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

ASSESSMENT OF THE PRESENT STATE OF MICROBIAL POLLUTION
IN THE MEDITERRANEAN SEA AND PROPOSED CONTROL MEASURES

In co-operation with:



WORLD HEALTH ORGANIZATION

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INTRODUCTION

1. There are two principal kinds of human exposure to microbial pollutants in the Mediterranean Sea: consumption of contaminated seafood and direct contact with contaminants while bathing, in the latter case mainly through ingestion of sea water. 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 in search of recreation. The consumption of shellfish raised in the Mediterranean is estimated as about ... 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 facts that the most popular recreational amenity along all the Mediterranean coastline is the bathing beach, and that the relatively warm temperatures result in bathers spending more time in the water than would normally be the case in temperate regions, thus providing a longer exposure period to potential contamination. Furthermore, 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 recreational ones.

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), entailed the regular monitoring of coastal recreational waters, shellfish-growing waters and shellfish flesh by a network of 30 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 recreational and shellfish-growing waters 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 bathing waters, shellfish and shellfish-growing waters surveyed during MED POL VII. The results of the pilot project 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 have recently been initiated, with the aim of actually correlating sea water quality with health effects in the exposed sectors of Mediterranean populations.

7. The scope of this document is to assess the present 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.

ASSESSMENT OF MICROBIAL POLLUTION IN THE MEDITERRANEAN

Introduction

8. 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 recreational waters and in the shellfish-growing areas of the Mediterranean.

9. The results obtained by the collaborating laboratories during the execution of MED POL VII have provided a sound basis for the first regional assessment of the microbiological quality of the recreational waters and shellfish-growing areas in the Mediterranean.

10. 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.

11. 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

12. 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.

13. 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.

14. 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.

15. 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

16. 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).

17. 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).

18. 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).

19. 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.

20. 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.

21. 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.

22. 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).

23. 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).

24. 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.

25. 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).

26. 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).

27. 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.

28. 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.

29. 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).

30. 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 recreational waters

31. Monitoring of recreational waters in the Mediterranean has been conducted by 30 collaborating laboratories, in 14 Mediterranean States, under WHO's coordination and according to the agreed procedures summarized in the final report of MED POL VII (WHO/UNEP, 1981).

32. Monitoring started in late 1976, with the formal approval of a major group of collaborating laboratories designated by the Mediterranean states, and it has been increasing steadily, with additional participation, and periodical evaluation of the activities carried out during the meetings of principal investigators. MED POL VII ended in March 1981, though in some cases collaborating institutes continued to send data for later months, showing clearly the momentum and interest achieved by the project.

33. A minimum programme was applied by all participating laboratories to promote comparability of results. Actual monitoring programmes of individual laboratories were generally larger than required, by including parameters other than the minimum specified in the MED POL VII operational document (WHO/UNEP, 1976).

34. The sampling areas were selected primarily taking into account their importance as public recreational beaches (Figure 1). However, road access to the area, distance to the analytical laboratory, and local administrative requirements resulted in a great diversity of sampling locations and sampling strategies between collaborating laboratories, and occasionally between subsequent years for a given laboratory.

35. Under these conditions, the selection of sampling stations was not fully random, and consequently, the conclusions derived concerning the microbiological quality of the recreational waters in the Mediterranean cannot be strictly considered as applicable universally. However, considering the number and spatial distribution of the collaborating institutes, as well as the large number of water samples analysed, the conclusions derived from the results of MED POL VII can reasonably be considered as a valuable assessment of the present situation of recreational waters in the Mediterranean.

36. Table 1 summarizes the basic facts relevant to the assessment of the microbiological quality of recreational waters in the Mediterranean through MED POL VII.

Table 1

Basic facts relevant to the assessment of the microbiological quality of recreational waters in the Mediterranean through MED POL VII

<u>Category</u>	<u>Number</u>
Water sampling stations	700
Water samples analysed	12 500
Reference stations	164
Reporting forms from reference stations	1 164

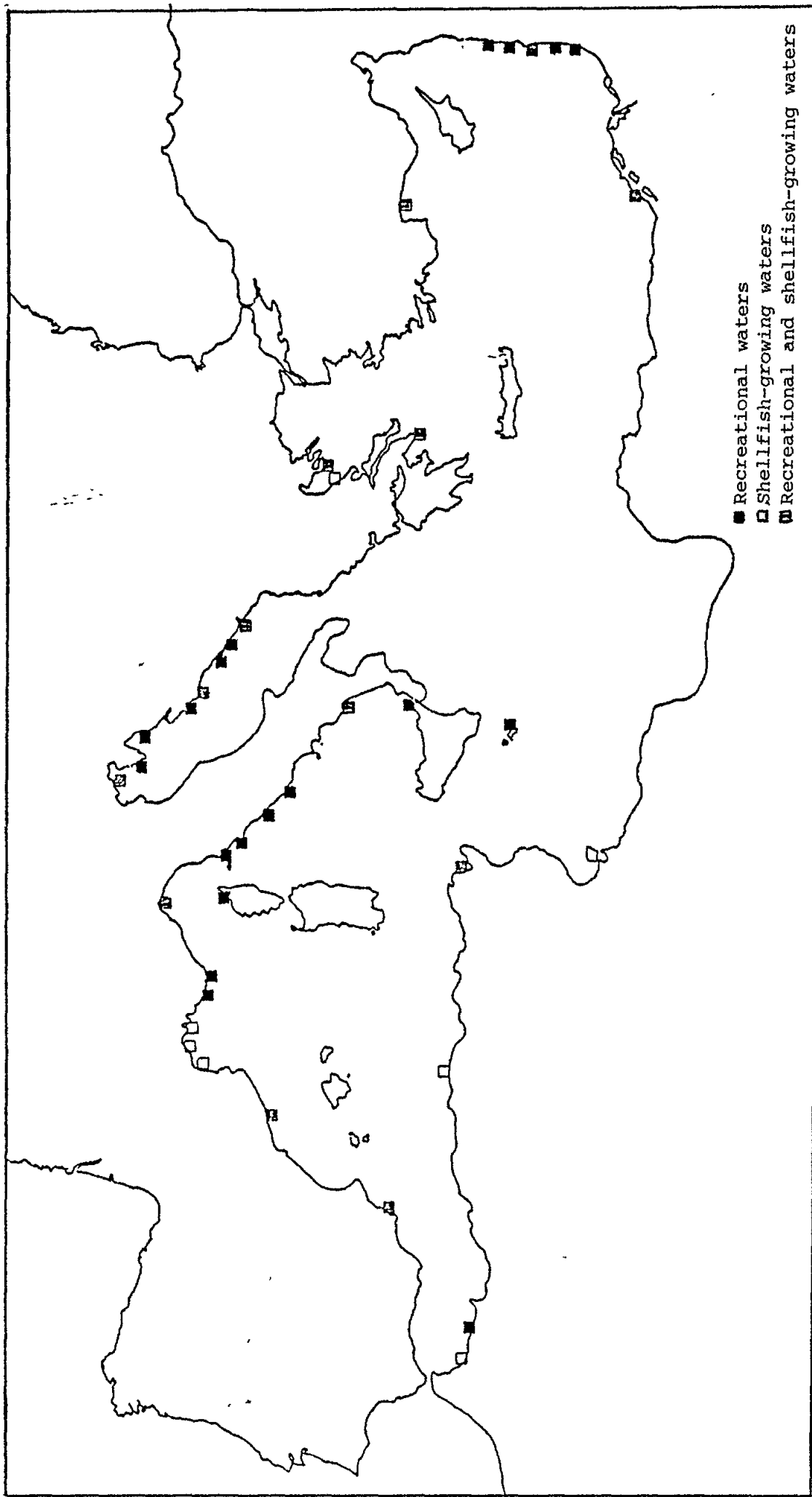


Figure 1. Areas monitored during the course of MED POL VII

37. Most of the monitoring was carried out during 1978, 1979 and 1980. The average number of water samples analysed at each sampling station was approximately 6 per year. However, this figure should only be considered as an overall regional average, as monitoring frequencies varied widely between sampling stations, both within areas monitored by the different individual laboratories, and, in a number of cases, within areas monitored by the same laboratory.

38. The basic parameters used for assessing the microbiological quality of recreational waters in the Mediterranean were the three indicators of microbial pollution: total coliforms, faecal coliforms and faecal streptococci. Additional parameters describing general conditions in the monitoring area at the time of sampling, and occasionally qualitative information on the presence of vibrios, salmonella, and viruses, was also recorded.

39. To ensure that the results obtained by different collaborating institutes would be comparable, the membrane filtration technique was adopted as the reference analytical method (WHO/UNEP, 1976). However, due to unforeseeable circumstances, and to the interest of including monitoring data otherwise obtained by nationally required procedures, the multiple dilution technique, with the most probable number (MPN) interpretation method, was also considered. This circumstance provided a good opportunity for carrying out a preliminary comparative study of the two methods at some collaborating laboratories.

40. The microbiological quality criteria for recreational waters recommended by the WHO/UNEP Expert Consultation (1977a) provided that "highly satisfactory bathing areas should show E. coli counts of consistently less than 100 per 100 ml, and to be considered acceptable, no more than 10% of at least 10 consecutive samples collected during the bathing season should exceed 1000 E.coli per 100 ml".

41. Faecal coliform counts were considered practically equivalent to E.coli counts for evaluation purposes by the MED POL VII Project Operational Document (WHO/UNEP, 1976). The "consistently" requirement of the previous text was considered equivalent to the median microbial concentration when evaluating the quality of beaches surveyed during the MED POL VII project (WHO/UNEP, 1979c). Furthermore, the faecal coliforms were considered as the best single indicator for the recreational quality of coastal Mediterranean waters.

42. As a result, the WHO/UNEP interim criteria, developed through MED POL VII by the laboratories participating in the project, considered for the present assessment of recreational coastal waters can be summarized as follows: "The faecal coliform concentrations, of at least 10 water samples collected during the bathing season, should not exceed: (a) 100 faecal coliforms per 100 ml in 50% of the samples, and (b) 1000 faecal coliforms per 100 ml in 90% of the samples" (UNEP, 1981).

43. Table 2 summarizes the results of an assessment of the microbiological quality of recreational waters in the Mediterranean, according to the WHO/UNEP interim criteria taking into account only the results from stations with at least 10 samples analysed per year.

Table 2

Summary assessment of the microbiological quality
of recreational waters in the Mediterranean
according to the WHO/UNEP interim criteria

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

<u>Year</u>	<u>Number of stations</u>			<u>Satisfactory by FC50 & FC90</u>
	<u>Surveyed</u>	<u>In accordance with</u>		
		<u>FC50</u>	<u>FC90</u>	
1976	21	16 (76%)	14 (67%)	14 (67%)
1977	40	38 (95%)	34 (85%)	34 (85%)
1978	33	30 (91%)	30 (91%)	28 (85%)
1979	133	124 (93%)	104 (78%)	104 (78%)
1980	86	79 (92%)	72 (84%)	69 (80%)
1981	0	0	0	0
Overall	313	287 (92%)	254 (81%)	249 (80%)

44. Results available show that approximately 80% of the sampling stations surveyed yearly are of satisfactory quality by meeting both requirements of the interim criteria. Moreover, the results point out the slightly more restrictive character of the second limit included in the WHO/UNEP interim criteria, as compared to the first one. Approximately 12% of the sampling stations in which 50% of the water samples contained less than 100 faecal coliforms per 100 ml, did not comply with the condition of having less than 1000 faecal coliforms per 100 ml in 90% of their water samples, and consequently could not be considered satisfactory according to both limitations. The limitation requiring less than 1000 faecal coliforms per 100 ml in 90% of the water samples, practically determines the overall classification of a water sampling station.

45. In an effort to broaden the basis for the assessment of the microbiological quality of the Mediterranean recreational waters, an additional analysis was made taking into account the results from all sampling stations with at least 6 samples per year (6 was the average sampling frequency in MED POL VII). The results are summarized in Table 3.

46. An analysis of Table 3 reveals a quite similar pattern to that revealed in Table 2, with only the absolute percentual values being approximately 5% lower than those of Table 2.

47. No attempt was made to use the results obtained from stations with less than 6 samples per year in the analysis of the microbiological quality of the Mediterranean recreational waters according to the WHO/UNEP interim criteria. The results obtained from such stations showed an increasing lack of homogeneity and therefore were considered unsuitable for statistical treatment.

Table 3

Summary assessment of the microbiological quality
of recreational waters in the Mediterranean,
according to the WHO/UNEP interim criteria
(MED POL VII sampling stations with at least 6 samples per year)

<u>Year</u>	<u>Number of stations</u>			
	<u>Surveyed</u>	<u>In accordance with</u>		<u>Satisfactory</u>
		<u>FC50</u>	<u>FC90</u>	<u>by FC50 & FC90</u>
1976	26	16 (62%)	15 (58%)	14 (54%)
1977	55	50 (91%)	46 (84%)	46 (84%)
1978	193	181 (94%)	164 (85%)	161 (83%)
1979	288	251 (87%)	201 (70%)	200 (69%)
1980	118	110 (93%)	100 (85%)	97 (82%)
1981	25	19 (76%)	20 (80%)	19 (76%)
Overall	705	627 (89%)	546 (77%)	536 (76%)

48. Considering that three Mediterranean countries, France, Greece, and Italy, belong to the European Economic Community (EEC), it has seemed appropriate to conduct a similar assessment of the microbiological quality of recreational waters in the Mediterranean, according to the provisions of the EEC Directive 76/160 concerning the quality of bathing waters (EEC, 1976) which considers imperative (I) and guide (G) limits for the microbiological and physico-chemical parameters. The Directive also indicates the minimum sampling frequency and the analytical or inspection methods for the parameters. From the article 5 of the Directive (see paragraph 92 and Table 13) the following limits are obtained:

I limits: less than 10 000 total coliforms/100 ml in 95% of samples
less than 2 000 faecal coliforms/100 ml in 95% of samples.

G limits: less than 500 total coliforms/100 ml in 80% of samples
less than 100 faecal coliforms/100 ml in 80% of samples
less than 100 faecal streptococci/100 ml in 90% of samples

49. Results obtained through MED POL VII from sampling stations with at least 10 samples per year, analysed according to the EEC criteria, are summarized in Table 4.

50. An analysis of Table 4 shows the relatively higher restrictive character of the guide (G) limits contained in the EEC Directive, as compared to the imperative (I) limits, and more specifically, the restriction imposed by the 100 faecal streptococci per 100 ml concentration not to be exceeded in 90% of the samples. While approximately 80% of the sampling stations satisfy the imperative (I) limits for total and faecal coliforms, only approximately 65% of the stations satisfy the guide (G) limits for the same two organisms, the percentage of stations satisfying the guide (G) limit for faecal streptococci being even lower, 47% approximately. As a result, the overall percentage of MED POL VII sampling stations considered satisfactory, according to the EEC Directive, is rather low, approximately 38%, with yearly values ranging from 26 to 53%.

Table 4

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

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

<u>Year</u>	<u>Surveyed</u>	<u>Number of stations</u>							<u>Satisfactory</u>
		<u>In accordance with</u>							
		<u>TC80</u>	<u>TC95</u>	<u>FC80</u>	<u>FC95</u>	<u>FS90</u>			
1976	21	13 (62%)	16 (76%)	10 (48%)	14 (67%)	13 (62%)	10 (48%)		
1977	40	32 (80%)	36 (90%)	29 (73%)	34 (85%)	20 (50%)	17 (43%)		
1978	33	25 (76%)	30 (91%)	21 (64%)	28 (85%)	15 (45%)	13 (39%)		
1979	133	65 (49%)	94 (71%)	84 (63%)	100 (75%)	50 (38%)	34 (26%)		
1980	81	59 (73%)	69 (85%)	52 (64%)	65 (80%)	48 (59%)	43 (53%)		
1981	0	0	0	0	0	0	0		
Overall	308	194 (63%)	245 (80%)	196 (64%)	241 (78%)	146 (47%)	117 (38%)		

Table 5

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

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

<u>Year</u>	<u>Surveyed</u>	<u>Number of stations</u>							<u>Satisfactory</u>
		<u>In accordance with</u>							
		<u>TC80</u>	<u>TC95</u>	<u>FC80</u>	<u>FC95</u>	<u>FS90</u>			
1976	35	19 (54%)	28 (80%)	16 (46%)	21 (60%)	19 (54%)	15 (43%)		
1977	56	44 (79%)	49 (88%)	39 (70%)	46 (82%)	28 (50%)	23 (41%)		
1978	194	128 (66%)	149 (77%)	137 (71%)	154 (79%)	103 (53%)	81 (42%)		
1979	286	134 (47%)	187 (65%)	161 (56%)	189 (66%)	114 (40%)	78 (27%)		
1980	112	72 (64%)	93 (83%)	73 (65%)	90 (80%)	54 (48%)	46 (41%)		
1981	24	16 (67%)	19 (79%)	15 (63%)	19 (79%)	15 (63%)	13 (54%)		
Overall	707	413 (58%)	525 (74%)	441 (62%)	519 (73%)	333 (47%)	256 (36%)		

51. The results of the analysis according to the EEC criteria using MED POL VII sampling stations with 6 or more samples per year are summarized in Table 5.

52. Table 5 shows a quite similar pattern to that discussed for Table 4. While the guide (G) limits for total and faecal coliforms are notably more restrictive than the corresponding imperative (I) limits, the guide (G) limit for faecal streptococci appears as the most restrictive of the five. Overall percentage values in Table 5 are slightly lower than the corresponding values in Table 4, with only a 2% difference in the number of satisfactory stations.

53. Data reported by the Ministère de la Santé of France (1981) on the microbiological quality of coastal recreational waters of France, when analysed according to the EEC criteria (Table 6), show a pattern similar to that obtained through MED POL VII for the whole Mediterranean region (Table 5) analysed according to the same (EEC) criteria. Assuming that the data used in this analysis represent a good average for the French coastal waters, it is interesting to note that the quality of the Mediterranean recreational areas is higher than that of the Atlantic coast and that there is a significant improvement of this quality in the three-year period (1979-81) covered by the analysis.

Table 6

Summary assessment of the microbiological quality of recreational areas of France, according to EEC criteria

(sampling stations with at least 10 samples per year)

<u>Coastal area</u>	<u>1979</u>		<u>1980</u>		<u>1981</u>	
	<u>Stations surveyed</u>	<u>Stations satisfactory</u>	<u>Stations surveyed</u>	<u>Stations satisfactory</u>	<u>Stations surveyed</u>	<u>Stations satisfactory</u>
Mediterranean stations only	298	66 (22%)	358	150 (42%)	357	171 (48%)
All coastal areas of France	-	-	1240	313 (25%)	1006	341 (34%)

Microbiological quality of shellfish and shellfish-growing waters

54. Monitoring of shellfish-growing areas in the Mediterranean has been 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).

55. 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.

56. 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.

57. Table 7 summarizes the basic facts relevant to the assessment of the microbiological quality of shellfish-growing waters in the Mediterranean.

Table 7

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

58. 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.

59. 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.

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

61. Although the MED POL VII operational document (WHO/UNEP, 1976) considered both flesh and intervalvar liquid for the microbiological examination of shellfish, the Guidelines for health-related monitoring of coastal water quality (WHO/UNEP, 1977b) recommended that the flesh alone be used. Following the recommendation of a working group, the meeting of MED POL VII principal investigators endorsed the Guidelines' provision, 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).

62. 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 intervalvar liquid (EEC, 1979).

63. The meeting of MED POL VII principal investigators referred to in paragraph 61 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):

(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 20% of the samples.

64. 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).

65. Table 8 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 8

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 satisfactory</u>
		<u>water</u>	<u>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%)

66. An analysis of Table 8 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 should be permitted without additional purification, is very low, approximately 3%.

67. 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 9) reveals a similar pattern. Only about 4% of these stations meet the criteria permitting direct sale of shellfish without additional purification.

Table 9

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 satisfactory</u>
		<u>water</u>	<u>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%)

68. According to the EEC criteria (EEC, 1979), the guide (G) value of 300 faecal coliforms per 100 ml of shellfish flesh and intervalvar 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, are summarized in Tables 10 and 11, taking into account stations with at least 10 and 6 shellfish analyses per year, respectively.

69. Table 10 reveals that the percentage of satisfactory stations, 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.

70. Tables 10 and 11 show an almost identical pattern, with approximately 40% of the stations surveyed being considered as satisfactory according to the EEC criteria. Comparison of Tables 9 and 11 reveals the considerably higher restrictive character of the WHO/UNEP interim criteria.

Table 10

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 satisfactory</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 11

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 satisfactory</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%)

71. 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 intervalvar liquid.

72. It should be noted that the WHO/UNEP and the EEC criteria are not directly comparable because the latter include also intervalvar liquid. 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-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).

Conclusions

73. The results obtained by the collaborating laboratories during the execution of the MED POL VII project have provided a sound basis for the first regional assessment of the microbiological quality of recreational waters, shellfish and shellfish-growing areas in the Mediterranean. Furthermore, results from the MED POL X project have provided an additional insight into the sources of the microbial pollution in the Mediterranean.

74. The results of MED POL VII during its operational period (1976-81) can be summarized as follows:

- (a) Monitoring of recreational waters in the Mediterranean has been conducted by 30 collaborating laboratories, in 14 Mediterranean states.
- (b) Monitoring of shellfish-growing areas in the Mediterranean has been conducted by 6 collaborating laboratories in 4 Mediterranean states.
- (c) Although the selection of sampling stations in recreational coastal waters was not fully random, and consequently the conclusions derived from them cannot be considered universally applicable, the number and spatial distribution of the sampling stations and water samples analysed provide a reliable assessment of the microbial quality of recreational waters in the Mediterranean.
- (d) 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 provides a good indication of the microbial quality of shellfish-growing areas in the Mediterranean.
- (e) A total of 700 sampling stations were monitored in coastal recreational waters, from which approximately 12 500 water samples were analysed. On average, 6 water samples were analysed from each sampling station per year.

- (f) 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.
- (g) Reference methods have been 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.
- (h) Interim criteria for the assessment of the microbiological quality of recreational waters, shellfish and shellfish-growing waters have been formulated by the principal investigators of MED POL VII. These criteria were used in preparing the first assessment of the microbiological quality of Mediterranean recreational waters, shellfish and shellfish-growing waters.
- (i) From 75 to 80% of the sampling stations surveyed in recreational waters of the Mediterranean were found to comply with the WHO/UNEP interim criteria for the microbiological quality recreational waters. Using the EEC criteria for recreational waters, only about 37% of these sampling stations have been found satisfactory for recreational use.
- (j) 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. Using the EEC criteria for shellfish-growing waters, about 40% of these sampling stations have been found satisfactory for direct consumption of shellfish.

MEASURES TO PREVENT AND CONTROL MICROBIAL POLLUTION IN THE MEDITERRANEAN SEA AREA

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

(a) National provisions

75. 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 recreational waters, 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 coastal recreational and/or shellfish-growing waters.

76. The available information on existing or proposed national standards dealing with recreational water quality in individual Mediterranean countries is summarized in Table 12. As this table illustrates, these standards show a considerable variation both in the type and numerical values assigned to the microbiological indicator used, and in the specified frequencies for sampling and standard-compliance. This disparity makes it difficult, at the very least, to obtain an adequate overall comparison between the national reports on the the actual quality of the relevant recreational waters.

77. The three main indicators used are total coliforms, faecal coliforms and E. coli. Most countries use only one of the three. In others, two or even three indicators are used concurrently (as in Libya and France respectively). In others (e.g. Turkey), only one indicator is used as a matter of routine, but in the case of special studies, other indicators as well as pathogenic organisms are utilized. The method most frequently used in Mediterranean countries is the most probable number (MPN) method.

78. 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.

79. Very few countries in the Mediterranean region possess appropriate legislation or have developed quality criteria for shellfish-growing waters, or for shellfish flesh. Furthermore, existing legislation and quality criteria differ significantly among the various countries. The bulk of information available is from France, Italy and Spain.

80. In some countries, the main emphasis is placed upon categorization of shellfish-growing areas. Such categorization is based on detailed investigations of both the topography of the area and the water quality itself. This is the case in Italy, where the quality of shellfish-growing areas is determined on the basis of E. coli in the water itself, utilizing the MPN method. Approval for shellfish growing is subject to the requirement that water samples taken over 12 consecutive months do not exceed 2 E. coli per 100 ml in 10% of the samples. In Spain, the general approach is similar, but the requirements are that concentrations of E. coli should not exceed 15 per 100 ml in 50% of the samples and should not exceed 50 per 100 ml in 90% of the samples.

81. In France, shellfish-growing areas are classified into four categories according to the concentration of E. coli in the water. 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

Table 12

Microbiological water quality standards for recreational water
of some Mediterranean countries

Country	Year of proposal	Water quality indicator	STANDARD				Notes
			Analytical method	Sampling frequency	Compliance frequency	Indicator concentration per 100 ml	
Algeria	*	*	*	*	*	*	
Cyprus	-	-	-	-	-	-	
Egypt	*	*	*	*	*	*	
France (*)	1973	Total coliforms Faecal coliforms Faecal streptococci	* * *	* * *	100% 100% 100%	2000 500 100	
Greece (*)	1965	Total coliforms Total coliforms Total coliforms Total coliforms	MPN MPN MPN MPN	* * * *	* * * *	0-50 51-500 501-1000 >1000	safe for bathing acceptable not recommended unsafe
Israel	1950	Faecal coliforms	MPN	over 30 days	90%	1000	
Italy (*)	1973	<u>E. coli</u>	MPN	*	100%	100	
Lebanon	*	*	*	*	*	*	
Libya	1975	Total coliforms Faecal coliforms	MPN MPN	* *	100% 100%	1000 100	
Malta		<u>E. coli</u>	MPN	*	100%	1000	
Monaco	*	*	*	*	*	*	
Morocco	*	*	*	*	*	*	
Spain	1977	<u>E. coli</u>	MPN or MF	over 30 days	50% 90%	200 1000	
Syria	*	*	*	*	*	*	
Tunisia	*	*	*	*	*	*	
Turkey	1978	<u>E. coli</u> <u>E. coli</u> <u>E. coli</u>	MPN MPN MPN	* * *	* * *	< 100 < 1000 > 1000	very suitable suitable polluted
Yugoslavia	1978	Total coliforms	MPN	24 per year	100%	500	

- No standards at present
* No information available

82. In a number of countries, the major emphasis is placed on the quality of shellfish taken from an area, although in assessing the suitability of any area for shellfish production, both topographical factors and the bacteriological quality of the water are taken into account. This method is used in France, where the quality of shellfish-growing areas is determined on the basis of the faecal coliform content of shellfish living in the respective area. Evaluation of the contamination is expressed as the MPN of faecal coliforms per 100 ml of flesh smashed and diluted according to a prescribed methodology and is based on 26 samplings spread over 12 consecutive months. Shellfish-growing areas are classified as "sanitary" when the MPN of faecal coliforms in shellfish flesh does not exceed 300 per 100 ml. This standard is considered to be met when the number of samples with an MPN of faecal coliforms higher than 300 per 100 ml does not exceed 5 during a period of 12 consecutive months. However, the MPN of faecal coliforms in 3 out of these 5 samples should not exceed 1000 per 100 ml, and the remaining 2 should not exceed 3000 per 100 ml. Waters where the above standards are not met are classified as unsuitable for shellfish growing, and collection of shellfish from such areas is forbidden, except in cases where purification is intended to be undertaken, such operations being subject to approval by the relevant authorities. In those cases where the MPN of faecal coliforms in shellfish flesh exceeds 10 000 per 100 ml in 25% of the samples, such approval requires confirmation by the sanitary Directorate.

83. Apart from determination of the quality of shellfish-growing waters, whether on the basis of the actual water quality itself or on that of the quality of shellfish, standards for shellfish themselves in relation to their suitability or otherwise for human consumption are relatively sparse throughout the region, and where they do exist, vary from country to country. In Italy, for example, the quality criteria for shellfish from approved growing areas are subject to the requirement that samples taken during 12 consecutive months do not exceed an MPN of 160 E. coli per 100 ml of shellfish in 90% of the samples and do not exceed a MPN of 500 E. coli per 100 ml in 10% of the samples. The values proposed above do not presume any judgement on the work of the European Economic Community on this subject, as contained in the Directive 79/923.

84. 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

85. 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".

86. Provided that 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.

87. The present situation regarding national legislation for control of coastal recreational water quality in the Mediterranean region as a whole obviously leaves room for considerable improvement, apart from eventual harmonization, although the basic measures are being applied to varying extents in most countries.

88. In the case of shellfish-growing areas, the great majority 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.

89. 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

90. With regard to the quality of coastal recreational waters, an effort towards the development of an internationally harmonized approach has been made by the European Economic Community (EEC). In this context, the Council of the European Communities adopted in 1975 the Directive (EEC 1976) concerning the quality of bathing water. The Directive takes into consideration the physicochemical and microbiological parameters applicable to bathing waters, the minimum sampling frequencies, and the reference methods of analysis for the proposed parameters.

91. According to this Directive, the EEC Member States shall take all necessary measures to ensure that, within 10 years following the notification of the Directive, the quality of bathing waters conforms to the limit values set in accordance with Article 3 of the Directive. Furthermore, Member States shall, four years following the notification of the Directive, and at regular intervals thereafter, submit a comprehensive report to the Commission on their bathing water and the most significant characteristics thereof.

92. The microbiological parameters considered by the Directive are: total coliforms, faecal coliforms, faecal streptococci, Salmonella and enteroviruses (Table 13). Bathing water shall be deemed to conform to the relevant parameters if the samples of that water, taken at the same sampling point and at the intervals specified in Table 13, show that it conforms to the parametric values for the quality of the water concerned, in the case of:

- 95% of the samples for parameters corresponding to those specified in column "I" of Table 13;
- 90% of the samples in all other cases with the exception of the "total coliform" and "faecal coliform" parameters, where the percentage may be 80%;

and if, in the case of the 5, 10 or 20% of the samples which do not comply:

- the water does not deviate from the parametric values in question by more than 50%, except for microbiological parameters, pH and dissolved oxygen;
- consecutive water samples taken at statistically suitable intervals do not deviate from the relevant parametric values.

93. France, Greece and Italy are members of the European Economic Community (EEC), and as such are bound to comply with the provisions contained in the EEC Council Directive concerning the quality of bathing water.

Table 13

Microbiological quality requirements for bathing water of the European Economic Community (EEC, 1976)

<u>Parameters</u>	<u>G</u> (1)	<u>I</u> (1)	<u>Minimum sampling frequency</u>	<u>Method of analysis and inspection</u>
Total coliforms per 100 ml	500	10 000	Fortnightly (2)	Enumeration in multiple tubes. Sub-culturing of the positive tubes on a confirmation sodium. Count according to MPN (most probable number) or membrane filtration and culture on an appropriate medium such as Tergitol lactose agar, endo agar, 0-4% Teepol broth, subculturing and identification of the suspect colonies.
Faecal coliforms per 100 ml	100	2 000	Fortnightly (2)	In the case of 1 and 2, the incubation temperature is variable according to whether total or faecal coliforms are being investigated.
Faecal streptococci per 100 ml	100	-	(3)	Litsky method. Count according to MPN (most probable number) or filtration on membrane. Culture on an appropriate medium.
Salmonella per litre	-	0	(3)	Concentration by membrane filtration. Inoculation on a standard medium. Enrichment - subculturing on isolating agar - identification.
Enteroviruses PFU per litre	-	0	(3)	Concentrating by filtration, flocculation or centrifuging and confirmation.

(1) G = Guide; I = Imperative (mandatory)

(2) When a sampling taken in previous years produced results which are appreciably better than those in this table and when no new factor likely to lower the quality of the water has appeared, the competent authorities may reduce the sampling frequency by a factor of 2.

(3) Concentration to be checked by the competent authorities when an inspection in the bathing area shows that the substance may be present or that the quality of the water has deteriorated.

94. 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-growing 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 96.

95. 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.

96. The requirements established by the Directive, as far as the microbiological and related parameters of shellfish-growing waters are concerned (reproduced in Table 14) 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.

97. 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.

98. 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.

99. The Directive on the quality required for shellfish waters concerns at present three Mediterranean countries: France, Greece and Italy. 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.

100. 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.

Table 14

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.

101. 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.

102. 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.

103. In view of the necessity to harmonize the methodologies used for the assessment of the quality of the Mediterranean coastal recreational waters, 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.

104. During the course of MED POL VII, laboratories were oriented towards the membrane filtration culture (MF) method, and in most of these laboratories, both methods were used concurrently. The 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 recreational area 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) is providing both for the development of reference methods using the MPN technique for the same three parameters (total coliforms, faecal coliforms and faecal streptococci), and for comparative studies on the MF and MPN methods. In the latter case, some evidence already exists that results obtained by either of the two methods have a satisfactory degree of comparability at critical levels.

105. Likewise, reference methods for the microbiological parameters to be used in connexion with the survey of shellfish and shellfish-growing waters were developed by WHO/UNEP through MED POL VII (UNEP/WHO, 1983a, 1983b, 1983d).

106. The World Health Organization (WHO), and the United Nations Environment Programme (UNEP), within the framework of the WHO/UNEP Pilot Project on Coastal Water Quality Control in the Mediterranean (MED POL VII) convened a group of experts in Athens in 1977 to initiate a scientific study concerning health criteria and epidemiological studies related to coastal water pollution (WHO/UNEP, 1977a). The purpose of the meeting was:

- to review the epidemiological factors and health criteria on which quality standards for recreational and coastal waters are based, and
- to develop a methodology for epidemiological research programmes intended to provide reliable data for practical application.

107. After examining the available evidence, the Working Group came to the conclusion that there was not as yet enough epidemiological basis for recommending changes in the conclusions reached by the WHO Working Group on Guidelines and Criteria for Recreational Quality of Beaches and Coastal Waters (WHO, 1975). Thus the following interim water quality criteria were adopted: "highly satisfactory bathing areas should, however, show E. coli counts of consistently less than 100 E- coli per 100 ml, and to be acceptable, bathing waters should not give counts consistently greater than 1000 E. coli per 100 ml".

108. The Working Group felt that, while it may be justified that water quality control at existing recreational facilities be based on the more lenient criterion of 1000 E. coli per 100 ml limit, the more strict value of 100 E. coli per 100 ml should be considered for new recreational facilities, and for long-term decisions on water quality management involving large investments.

109. The Working Group felt that the above numerical limitation of 1000 E. coli should be more closely defined statistically, and recommended the following expression:

"no more than 10% of at least 10 consecutive samples collected during the bathing season should exceed 1000 E. coli per 100 ml".

110. 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.

111. In considering microbiological limits, the seminar took into account the limits adopted by several countries, and recommended the following interim standards:

for shellfish

- as faecal coliforms (FC) per gram (g) of shellfish flesh

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

for water of shellfish culture areas

- less than 10 faecal coliforms per 100 ml in 80% of samples
- less than 100 faecal coliforms per 100 ml in 20% of samples

112. 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.

Scientific rationale for establishment of environmental quality criteria for recreational waters in the Mediterranean

113. Studies carried out within the framework of MED POL VII have pointed out the need for a thorough analysis of the scientific and technical information available on which to base environmental quality criteria applicable to the Mediterranean coastal recreational waters. Accordingly, the attention of scientists participating in this pilot project was focused on discussing and elaborating the scientific rationale, and the relevant considerations needed for the establishment of water quality criteria applicable to Mediterranean coastal waters, specifically as regards public health protection.

114. Although literature available on the subject contains a considerable number of contributions, particularly dealing with analytical methods and the adequacy of the various microbial indicators, the conclusions reached are sometimes contradictory, apparently due to the specific conditions under which individual studies were carried out.

115. As a result, in the following discussion, particular attention has been given to experimental results reported by investigators working on Mediterranean coastal waters, while keeping in perspective the relevant information available in global literature.

116. The modern approach to the process of developing water quality criteria is to accord specific consideration to at least the following aspects (discussed in paragraphs 116-153):

- the type and nature of the quality indicator or indicators;
- the indicator concentration which should not be exceeded;
- the analytical methods involved;
- the method and the frequency of sampling;
- the frequencies of compliance for a given indicator concentration;
- the interpretation method; and
- all additional factors peculiar to the water mass and to the beneficial uses considered.

(a) Quality indicators

117. The assumption that discharges of animal and human faecal wastes into water bodies used primarily for recreational purposes, bathing and swimming, are a source of potential public health hazards, has developed historically into the search for a microbial indicator capable of characterizing the risk involved in the intended use of the water.

118. The coliform group was originally selected, in accordance with both analytical methodology and field experience available. A better understanding of the source and significance of the different components of the coliform group brought about the consideration of the faecal coliform group, and particularly of E. coli, as a good quality indicator for recreational waters. Development of analytical techniques, as well as increased knowledge of the ecological behaviour of faecal micro-organisms, have aroused interest in the possible consideration of other microbial indicators, among them some pathogens, such as faecal streptococci, Clostridium perfringens, Pseudomonas aeruginosa, Salmonella, and selected enteric viruses and bacteriophages.

119. Any potential microbiological quality indicator relevant to public health should fulfil the following requirements:

- be consistent and exclusively associated with the source of pathogens of concern;
- be present in sufficient numbers to provide an accurate density estimate whenever the level of the pathogen is capable of producing a detectable effect;
- approach the behaviour and resistance, under disinfection and environmental stresses, of the most resistant pathogen expected to be present at significant levels in the source;
- be identifiable and quantifiable by reasonably easy and economical methods, with sufficient specificity, accuracy and precision.

120. Taking into account both the general conditions of most laboratories in charge of microbiological control of coastal water quality, and the different indicators available, faecal coliforms (particularly E. coli) were considered to be the most sensitive indicators of the degree of sewage pollution (WHO, 1975), and were subsequently proposed for routine monitoring of coastal water quality within the framework of MED POL Phase II (WHO/UNEP, 1981).

121. Epidemiological studies carried out by the United States Environmental Protection Agency (USEPA, 1980) and by two MED POL VII collaborating institutes, Malaga and Tarragona (UNEP, 1981b), have indicated a potential interest in faecal streptococci as an additional water quality indicator for public health protection.

122. The results of the epidemiological study conducted by the USEPA (1980) showed that:

- gastro-intestinal symptoms and, to a lesser extent, fever, were found to be both swimming-associated and pollution-related;
- faecal streptococci and, to a lesser degree, E. coli, were the two quality indicators that best correlated with the morbidity rate of gastro-intestinal symptoms.

123. The results from the study carried out in two MED POL VII pilot zones (UNEP, 1981) indicated that:

- skin, ear and eye ailments were the most frequently suffered by swimmers in coastal recreational waters;
- the habit of immersing the head in the water while swimming followed a statistically significant association with the occurrence of ear and eye ailments;
- the fact that faecal streptococci predominated over faecal coliforms in waters considered satisfactory by the WHO/UNEP interim water quality criteria was consistent with the different inactivation behaviour of both indicators. The statistically significant association obtained between the morbidity rate for ear ailments and faecal streptococci concentrations could be used as a valuable additional quality criteria for coastal recreational waters.

124. Pending the development and implementation of epidemiological studies on public health effects derived from recreation in coastal waters, and the establishment of definite cause-effect relationships, the presently available information and the results of MED POL VII justify the establishment of adequate interim criteria for coastal water quality management aimed at efficiently protecting public health.

(b) Concentration limits for indicators

125. The essential component of a water quality criterion is the quantitative exposure-effect relationship between the density of the quality indicator and the level of protection provided for a given beneficial use. Although the information presently available is far from complete, considerable progress has been achieved during the last few years. The more recent results are summarized below.

126. Morbidity rates for gastro-intestinal symptoms approaching 1% are associated with both mean concentrations of approximately 10 faecal streptococci per 100 ml, and mean E. coli concentrations in the range of 10 to 100 E. coli per 100 ml. A 5% morbidity rate for the same symptoms is associated with mean concentrations of approximately 1000 faecal streptococci per 100 ml (USEPA, 1980).

127. Morbidity rates for ear ailments approaching 1% are associated with mean concentrations of 10 faecal streptococci per 100 ml, while a 2% morbidity rate was associated with a mean concentration of approximately 1000 faecal streptococci per 100 ml (UNEP, 1981b).

128. In studies carried out in the coastal waters of the Tyrrhenian Sea (UNEP, 1981), viruses were detected in all water samples exceeding 920 E. coli per 100 ml. A significant correlation was also found between the number of E. coli and that of enteroviruses.

129. In studies carried out on beaches in the area of Tel-Aviv, Israel (WHO/UNEP, 1980), enteroviruses were detected on all three beaches surveyed even when coliform levels were below the 1000 faecal coliform per 100 ml level stipulated in the proposed interim criteria.

130. Finally, results obtained on the relative survival of microbial indicators in a number of MED POL VII pilot zones revealed that faecal streptococci are more persistent in seawater than faecal coliforms, and approach the resistance behaviour of some enteric viruses. Also, the gradual variation of the faecal coliforms to faecal streptococci ratio, as the faecal coliforms vary over a wide range of concentrations, could explain the different regression lines obtained by investigators working within a short range of faecal coliform concentrations.

(c) Analytical methods

131. Two basic methods can be used for the determination of microbial indicators:

- the multiple tube dilution technique, followed by the interpretation by the Most Probable Number (MPN); and
- the Membrane Filtration technique (MF).

132. Selective media and incubation procedures are available for the most common microbial indicators.

133. There has been considerable discussion as to which of the above two methods gives more accurate results. From the strictly theoretical viewpoint, the MPN method relies on the statistical hypothesis that the microbial concentration assigned to a given number is that which makes the observed combination of positive tubes the combination with the highest probability of occurrence. The inclusion of the 95% confidence interval in the MPN method tabulation is precisely directed to inform the user that other microbial concentrations can result in the same combination of positive tubes. Although the MF method is based on the direct counting of visible colonies, there are also a series of working hypotheses that can reduce the number of visible organisms from those originally present.

134. There are two specific conditions of coastal water quality control that should greatly help in solving the existing discussion on analytical methodology. In the first place, the degree of uncertainty with which microbial standards are currently determined is not proportional to the degree of accuracy and precision of either method of analysis. Although this assertion should not be used as a ground for discontinuing further improvement and development of analytical techniques, it stresses the need for practical considerations when dealing with microbial concentrations measured in coastal recreational waters.

135. Secondly, results gathered both in the Mediterranean pilot zones and in other coastal areas in the world show that statistical interpretation of microbiological values is an adequate and strong tool in the evaluation of coastal water quality. The simultaneous consideration of microbial values obtained during several weeks allows for a balancing of the intrinsic spatial and temporal variation of coastal water quality. Furthermore, current results available indicate that the overall evaluation of microbial quality of coastal waters through a statistical approach is practically constant, regardless of the analytical technique used for microbial estimations.

136. The wide application of a lognormal probability distribution method, as considered by some European countries (WHO, 1975) and recommended for use within the MED POL VII Pilot Project (WHO/UNEP, 1977b, 1979b) should provide valuable information on its validity, as well as on its ability to overcome the dual methodology discussion.

(d) Method and frequency of sampling

137. The overall objective of MED POL VII was specifically addressed to the evaluation of the public health implications of coastal water quality. The sampling methodology recommended for use by participants in this pilot project was to obtain water samples in recreational areas with a high user-population density. As a compromise between the accessibility of the sampling points and coverage of the areas more frequented by bathers, the recommended procedure was to collect a water sample from 10 to 20 cm below the surface at a point with 1.0 to 1.5 m depth.

138. Although operational difficulties were experienced by several participants, and some alternative procedures were used, there is no conclusive evidence to warrant a modification of the proposed procedure. Thus the long-term programme (MED POL Phase II) gives specific attention to this point.

139. Water samples should be collected in sterilized wide-mouthed bottles, and protected from sunlight and heat until the time of analysis. A maximum of 8 hours should elapse from the collection of the sample until the actual analysis. Water samples analysed after that period of time result in microbial concentrations with inadequate accuracy and precision.

140. An adequate evaluation of microbial quality for coastal waters, be it statistically or otherwise, requires a minimum number of samples to obtain a meaningful result. From the analysis of data collected at various pilot zones, it appears that at least 10 consecutive samples should be collected during a given period to allow a meaningful interpretation of the results. This requirement translates into a one per week sampling frequency during the summer period, considering that the bathing season in most Mediterranean coastal areas is about three months.

141. It has to be pointed out that microbiological quality is but one element in judging the public health implications of recreation in coastal waters. A detailed technical inspection is also an important and necessary complement for the evaluation of the sanitary conditions of coastal recreational areas.

142. Sampling should be carried out systematically, by observing a regular time interval between consecutive samples, and by consistently sampling at the same periods during the day. Although the influence of sunlight and temperature has been documented as the main factor affecting the survival of microbial indicators, the selection of the sampling time is also dependent on the recreational pattern in the waters of concern, as well as on the means of sample collection, transportation and analysis. As a tentative schedule, the period between 10.00 and 15.00 hours was adopted by many laboratories during the course of the pilot project.

143. Sampling requirements are detailed in the bacteriological reference methods developed by WHO/UNEP through MED POL VII for coastal recreational areas (UNEP/WHO, 1983a, 1983b, 1983c). Studies to determine the comparative results obtained from samples collected during different periods of the day are currently in progress within the framework of the research component of MED POL Phase II.

(e) Frequencies of compliance

144. Quality control techniques quite often rely on statistical methods. The interest and validity of this approach, for the study of the microbiological quality of coastal waters, is reflected in the increasing number of national and international standards which include upper concentrations of indicators not to be exceeded in a given percentage of a set of consecutive samples.

145. A major outcome of the MED POL VII Pilot Project has been the verification that microbial concentrations in coastal waters can be adequately interpreted by a lognormal probability distribution model. Furthermore, standard deviations for most water sampling stations are within a short interval, regardless of the microbial indicators considered, which includes the standard deviation value implied by the proposed interim criteria.

146. The immediate consequences of this practical observation are that:

- two pairs of microbial concentration versus frequency of compliance completely define the water quality characteristics of a sampling station, since two parameters determine a lognormal distribution; and
- two pairs of microbial concentration versus frequency of compliance, whose implied standard deviation is not within the experimentally observed interval, are bound to be imbalanced, one of them becoming the most restrictive for all practical purposes, and the other becoming practically inoperative.

147. A further benefit from a balanced set of standards concerns the design of facilities for wastewater treatment and disposal at sea. A receiving water quality standard, defined by a pair of balanced limits, assures the designer that if the median limitation is met, it is most likely that the other limitation will also be met.

148. From the experimental results gathered in those Mediterranean coastal waters studied during the course of the MED POL VII project, it appears that a 10-fold ratio between the concentration not to be exceeded 90% of the time, and that not to be exceeded 50% of the time, comes quite close to the naturally-observed variation of microbial quality. Simple calculations will enable the ratio associated to a different pair of frequencies to be obtained.

149. Detailed analysis of the microbiological quality of coastal waters during MED POL Phase II should provide very valuable information on which to validate and extend the criterion proposed above.

(f) Interpretation methods

150. The ultimate purpose of the microbiological results gathered at a sampling station is to determine compliance with a proposed quality standard. The practical process results in a comparison of the set of values defined by the standard with the corresponding parameters of the experimental data.

151. A graphical interpolation of a straight line on the experimental points drawn on a lognormal probability paper seems to be a simple and effective method for verifying the agreement of the data with the proposed model, as well as for the estimation of the water quality parameters concerned (UNEP/WHO, 1977b). The graphical method is being suggested over the numerical method because of the better insight it provides, particularly on the possible discrepancies with the model and the values lying outside the general trend.

(g) Additional factors

152. Among the additional factors to be considered in the development process of coastal water criteria and standards for the Mediterranean Sea, the following should be mentioned.

153. Mediterranean coastal waters can be characterized by high salinity values and low tide fluctuations. Current patterns, within the 3 km range from the coastline, predominantly follow a direction parallel to the coast. Furthermore, the summer season is characterized by high solar radiation levels and warm temperatures, both ambient and in the water.

154. Bathing and recreation in Mediterranean coastal waters is characterized by long periods of water contact intermixed with intensive sun exposure. Between 60 and 70% of populations visiting Mediterranean coastal areas stay at a given resort town for up to three weeks, with almost daily recreation on the beach. Recreation in coastal areas takes place mainly during July, August and September, and generally concentrates around widely-known beaches, where high population densities are reached.

Proposed environmental quality criteria for recreational bathing waters in the Mediterranean

155. Based on the results and experience gathered from the pilot phase of the Joint WHO/UNEP Project on Coastal Water Quality Control in the Mediterranean (MED POL VII), and in accordance with the above review of the scientific rationale presently available. The following is proposed as the WHO/UNEP environmental quality criteria for interim application to coastal recreational waters of the Mediterranean, as far as public health protection is concerned.

"The coastal bathing waters of the Mediterranean will be considered as satisfactory and safe for use by the general public if the concentrations of faecal coliforms (FC) and faecal streptococci (FS) in at least 10 representative water samples collected during the bathing season at intervals of not more than 14 days do not exceed 100 FC or FS per 100 ml in 50% of the samples and 1000 FC or FS per 100 ml in 90% of the samples. The concentration of FC and FS should be determined by agreed reference methods or by methods yielding comparable results, proved by intercalibration with the relevant reference methods."

156. The proposed interim criteria are summarized in Table 15.

Table 15

Proposed interim environmental quality criteria for recreational waters
in the Mediterranean Sea

<u>Para- meter</u>	<u>Concentrations per 100 ml not to be exceeded</u>		<u>Minimum number of samples</u>	<u>Analytical method</u>	<u>Interpretation method</u>
	<u>50%</u>	<u>90%</u>			
Faecal coliforms	100	1000	10	Membrane filtration, m-FC broth or agar incubated at 44.5±0.2°C for 24h	Graphical or analytical adjustment to a lognormal probability distribution
Faecal streptococci	100	1000	10	Membrane filtration, M-enterococcus agar incubated at 35±0.5°C for 48 h	

157. The proposed faecal coliform criteria were unanimously recommended by the principal investigators of the collaborating institutes in the pilot phase of the MED POL VII project. In view of the experimental results gathered during the pilot phase, faecal streptococci have also been included in the proposed interim quality criteria.

158. The proposed WHO/UNEP interim criteria represent a major step in eventually ensuring an appropriate level of protection for those bathing in the coastal waters of the Mediterranean. They could be considered as temporarily adequate requirements that can be applied in all Mediterranean states as the minimum common safeguard. Subject to availability of further scientific and technical information, the proposed criteria should be periodically reviewed and revised, as appropriate. Such information, planned to be obtained, in particular through MED POL Phase II, includes:

- epidemiological studies on bathers in Mediterranean coastal waters to appraise the public health implications of bathing and their potential association with water quality;
- further studies on the inactivation processes of faecal micro-organisms in the sea, to better understand the parameters governing these processes and the technical principles available to promote them;
- further studies on the adequacy and efficacy of analytical methods for detecting microbial indicators;
- a quality control programme established and implemented by all laboratories participating in the MED POL Phase II monitoring programme, in order to ensure the accuracy, precision, and comparability of the results of the microbiological analyses.
- further studies on suitability of other indicators, such as Salmonella and several enteric viruses, as reliable, consistent, representative and economic water quality indicators of faecal pollution.

159. Reference methods recommended for adoption in connexion with the proposed environmental quality criteria are:

- UNEP/WHO: Determination of faecal coliforms in seawater by the membrane filtration culture method. Reference Methods for Marine Pollution Studies No. 3, Rev.1, UNEP, 1983.
- UNEP/WHO: Determination of faecal streptococci in seawater by the membrane filtration culture method. Reference Methods for Marine Pollution Studies No. 4, Rev.1, UNEP, 1983.

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

160. 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.

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

162. 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.

163. 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.

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

165. 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 correlation between water quality and health effects, 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 studies have provided the basis for firmer definitive measures.

166. 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.

167. 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.

168. 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.

169. 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.

170. 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 use by Mediterranean laboratories is the MPN method (UNEP/WHO, 1983d). A reference method utilizing the MF method is also being developed and the comparability of the two methods is currently under investigation.

171. 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.

172. 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 flesh will also be needed in this respect.

173. In view of the absence of epidemiological studies performed within the Mediterranean region to back any criterion, the proposed interim criteria for shellfish-growing 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.

174. 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 environmental quality criteria for shellfish and shellfish-growing waters in the Mediterranean

175. Based on the results and experience gathered from the joint WHO/UNEP Pilot Project on Coastal Water Quality Control in the Mediterranean (MED POL VII), and in accordance with the above review of the scientific rationale presently available, the following is proposed as the WHO/UNEP environmental quality criteria for application on an interim basis to shellfish and shellfish-growing waters in the Mediterranean:

"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 (soft 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."

176. The proposed interim criteria are summarized in Table 16.

177. The proposed faecal coliform criteria were unanimously adopted by the principal investigators of the institutes collaborating in the MED POL VII project. However, not only faecal coliforms were investigated during the pilot phase, but also total coliforms, faecal streptococci and total heterotrophic bacteria were included in the list of compulsory indicators. Moreover, Vibrio parahaemolyticus, Salmonella and enteric viruses were also investigated on a voluntary basis as potential faecal indicators (WHO/UNEP, 1977b, 1980; UNEP, 1981).

Table 16

Proposed interim criteria for shellfish and shellfish-growing areas
in the Mediterranean Sea

SHELLFISH-GROWING WATERS

<u>Parameters</u>	<u>Concentration per 100 ml not to be exceeded</u>		<u>Minimum Sampling Frequency</u>	<u>Analytical method</u>	<u>Interpretation method</u>
	<u>80%</u>	<u>100%</u> <u>of the time</u>			
Faecal coliforms	10	100	In winter: monthly In summer: fortnightly	Membrane filtration m-FC broth or agar incubated at 44.5±0.2°C for 24h	Graphical or analytical adjustment to a lognormal probability distribution

SHELLFISH FLESH

<u>Parameters</u>	<u>Concentration per gram of flesh</u>	<u>Minimum sampling frequency</u>	<u>Analytical method</u>	<u>Interpretation method</u>
Faecal coliforms	2 Sale permitted Between 3 & 10 temporary prohibition of sale 10 Sale prohibited	In winter: monthly In summer: fortnightly	Multiple tube fermentation and counting according to MPN (Most Probable Number) incubated at 35±0.5°C for 24h and then at 44.5±0.2°C for 24h	By individual results histograms or graphical adjustment of a lognormal probability distribution MacConkey broth

178. In reviewing the results obtained from the shellfish monitoring programme, the participating principal investigators agreed that the compulsory microbiological parameters to be used for shellfish monitoring should be limited to faecal coliforms and faecal streptococci (WHO/UNEP, 1980). On the other hand, other potential indicators, such as Salmonella and enteric viruses, should be further investigated.

179. Reference methods recommended for adoption in connexion with the proposed environmental quality criteria are:

- UNEP/WHO: Determination of faecal coliforms in seawater by the membrane filtration culture method. Reference Methods for Marine Pollution Studies No. 3, Rev.1, UNEP, 1983.
- UNEP/WHO: Determination of faecal coliforms in bivalves by the multiple test-tube method. Reference Methods for Marine Pollution Studies No. 5, Rev.1, UNEP, 1983.

180. The proposed WHO/UNEP interim criteria represent a major step in eventually ensuring an appropriate level of protection for consumers of shellfish grown in coastal areas of the Mediterranean. They could be considered as temporarily adequate requirements that can be applied in all Mediterranean States as the minimum common safeguard. Subject to availability of further scientific and technical information, the proposed criteria should be periodically reviewed, as appropriate. Such information, planned to be obtained in particular through MED POL Phase II, includes:

- epidemiological studies on permanent and seasonal populations of Mediterranean coastal areas to appraise the public health implications of shellfish consumption;
- studies on the influence of the natural self-purification processes of shellfish on the accumulation of faecal micro-organisms, to understand the parameters governing these processes, and to evaluate the relationship between growing-water quality and shellfish flesh quality;
- further studies on the adequacy and efficacy of analytical methods for detecting microbial indicators, as well as on the influence of intervalvular liquid on analytical results;
- a quality control programme established and implemented by all the laboratories participating in the MED POL Phase II monitoring programme, in order to ensure the accuracy, precision and comparability of the results from microbiological analyses; and
- the systematic evaluation of microbiological data, from both shellfish-growing waters and shellfish flesh, following that conducted on data obtained in MED POL VII, should be continued preferably by graphical adjustment of a lognormal probability distribution, to attain a better understanding of the factors influencing the quality of both the shellfish flesh and the shellfish-growing waters of the Mediterranean.

MEASURES PROPOSED FOR ADOPTION BY THE CONTRACTING PARTIES

181. On the basis of the results of MED POL VII (including the assessment of the quality of Mediterranean recreational waters, shellfish and shellfish-growing waters) and the analysis of the present national and international regulations related to the environmental quality criteria of the Mediterranean recreational waters, shellfish and shellfish-growing waters, the following proposals are submitted for the consideration of the Working Group, with a view to their transmission by UNEP to the Contracting Parties.

- (a) Adopt the proposed WHO/UNEP interim environmental quality criteria for coastal recreational waters, shellfish and shellfish-growing areas (see paragraphs 154-155 and 174-175) and implement them to the extent possible by appropriate national legal and/or administrative measures as the minimal common measures safeguarding the general public.
- (b) Adopt the methods for marine pollution studies developed to support the proposed interim environmental quality criteria (see paragraphs 158 and 178) as the reference methods to be used in connexion with these criteria.
- (c) Include, to the extent possible, all public coastal recreational beaches and shellfish-growing areas in their national monitoring programmes within the framework of MED POL Phase II.
- (d) Provide the secretariat to the Convention with the fullest information possible on:
 - present legislation and administrative measures on existing national criteria for coastal recreational and shellfish-growing waters;
 - measures taken on (a) and (c) above;
 - relevant monitoring data from (c) above.
- (e) 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 recreational waters, shellfish and shellfish-growing waters (see paragraphs 157, 172 and 179), in particular to:
 - studies on the intercomparison of various analytical techniques for the main microbiological indicators;
 - development of sampling and analytical techniques for pathogenic organisms in coastal recreational and shellfish-growing waters;
 - studies on the survival of pathogenic organisms in seawater;
 - expansion of the present programme of epidemiological studies to achieve the necessary information on the relationship between water quality and health effects.

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