. While total coliforms and faecal coliforms seem to be inactivated in seawater rather quickly and progressively under natural conditions, faecal streptococci show a lower inactivation rate, as well as a smaller long-term percentual reduction.

28. The physicochemical processes of flocculation of microbial cells, and their subsequent sedimentation to the sea bottom, are being considered as the mechanism responsible for the microbial enrichment of sediments in the areas surrounding wastewater discharges (Mitchell, 1975).

29. Natural turbulence and sea currents can become a plausible mechanism by which the contaminated sediments can be resuspended, with the consequent impairment of the microbiological quality of the overlying seawaters (Volterra, 1980; Velescu, 1982).

30. However, the sea bottom not being the natural environment for most of the micro-organisms contributed by wastewater effluents, it can be expected that by termination or improvement of the discharge, and subsequent depletion of organic substrates, the survival of those micro-organisms will be highly impaired.

31. Similarly to what happens in circulating seawaters, results from field studies have pointed out the ability of faecal streptococci to survive for longer periods than faecal coliforms, to the point of outnumbering them, in contrast to what is normally observed in raw municipal wastewater effluents (Volterra, 1980; Velescu, 1981).

32. All previous findings provide strong support for the inclusion of faecal streptococci, as an additional indicator of faecal pollution, for use in routine monitoring programmes. Present knowledge supports the usage of both faecal coliforms and faecal streptococci as routine parameters for monitoring of coastal water quality, because of their significance as individual indicator, as well as the valuable additional information they provide, when compared, on the origin and the residence time in the sea of the wastewater effluent (Geldreich, 1976).

33. Most shellfish are filter feeders, and thus only grow in areas relatively rich in phytoplankton, or in areas having important amounts of particulate matter. Two other environmental factors markedly influencing their feeding rate are the salinity and the temperature of the water. While feeding can be suppressed at low temperatures, there is a lower limit of salinity below which most molluscs will not feed.

34. The feeding mechanism of shellfish involves the filtering of considerable amounts of seawater, up to 4 litres per hour by some oysters. Consequently, any micro-organisms present in the surrounding waters will be concentrated in the shellfish body, and may pose a public health danger when their flesh is directly consumed by humans.

35. The same filtering process is used in practice for the self-purification of shellfish from microbial pollution, when immersed in clean seawater. Although it is generally considered that the microbial content acquired by live shellfish is not significantly affected during its stay in the shellfish

body, the self-purification process is quite effective, and may bring the microbial content of shellfish to very low values within a matter of days (Mahoney, 1974; Wood, 1976).

36. The time lapse required for microbial self-purification of shellfish, from the time they are immersed in clean seawater, explains the frequent discrepancies between the microbiological quality of shellfish, naturally attached to a fixed substrate, and that of the surrounding waters, experiencing variable circulating patterns and, consequently, microbial contents.

Microbiological quality of shellfish and shellfish-growing waters

37. Monitoring of shellfish-growing areas in the Mediterranean was conducted by six collaborating laboratories, in four Mediterranean states, under WHO's coordination and according to agreed procedures summarized in the final report on MED POL VII (WHO/UNEP, 1981).

38. Monitoring started in late 1976, simultaneously with monitoring of recreational waters, and ended in March 1981. A minimum programme was applied by all participating laboratories to promote comparability of results. Actual monitoring programmes were generally above those required, by including parameters other than the minimum specified.

39. The selection of sampling areas, as well as the number of sampling stations (Figure 1), was mainly determined by the location and organization of existing shellfish-growing areas. As a result, the conclusions from the monitoring programme carried out on shellfish-growing areas in the Mediterranean cannot be considered as of general application. However, considering the number and spatial distribution of the collaborating laboratories, the conclusions derived therefrom can be reasonably considered as a valuable indication of the present situation of shellfish-growing areas in the Mediterranean.

40. Table 1 summarizes the basic facts relevant to the assessment of the microbiological quality of shellfish-growing waters in the Mediterranean.

Table 1

Basic facts relevant to the assessment of the microbiological quality of shellfish-growing areas in the Mediterranean through MED POL VII

<u>Category</u>	<u>Number</u>
Sampling stations	50
Water/shellfish samples analysed	2 300

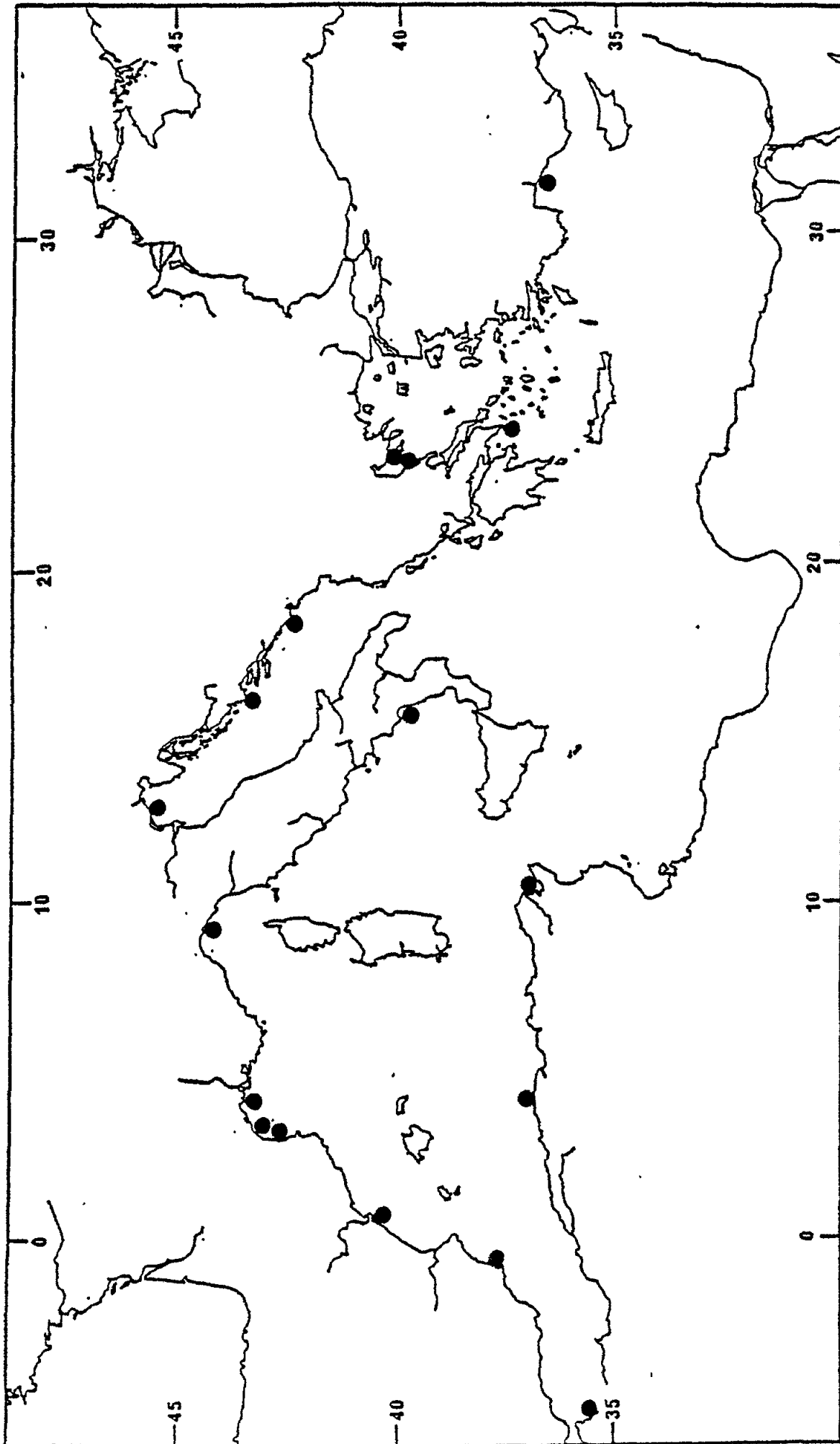


Figure 1. Shellfish growing areas monitored during the course of MED POL VII

41. The average number of water samples analysed at each sampling station was estimated as 10 per year, although the sampling frequencies varied widely among sampling stations, both within the area monitored by a given laboratory, and within areas of different laboratories. The considerably higher average number of samples analysed yearly at each sampling station, compared with the average number of samples analysed for the quality of recreational waters, clearly points out the more continuous and systematic monitoring performed by the laboratories involved in the monitoring of shellfish-growing areas.

42. The basic parameters used in assessing the microbiological quality of shellfish-growing waters were the four indicators of microbial pollution: total coliforms, faecal coliforms, faecal streptococci and total heterotrophic bacteria. Additional microbiological parameters were used, such as qualitative and quantitative analyses of vibrios, Vibrio parahaemolyticus, Salmonella and viruses. Furthermore, parameters describing general conditions in the monitoring area at the time of sampling were also recorded.

43. The method used for the analysis of the microbiological quality of shellfish-growing waters was the membrane filtration techniques, similar to those used for the analysis of recreational waters. The method used for the analysis of the microbiological quality of shellfish was the multiple test tube technique and the most probable number (MPN) interpretation method (WHO/UNEP, 1976).

44. The original MED POL VII operational document (WHO/UNEP, 1976) considered both the flesh and the intervalvular liquid for the microbiological examination of shellfish. However, a group of experts jointly convened by WHO and UNEP in April 1977 to produce comprehensive guidelines for health-related monitoring of coastal water quality (WHO/UNEP, 1977b) recommended that the flesh alone be used. Following a thorough review of the principles involved, the meeting of principal investigators of MED POL VII endorsed this recommendation, on the basis that the inclusion of the liquid in the sample would introduce a factor of variability which could reduce the comparability of results (WHO/UNEP/1978).

45. This methodological requirement posed some administrative difficulties for those collaborating laboratories in Mediterranean states belonging to the EEC, since the applicable EEC Directive explicitly requires that analyses be carried out in shellfish flesh and intervalvular liquid (EEC, 1979).

46. The same meeting of MED POL VII principal investigators referred to in paragraph 42 discussed the limits adopted by several countries for microbiological quality of shellfish, and recommended the following interim criteria for shellfish-growing areas (WHO/UNEP, 1978) to be applied for the assessment of the pilot project's results in terms of water and shellfish quality:

(a) for shellfish, in terms of faecal coliforms (FC) per gram of shellfish flesh:

0 - 2 FC/g	sale permitted
3 - 10 FC/g	temporary prohibition of sale
above 10 FC/g	sale prohibited

(b) for satisfactory water of shellfish-growing areas, in terms of faecal coliforms (FC) per 100 ml of water:

less than 10 FC/100 ml in 80% of the samples
less than 100 FC/100 ml in the remaining 20% of the samples.

47. These WHO/UNEP interim criteria were applied for the assessment of the microbiological quality of shellfish and shellfish-growing waters during the execution of MED POL VII, and were recommended for adoption in the final report of the project (WHO/UNEP, 1981). Further details of their elaboration are given in paras 80 to 82.

48. Table 2 summarizes the assessment of the microbiological quality of shellfish and shellfish-growing areas in the Mediterranean according to these WHO/UNEP interim criteria. Specifically, the 2 faecal coliforms per gram of shellfish flesh was considered as the limiting factor in the evaluation process. Consequently, any sampling stations satisfying this limitation, and the corresponding criteria for the quality of shellfish-growing waters was considered as satisfactory for direct sale of shellfish, without additional purification or depuration, during the yearly period considered. Only those stations with 10 or more shellfish analyses per year were included in the analysis.

Table 2

Summary assessment of the microbiological quality of shellfish and shellfish-growing waters in the Mediterranean, according to WHO/UNEP interim criteria

(MED POL VII sampling stations with at least 10 shellfish analyses per year)

<u>Year</u>	<u>Stations surveyed</u>	<u>Stations with satisfactory</u>		<u>Stations with</u>
		<u>water</u>	<u>shellfish</u>	<u>satisfactory</u>
				<u>water and shellfish</u>
1976	15	10 (67%)	0 (0%)	0 (0%)
1977	12	7 (58%)	0 (0%)	0 (0%)
1978	21	14 (67%)	2 (10%)	2 (10%)
1979	19	9 (47%)	4 (21%)	1 (5%)
1980	21	16 (76%)	0 (0%)	0 (0%)
1981	-	-	-	-
Overall	88	56 (63%)	6 (7%)	3 (3%)

49. An analysis of Table 2 clearly shows the marked influence that the microbiological limitation on shellfish flesh has in the evaluation of a sampling station. While approximately 63% of the sampling stations could be considered satisfactory from the point of view of the microbiological quality of their water, only approximately 7% could be considered satisfactory from the point of view of the microbiological quality of the shellfish flesh collected at these stations. Taking into account both limiting factors (i.e. the quality of water and shellfish), the number of sampling stations from which, according to the WHO/UNEP interim criteria, shellfish sale would be permitted without additional purification, is very low, approximately 3%.

50. An additional analysis, according to the WHO/UNEP interim criteria, taking into account all sampling stations with 6 or more samples of both water and shellfish analysed per year (Table 3) reveals a similar pattern. Only about 4% of these stations meet the criteria permitting direct sale of shellfish without additional purification.

Table 3

Summary assessment of the microbiological quality shellfish and of shellfish-growing waters in the Mediterranean, according to WHO/UNEP interim criteria

(MED POL VII sampling stations with at least 6 analyses per year)

<u>Year</u>	<u>Stations surveyed</u>	<u>Stations with satisfactory</u>		<u>Stations with satisfactory</u>
		<u>water</u>	<u>shellfish</u>	<u>water and shellfish</u>
1976	18	12 (67%)	0 (0%)	0 (0%)
1977	13	8 (62%)	0 (0%)	0 (0%)
1978	24	17 (71%)	3 (13%)	2 (8%)
1979	33	17 (52%)	6 (18%)	3 (9%)
1980	21	14 (67%)	0 (0%)	0 (0%)
1981	7	6 (86%)	0 (0%)	0 (0%)
Overall	116	74 (64%)	9 (8%)	5 (4%)

51. According to the EEC criteria (EEC, 1979), the guide (G) value of 300 faecal coliforms per 100 ml of shellfish flesh and intervalvular liquid cannot be exceeded in 75% of the samples collected at a minimum quarterly frequency, at a given sampling point and during a 12-month period. The results of MED POL VII, analysed by these criteria, and assuming 100 g of shellfish flesh as equivalent to 100 ml of flesh plus intervalvular fluid, are summarized in Tables 4 and 5, taking into account stations with at least 10 and 6 shellfish analyses per year, respectively.

52. Table 4 reveals that the percentage of satisfactory stations (i.e. those with satisfactory shellfish), having at least 10 shellfish analyses per year, varies widely, between 0 and 71%, with an overall 40% of satisfactory stations for the 1976-1980 period.

53. Tables 4 and 5 show an almost identical pattern, with approximately 40% of the stations surveyed being considered as satisfactory according to the EEC criteria. A comparison of Tables 2 and 3 on the one hand and 4 and 5 on the other, reveals the considerably higher restrictive character of the WHO/UNEP interim criteria.

Table 4

Summary assessment of the microbiological quality of shellfish
in the Mediterranean, according to the EEC criteria

(MED POL VII sampling stations with at least 10 shellfish analyses per year)

<u>Year</u>	<u>Stations surveyed</u>	<u>Stations with satisfactory shellfish</u>
1976	15	4 (27%)
1977	12	0 (0%)
1978	21	11 (52%)
1979	20	6 (30%)
1980	21	15 (71%)
1981	0	0
Overall	89	36 (40%)

Table 5

Summary assessment of the microbiological quality of shellfish
in the Mediterranean, according to the EEC criteria

(MED POL VII sampling stations with at least 6 analyses per year)

<u>Year</u>	<u>Stations surveyed</u>	<u>Stations with satisfactory shellfish</u>
1976	18	4 (22%)
1977	13	0 (0%)
1978	24	14 (58%)
1979	34	11 (32%)
1980	21	15 (71%)
1981	7	4 (57%)
Overall	117	49 (42%)

54. The considerably higher percentage of satisfactory stations obtained when applying the EEC criteria (approximately 40%) as compared with the percentage of satisfactory stations according to the WHO/UNEP interim criteria (approximately 5%), can be explained by the less restrictive character of the former criteria. While the WHO/UNEP interim criteria require that all the samples have at most 2 faecal coliforms per gram of shellfish flesh, the EEC criteria require that 75% of the samples have at most 3 faecal coliforms per millilitre of shellfish flesh and intervalvular liquid.

55. It should be noted that the WHO/UNEP and the EEC microbiological criteria are not directly comparable for a number of reasons:

- the former include both shellfish-growing waters and the shellfish themselves. The latter are limited to shellfish.
- for the analysis of shellfish, the former utilize the flesh only, while the latter utilize both the flesh and the intervalvular fluid.
- while the WHO/UNEP interim criteria have the primary objective of protecting public health through prevention of infection through shellfish consumption, the main objective of the EEC criteria is to protect the quality of shellfish through protection of the quality of shellfish-growing waters. Nonetheless, the EEC Directive specifying the criteria recommends that, pending the adoption of a Directive on the protection of consumers of shellfish products, it is essential that the above value be observed in waters in which shellfish directly edible by man are living (EEC, 1979). The value can therefore be considered as imperative, not guide, insofar as edible shellfish are concerned.

Conclusions

56. The results of MED POL VII during its operational period (1976-81) insofar as these concern shellfish and shellfish-growing waters, can be summarized as follows:

- (a) Monitoring of shellfish-growing areas on a regular basis throughout the course of the project was conducted by 6 collaborating laboratories in 4 Mediterranean states.
- (b) The selection of sampling stations in shellfish-growing areas was mainly determined by the location and management of existing shellfish-growing areas, and did not have a random character. Nonetheless, the conclusions derived from the monitoring results in the areas surveyed provide a good indication of the microbial quality of shellfish-growing areas in the Mediterranean.
- (c) A total of 50 sampling stations were monitored in shellfish-growing areas, from which approximately 2300 samples of both water and shellfish were analysed. On average, 10 dual samples were analysed from each sampling station per year, which indicates a continuous and systematic monitoring programme performed by the collaborating laboratories.
- (d) Reference methods were developed by the principal investigators of MED POL VII and used for the analysis of the microbiological quality of recreational waters, shellfish and shellfish-growing waters. The use of these methods made the comparison of results obtained by various laboratories possible on the Mediterranean scale.

- (e) Interim criteria for the assessment of the microbiological quality of shellfish and shellfish-growing waters were formulated by the principal investigators of MED POL VII. These criteria were used in preparing the first such assessment.
- (f) Only 3-4% of the monitored stations in the shellfish-growing areas were found to be suitable for direct consumption of shellfish, according to the WHO/UNEP interim criteria for the quality of shellfish and shellfish-growing waters. For comparison purposes, on the basis of the EEC criteria for shellfish-growing waters, about 40% of these sampling stations would have been found satisfactory for direct consumption of shellfish.

MEASURES TO PREVENT AND CONTROL MICROBIAL POLLUTION OF SHELLFISH AND SHELLFISH-GROWING WATERS IN THE MEDITERRANEAN SEA AREA

Existing national provisions and international arrangements and agreements relevant to the Mediterranean Sea

(a) National provisions

57. Although all Mediterranean countries have comprehensive regulatory mechanisms for ensuring strict quality control for drinking water, the situation has still not reached the same level in the case of shellfish and shellfish-growing waters. Practically all countries have had umbrella-type general public health legislation, under which the deterioration of any medium likely to result in hazards to human health could be controlled, for a number of years. The need for pollution control of coastal waters as a routine measure is being increasingly recognized in Mediterranean countries, and the majority of these now have specific legislation either in force or under consideration. However, from information currently available, only a limited number of Mediterranean countries have issued norms or standards on the quality of shellfish-growing waters, or shellfish prior to removal from such waters for consumption, though shellfish in markets and food-stores normally come under general food-safety regulations (which are outside and beyond the scope of the proposed criteria).

58. In the case of shellfish-growing waters, some Mediterranean countries in which a substantial shellfish industry exists have evolved adequate control systems, covering all aspects of production, from the growing area to the processing facilities, as well as to the wholesale and retail markets. In other countries also possessing a substantial shellfish industry, such control systems have yet to be developed. The elaborate control systems and support services required to ensure the production and distribution of shellfish free from contamination appear to be feasible only in a limited number of Mediterranean states, as the economic justification would depend on the size of the industry.

59. A number of countries in the Mediterranean region have developed statutory and/or administrative criteria for the microbiological and related quality of shellfish waters. Legislation and actual criteria differ in the various countries. Information currently available is from France, Greece, Italy, Spain and Yugoslavia.

60. In France, the statutory requirement for the microbiological quality of shellfish waters is that shellfish from such waters, on the basis of 26 samplings during 12 consecutive months, must contain less than 300 faecal coliforms per 100 ml in at least 21 of such samples. Of the remaining five, three should not exceed 1000 per 100 ml, and the other two should not exceed 3000 per 100 ml. Although the intervalvular fluid is added to the flesh as part of the diluent, estimations are made in terms of the flesh.

61. In addition, an internal (non-statutory) system of classification divides shellfish areas into four categories, on the basis of concentrations of E. coli in the water itself. These categories are as follows:

Class I:	satisfactory	0 per 100 ml
Class II:	acceptable	1-60 per 100 ml
Class III:	doubtful	60-120 per 100 ml
Class IV:	unfavourable	120 per 100 ml

62. Up to 1986, the law in Greece classified Shellfish waters into three categories on the basis of Total coliform concentrations in the water itself, as follows:

Suitable (clean)	:	0- 70/100 ml
Moderately infected	:	71-700/100 ml
Unsuitable (heavily infected):	:	above 700/100 ml

New legislation defines shellfish waters as suitable if the total coliform concentration is 0-70 per 100 ml in 75% of the samples collected over a period of one year, based on a minimum sampling frequency of once every three months. There are also provisions for shellfish from areas containing 700 or more total coliforms per 100 ml seawater to be subject to depuration in ad hoc stations. The new law also stipulates that the acceptable concentrations for shellfish is 300 faecal coliforms per 100 ml of flesh plus intervalvular fluid, also for 75% of the samples collected over one year on the basis of a minimum frequency of once every three months.

63. In Italy, the law distinguishes between two types of shellfish-growing waters or areas: approved areas and conditioned areas. Microbiological quality requirements are as follows:

"Approved" areas

(a) Water

Not more than 2 E. Coli per 100 ml. A maximum of 7 E. Coli per 100 ml in 10% of the samples is admissible, on condition that shellfish from these areas do not exceed the stipulated microbiological limits for this matrix.

(b) Shellfish

Not more than 4 E. coli per ml of flesh plus intervalvular fluid, and Salmonella absent in 25 ml.

"Conditioned" areas

(a) Water

Not more than 34 E. Coli per 100 ml. A maximum of 49 E. Coli per 100 ml in 10% of the samples is admissible provided that shellfish do not exceed the stipulated microbiological limits for this matrix.

(b) Shellfish

Not more than 39 E. coli per ml of flesh plus intervalvular fluid.

64. In Spain, the law regarding the microbiological quality of Shellfish waters stipulates the following limits:

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

65. In Yugoslavia, a general water quality law classifies areas as suitable for shellfish growing of, inter alia, the concentration of total coliforms in the water does not exceed 10 per 100 ml.

66. An analysis of the situation from the statutory requirements of the various countries as described in paras 59 to 65 above shows a wide variation both in approach and in methodology. There are three different modes of approach in determination of the suitability or otherwise of aquatic areas for shellfish growing - (a) the quality of the actual water itself, (b) the quality of the shellfish in the area and, (c) a combination of both the above. In addition, the microbiological parameters differ in the various countries. For seawater itself, Greece and Yugoslavia utilise total coliforms. Italy, France (in its "internal" classification) and Spain utilise E. coli. For the shellfish in the growing areas, France and Greece utilise faecal coliforms, Italy E. coli, while Spain and Yugoslavia do not utilise this particular matrix. While the predominant methodology employed is the Most Probable Number (MPN) technique, there are differences as to whether, in the case of shellfish, the flesh alone or the flesh plus intervalvular fluid is analysed.

67. The establishment of acceptable quality criteria for Shellfish waters, whether on the basis of the waters themselves or on the shellfish present in such waters, does not automatically imply that shellfish from approved or similarly designated areas are suitable for direct human consumption, as prior to this, they would normally have to be processed through sanitary control mechanisms and procedures, either under general food safety regulations or under specific provisions regarding shellfish. While criteria for the suitability or otherwise of shellfish for direct human consumption are outside the scope of this document, some information on the standards utilised in various Mediterranean countries is given below, mainly to demonstrate that the achievement of satisfactory Shellfish waters is only the first step in the eventual assurance of a bacteriologically safe product when it finally reaches the consumer.

68. In Italy, the law on shellfish-growing waters also stipulates further conditions prior to sale. In the case of shellfish from approved zones, deposable species (a) have to be treated in a special deputation establishment and (b) must also undergo sorting, washing, conditioning and labelling operations. Non-deposable species must only undergo the operations at (b) above. In the case of conditioned areas, samples of shellfish must not exceed an MPN of 39 E. coli per ml (flesh plus intervalvular fluid), otherwise the areas is prohibited. Deposable species are subject to the same conditions as those from approved areas. Non-deposable species have to be sterilized prior

to canning, or conditioned after cooking. Normally, shellfish from "conditioned" areas are not sold for direct human consumption.

69. In France, the microbiological criteria relevant to shellfish for human consumption are as follows:

- Aerobic micro-organisms, 30°C	100 000 per gram
- Faecal coliforms	300 per 100 ml
- Faecal streptococci	2 500 per 100 ml
- Staphylococcus aureus	100 per gram
- Anaerobic Sul. reducing bacteria, 46°C	10 per gram
- <u>Salmonella</u>	absent in 25 grams

70. Interpretation of the results provides three categories of contamination, namely:

- the contamination does not exceed the above described criteria "m";
- the contamination is between the above criteria "m" and an upper level "M" equal to 10 times the criteria "m", when the enumeration of micro-organisms is made on solid media, or a level equal to 30 times the criteria "m" when the enumeration is made on liquid media;
- the contamination exceeds the level "M".

71. Provided that if any of the results included in a given sampling exceeds "M", the quality of the sampling is considered:

- satisfactory, when the observed values are:
 - < 3m for solid culture media, or
 - < 10m for liquid culture media;
- acceptable, when the observed values are:
 - between 3m and 10m for solid culture media, or
 - between 10m and 30m for liquid culture media, and
 - $\frac{c}{n} < \frac{2}{5}$, where "c" is the number of units composing the sample,
 - which give results between m and M, and "n" is the number of units per sample, depending on the adopted interpretation plan;
- unsatisfactory, when:
 - $\frac{c}{n} > \frac{2}{5}$, for the values specified above, or whenever a value above the "M" criteria is observed.

72. In Greece, the law formerly classified shellfish for consumption into three categories, as follows:

First class	:	up to 500 <u>E. coli</u> /100 ml flesh
Second class	:	501 - 1500 " " " " "
Third class	:	above 1500 " " " " "

Those have now been replaced by the provisions of the new legislation which covers both Shellfish waters and Shellfish.

73. In Spain, depurated shellfish destined for consumption must conform with the following quality standards:

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

74. There is no specific law determining the microbiological and related quality of shellfish in Yugoslavia. These, however, are covered by general market quality standards, which stipulate:

<u>Salmonella</u>	absent in 25 g or ml
Coagulase-positive Staphylococci	" " 0.01 g or ml
Sulphite-reducing Clostridia	" " 0.01 g or ml
<u>Proteus</u> species	" " 0.001 g or ml
<u>E. coli</u>	" " 0.001 g or ml

75. An analysis of the present situation regarding national legislation for control of water quality of shellfish-growing areas in the Mediterranean region as a whole shows that at least some of Mediterranean countries are concerned with shellfish production without having adequate facilities for ensuring a safe product, including the treatment and handling of polluted products. In these cases, the appropriate surveillance of shellfish production areas can be a critical measure for ensuring that shellfish leave such areas in a safe and wholesome state. Sanitary control could therefore be ensured by the appropriate authorities through normal food hygiene procedures.

76. While the quality criteria developed by each Mediterranean country may fulfil its relevant needs, harmonization or coordinated action will obviously be difficult to promote under the conditions currently prevailing. The various methodologies and criteria presently in use still have to be comprehensively compared. Obviously, when assessment of the suitability of an area for shellfish production is based on examination of the actual growing waters, one difficulty is the lack of a direct relationship between the bacterial content of the water and that of the shellfish grown in it. However, effective control has been exercised in some countries through such a mode of assessment.

(b) International arrangements and agreements

77. With regard to the quality of shellfish-growing areas, international arrangements and agreements satisfactorily covering the Mediterranean as a whole do not exist. However, some international arrangements have been or are being developed, concerning the quality of shellfish waters and the sanitary requirements for shellfish intended for human consumption. These arrangements promulgated by EEC and the Codex Alimentarius Commission, are summarized in paragraph 79.

78. The Council of the European Communities adopted in 1979 the Directive on the quality required in shellfish-growing waters (EEC, 1979). The Directive takes into consideration the physicochemical and microbiological parameters to be applied to shellfish-growing waters, the relevant (G) guide values, the (I) imperative or mandatory values, the reference methods of analysis, and the minimum sampling and measuring frequencies.

79. The requirements established by the Directive, as far as the microbiological and related parameters of shellfish-growing waters are concerned (reproduced in Table 6) include a stipulated guide value of an MPN concentration of less than or equal to 300 faecal coliforms per 100 ml of shellfish flesh and intervalvular liquid. However, pending the adoption of a Directive on the protection of consumers of shellfish products, the current Directive states that it is essential that the above value be observed in waters containing shellfish directly edible by man.

80. EEC Member States shall, initially within a two-year period following the notification of the Directive, designate shellfish-growing waters. Further provisions are made for additional designations and for revisions. Member States shall establish programmes to ensure that the designated waters conform, within six years following designation, to both the values set by the Member States and the comments contained in columns G and I of the Annex of the Directive.

81. Designated waters shall be deemed to conform to the microbiological provisions of the Directive if samples of such waters, taken at a minimum quarterly frequency, at the same sampling point, and over a period of 12 months, show that they conform to both the values set by the Member States and the guide value described above, as regards 75% of the samples.

82. The Directive on the quality required for shellfish waters concerns at present four Mediterranean countries: France, Greece, Italy and Spain. Under this condition the Directive does not help to avoid the shortcomings experienced in the Mediterranean, which are due to the different criteria of individual countries.

83. A proposed draft code of hygiene practice for molluscan shellfish has been prepared by the Codex Alimentarius Commission (1978). The draft code in its Appendix III provides general environmental sanitation recommendations. These concern:

- sanitary disposal of human and animal wastes;
- determination of pollution types and sources;
- classification of shellfish-growing areas;
- control of shellfish-growing areas;
- reclassification of shellfish-growing areas;
- animal, plant, pest and disease control.

84. A list of current laboratory procedures and standards are given in an annex to Appendix III of the Code. This list includes bacteriological standards and methods currently employed in several developed countries. France and Italy are the only Mediterranean countries in the above-mentioned list.

85. The Committee on Food Hygiene considered that:

- successful shellfish control programmes have been in operation in a number of Member States for many years, using a wide range of bacteriological standards and methods; and
- that it was virtually impossible to reach agreement at this time on any specific set of standards and methods.

Table 6

Microbiological and related quality requirements for shellfish-growing waters of the European Economic Community (EEC, 1979)

Parameter	Guide (G)	Imperative (I) (mandatory)	Reference methods of analysis	Minimum sampling and measuring frequency
10. Faecal coliforms/100 ml	> 300 in the shellfish flesh and intervalvular liquid(*)		Method of dilution with fermentation in liquid substrates in at least 3 tubes in 3 dilutions. Subculturing of the positive tubes on a confirmation medium. Count according to MPN (most probable number). Incubation temperature 44°C±0.5°C	Quarterly
4. Suspended solids mg/l		A discharge affecting shellfish waters must not cause the suspended solid content of the waters to exceed by more than 30% the content of waters not so affected	- filtration through a 0-45 µm membrane, drying at 105°C and weighing - centrifuging (for at least 5 minutes with mean acceleration 2800 to 3200 g) drying at 105°C and weighing	Quarterly
6. Dissolved oxygen Saturation †	> 80%	- ≥ 70% longer (average value) - should an individual measurement indicate a value lower than 70%, measurements shall be repeated - an individual measurement may not indicate a value of less than 60% unless there are no harmful consequences for the development of shellfish colonies	- Winkler's method - electrochemical method	Monthly, with a minimum of 1 sample representative of low oxygen conditions on the day of sampling. However, where major daily variations are suspected, a minimum of 2 samples in 1 day shall be taken

(*) However, pending the adoption of a Directive on the protection of consumers of shellfish products, it is essential that this value be observed in waters containing shellfish edible by man.

86. In view of the necessity to harmonize the methodologies used for the assessment of the quality of Mediterranean shellfish and shellfish-growing waters along the lines requested by the Contracting Parties to the Barcelona Convention and its protocols, from the early stages of the MED POL VII project, efforts have been undertaken in this direction.

87. Prior to 1976, the main method used in Mediterranean laboratories for microbiological examination of seawater and shellfish was the multiple test tube or most probable number (MPN) method. During the course of MED POL VII, participating laboratories were oriented towards the membrane filtration culture (MF) method, and in most of these laboratories, both methods were used concurrently. The first reference methods for the main three microbial parameters (total coliforms, faecal coliforms and faecal streptococci), developed by WHO/UNEP through MED POL VII for eventual adoption by Mediterranean laboratories for coastal water monitoring, were based on the MF method (UNEP/WHO, 1983a, 1983b, 1983c). Nevertheless, the MPN method still remains to a very large extent the official method of bacteriological indicator analysis in most countries, and to ensure a greater degree of comparability, the Long-term Programme for Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase II) has also developed reference methods using the MPN technique for the same three parameters (total coliforms, faecal coliforms and faecal streptococci) (UNEP/WHO, 1985a, 1985b, 1985c). As a result of comparative studies on the MF and MPN methods, evidence has been obtained that results obtained by either of the two methods have a satisfactory degree of comparability at critical levels.

88. The MPN technique formed the basis of a reference method developed by WHO/UNEP for determination of faecal coliforms (the main microbiological parameter) in bivalves (UNEP/WHO, 1983d).

89. Also within the framework of MED POL VII, a seminar on coastal quality monitoring of recreational and shellfish areas was convened by WHO and UNEP in Rome in April 1978 (WHO/UNEP, 1978). With regard to monitoring of shellfish and shellfish-growing waters, it was considered by the seminar that the first two phases in assessing shellfish quality (the culture area and the shellfish in its natural surroundings) must comply with appropriate microbiological limits. It was, of course, understood that for a full assessment of shellfish quality as a food product, shellfish should also be examined at subsequent phases of handling (transport, processing and marketing). During the same seminar, the recommendations contained in the Guidelines for health-related monitoring of coastal water quality (WHO/UNEP, 1977b), that in shellfish the flesh alone should be utilized for microbiological analysis, was endorsed.

90. In considering microbiological limits, the seminar took into account the limits adopted by several countries, and recommended the interim criteria described in para 46 of this document. These recommendations were renewed by a meeting of principal investigators of MED POL VII convened by WHO and UNEP in Rome in November 1979, in which one of the subjects for discussion was the development of interim microbiological criteria. Following their proposal to the Contracting Parties in 1985, they could not, however, be adopted owing to the various different criteria already in force in a number of countries.

Scientific rationale for establishment of environmental quality criteria for shellfish and shellfish-growing waters in the Mediterranean Sea

91. The major health hazards associated with shellfish are those caused by the consumption of raw shellfish polluted by faecal material. The pollution

of shellfish-growing waters by pathogenic micro-organisms may occur whenever the water is contaminated by faeces, sewage outfalls, discharges from polluted rivers or drainage from polluted areas. Sewage outfalls constitute the major pollution source for shellfish-growing areas.

92. The marine environmental factors which affect the sanitary quality of shellfish may be divided into two broad groups: physical and biological.

93. The following physical factors determine the extent to which shellfish-growing areas are affected by pollution:

- the location of the sewage discharge relative to the shellfish-growing areas;
- the quantity and concentration of the sewage effluent;
- the type and degree of treatment of the sewage before discharge;
- the dilution and dispersion characteristics of the receiving waters.

94. The following biological factors determine the degree of contamination of shellfish-growing waters and of shellfish:

- the microbiological content of the sewage discharge;
- the viability of the different micro-organisms in the sea;
- the biological processes of shellfish;
- the development of toxic algae.

95. The present document deals only with the environmental quality criteria applicable to shellfish waters, in those aspects relevant to the protection of human health.

96. In those Mediterranean countries where national quality criteria exist and are applied, satisfactory results have generally been experienced as far as the protection of human health is concerned. However, taking into account (a) the relatively small number of Mediterranean countries actually possessing and applying such standards and criteria, (b) the small number of shellfish-growing areas monitored during the course of MED POL VII, as well as their unbalanced geographical distribution, and (c) the current lack of information within the region on the results of further monitoring performed since 1982, any criteria proposed on a regional scale would have to be of an interim nature until such time as extended monitoring programmes and appropriate epidemiological and related studies have provided the basis for firmer definitive measures.

97. The development of criteria applicable to Mediterranean shellfish-growing areas should be based on indicators, which are:

- consistently and exclusively present in faecal wastes in reasonably high densities;
- capable of survival, during sewage treatment and various types of transport, to an extent comparable to that of the pathogens potentially contained therein.

98. Moreover, considering that such criteria should be applicable to the Mediterranean as a whole, they should be based on:

- a minimum number of indicators;
- a simplified and limited number of analytical methods;
- the infrastructure and facilities available in each of the Mediterranean States.

99. Various indicators are being applied in those countries where shellfish-growing areas are routinely monitored. These indicators include E. coli, faecal coliforms, faecal streptococci, Cl. perfringens and Salmonella. However, in certain circumstances, such as those occurring after a disease incident involving shellfish, the range of tests should be expanded to include pathogens likely to be implicated. Among them are Salmonella typhi, other Salmonella species, V. parahaemolyticus and V. cholerae.

100. Considering the universality of faecal coliforms, these indicators have been retained for routine monitoring. However, the study of other practical indicators, both existent and new, should be included in any monitoring exercise, with the aim of further improving results by the constant selection of better indicators, simplified methods, and more economic procedures.

101. The methods usually applied include the MPN method, the MF method and the total plate count method. Similarly, the media used for recovery of the indicators are various. Here again the aim is to apply appropriate reference methods which will be used by all those concerned in the Mediterranean area, and will provide more satisfactory results. In this connexion, the current reference method recommended for used by Mediterranean laboratories is the MPN method (UNEP/WHO, 1983d).

102. In addition to simplification and applicability all over the Mediterranean, the proposed harmonization of quality criteria and methodology aims at promoting comparability, uniform evaluation, exchanging of knowledge and experience, gradual improvement of approach and of results, development of control methods, and certification and recognition of results. The interim quality criteria proposed would be subject to refinement and additions, in order to meet the requirements defined by different species of shellfish and/or conditions in the Mediterranean.

103. Purification of shellfish may also be expanded in the Mediterranean in the future. In this respect, quality criteria should be developed and applied for seawater in storage basins and in purification plants. Quality criteria for shellfish will also be needed in this respect.

104. In view of the absence of epidemiological studies performed within the Mediterranean region to back any criterion, the proposed interim criteria for shellfish waters are mainly based on the available experience in certain European countries and in the USA. It is the intention to assess the validity of these quality criteria through the performance of appropriate epidemiological studies, which should be conducted in one of the following ways (WHO/UNEP, 1977a):

- predictive models;

- retrospective epidemiological studies of case reports and disease outbreaks; and
- prospective controlled epidemiological and microbiological studies.

105. These studies are catered for in the research component of MED POL Phase II (Activity D). Following such evaluation, the proposed interim criteria would be confirmed, including such adjustments as may be required. The next step would be the establishment of national standards based on the criteria. At that stage a decision should be made as to the "acceptable risks" of symptoms of varying degrees of severity, or of specific diseases. This decision should take social, economic, political and health factors into account.

Proposed interim environmental quality criteria for Shellfish and Shellfish-growing waters in the Mediterranean Sea

106. A Consultation on Environmental Quality Criteria for Shellfish-growing waters and Shellfish in the Mediterranean jointly convened by WHO and UNEP in Athens on 26-27 March 1987, reviewed the relevant legislation currently in force in a number of Mediterranean countries, as well as the results of comparability studies on the various criteria and their supporting methodologies carried out over the past two years. The Consultation made the following recommendations regarding Shellfish water quality in the Mediterranean (WHO, 1987):

1. The term "Shellfish waters" should mean those coastal and brackish waters in which shellfish (bivalve and gastropod molluscs) live.
2. For the assessment of the microbiological quality of these waters, the shellfish themselves should be taken into account.
3. For the determination of microbiological parameters, preference should be given to analysis of shellfish flesh and intervalvular fluid, rather than flesh alone.
4. The results of analysis of microbiological quality should be expressed by the number of faecal coliforms recorded in 100 ml (FC/100 ml).

The method of analysis utilised 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.

Enumeration should be effected according to the Most Probable Number (MPN) method.

5. The minimum sampling and measurement frequency should be once every three months. This frequency should be increased as much as possible, in accordance with specific local conditions.
6. The concentration of faecal coliforms should be less than 300 per 100 ml of shellfish flesh and intervalvular fluid or of flesh alone.
7. 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.

MEASURES PROPOSED FOR ADOPTION BY THE CONTRACTING PARTIES

107. On the basis of the present assessment carried out by WHO/UNEP, and taking into account the rationale for ensuring the suitability of Shellfish waters in the Mediterranean, the currently existing situation as regards national legislation within the region, and the recommendations of the WHO/UNEP Consultation as given in para 106 above, the following interim environmental quality criteria for Shellfish waters are submitted for the consideration of the Working Group with a view to their transmission by the Secretariat to the next meeting of the Contracting Parties:

The Contracting Parties

- (a) Take into account the provisions of Article 7.1(c) of the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources, regarding the quality of seawater used for specific purposes that is necessary for the protection of human health, living resources and ecosystems.
- (b) Adopt as a minimum common requirement for the quality of Shellfish waters, the proposed WHO/UNEP interim environmental quality criteria, as detailed in (c) and (d) below and in the accompanying table.
- (c) For the purposes of such criteria, consider the term "Shellfish waters" to mean those coastal and brackish waters in which Shellfish (bivalve and gastropod molluscs) live.
- (d) Utilise the following in the application of such criteria:
 - for the assessment of the microbiological quality of Shellfish waters, the Shellfish themselves shall be taken into account;
 - for the determination of microbiological parameters, preference shall be given to analysis of shellfish flesh and intervalvular fluid, rather than flesh alone;
 - the results of analysis of microbiological quality shall be expressed by the number of faecal coliforms recorded in 100 ml (FC/100 ml);
 - the method of analysis utilised shall be incubation at 37 ± 0.5 °C with fermentation on a liquid substrate for a period of 24 to 48 hours, followed by a confirmation test at 44 ± 0.2 °C for 24 hours. Enumeration shall be effected according to the Most Probable Number (MPN) method;
 - the concentration of faecal coliforms should be less than 300 per 100 ml of shellfish flesh and intervalvular fluid or of flesh alone, in at least 75% of the samples, based on a minimum sampling frequency of once every three months.
- (e) Take any other complementary measures, such as increasing sampling frequency, including further parameters, and monitoring of the quality of the water itself in shellfish areas, as may be demanded by national or local circumstances for ensuring satisfactory quality of shellfish waters.

- (f) Include, to the extent possible, all Shellfish waters in their National Monitoring Programmes within the framework of MED POL Phase II.
- (g) Provide the Secretariat to the Convention with the fullest information possible on:
- present legislation and administrative measures on existing national criteria for Shellfish waters;
 - measures taken on (b) and (e) above;
 - relevant monitoring data from (f) above.
- (h) Continue to provide full support to the research and monitoring component of MED POL Phase II relevant to the assessment of the environmental quality of Shellfish waters, in particular to studies on the comparability of faecal coliforms and E. coli determinations, as well as on the usefulness of other indicator organisms.

Proposed interim criteria for shellfish waters in the Mediterranean

<u>Matrix</u>	<u>Parameter</u>	<u>Concentration</u>	<u>Minimum sampling frequency</u>	<u>Analytical method</u>	<u>Interpretation method</u>
Shellfish	Faecal coliforms	< 300 per 100 ml flesh + intervalvular fluid <u>or</u> flesh in at least 75% of the samples.	every 3 months (more frequently whenever local circumstances so demand).	Multiple tube fermentation and counting according to MPN (most probable number) method.	By individual results, histograms or graphical adjustment of a lognormal- probability distribution.

Incubation period:
37 + 0.5 °C for 24 h or
48 h followed by 44 +
0.2 °C for 24 h.

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