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

MICROBIOLOGICAL METHODS FOR COASTAL WATER QUALITY MONITORING

Report on a joint WHO/UNEP meeting

Barcelona
7 - 11 November 1983

WORLD HEALTH ORGANIZATION
Regional Office for Europe
Copenhagen, 1985

Original: French

ICP/RCE 211(3)
7911L

Note

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted, quoted or translated without the agreement of the World Health Organization Regional Office for Europe. Authors alone are responsible for views expressed in signed articles.

CONTENTS

FOREWORD

Within the framework of the Mediterranean Action Plan adopted by countries of the Mediterranean basin at Barcelona in February 1975, and in accordance with Article 10 of the Convention for the Protection of the Mediterranean Sea against Pollution, the Contracting Parties are currently establishing a system for pollution monitoring in the region, in close cooperation with the international agencies concerned.

The pilot phase of the joint coordinated Mediterranean pollution monitoring and research programme (MED POL I), carried out from 1976 to 1981, was aimed at providing a framework and the necessary knowledge for a monitoring system of this nature. During that time, activities were undertaken by the United Nations Environment Programme (UNEP), in close cooperation with the World Health Organization (WHO), in order to develop standard methods for sampling and bacteriological analysis. These methods were developed through a project on coastal water quality control in the Mediterranean (MED POL VII), which was jointly coordinated by WHO and UNEP and aimed mainly at studying bacteriological and related parameters for monitoring of coastal recreational waters as well as shellfish-growing areas.

Under the long-term programme for pollution monitoring and research in the Mediterranean (MED POL II), covering the period 1981-1990, and in accordance with the relevant articles of the Convention and its related protocols, most Mediterranean countries have already submitted national monitoring programmes or are in the process of finalizing them.

For comparison of the results and quality control of the analyses at both the national and the regional level, a series of intercalibration exercises is being conducted in the countries concerned. The exercises are directed to the laboratories of the host country which are participating in the monitoring programe, and to a few laboratories in other countries, with the aim of ensuring continuity of organization and participation.

Several reference methods for sampling and analysis of coastal recreational waters as well as shellfish-growing areas have been prepared for use by the Mediterranean countries as a common methodology for implementation of the national monitoring programmes. These methods, which form part of a complete series, have been or are being formulated by the UNEP Regional Seas Programme in collaboration with the specialized agencies of the United Nations and are designed to cover all the possible parameters set out in the annexes to the Convention and its related protocols; they are also intended for use in regions other than the Mediterranean.

The methods relating to bacteriological parameters were reviewed at the WHO/UNEP joint intercalibration exercise and consultation meeting on methods for monitoring selected pollutants in sewage effluents and coastal recreational waters (Rome, 22-26 November 1982).

The present intercalibration exercise was organized by WHO and UNEP in conjunction with the Escola Tècnica Superior d'Enginyers de Camins, Canals i Ports de Barcelona, within the framework of phase II of MED POL, and as part of the Spanish monitoring programme. The main purpose of the exercise was to enable the participants to make determinations of bacteriological parameters in identical samples of seawater, using the following recommended methods, as finalized after the meeting in Rome:

- determination of total coliforms in seawater by the membrane filtration culture method (Reference Methods for Marine Pollution Studies No. 2/Rev. 1, 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/WHO);
- determination of faecal streptococci in seawater by the membrane filtration culture method (Reference Methods for Marine Pollution Studies No. 4/Rev. 1, UNEP/WHO).

Further objectives of the consultation meeting were:

- to review the results of the previous consultation (Rome, 22-26 November 1982);
- to review the results of the intercalibration exercise in order to identify the technical problems with regard to both methodology and quality control;
- to draw up recommendations for performance of future intercalibration exercises in the series;

- to study draft guidelines for statistical analysis and evaluation of results of microbiological monitoring;

- to draw up recommendations concerning the long-term monitoring and research programme.

Representatives of the Spanish institutes participating in the microbiological aspects of the monitoring component of MED POL II and other national monitoring programmes were invited to take part in the intercalibration exercise and consultation meeting, as well as representatives of institutes in other Mediterranean countries that are involved in MED POL II (Algeria, France, Monaco, Morroco and Tunisia). To facilitate subsequent application of the reference methods in other regions, representatives from two non-Mediterranean countries were invited to take part in the exercise and the consultation meeting, i.e. from Senegal and the Ivory Coast, both of which are participating in the action plan for West Africa.

In addition, the following organizations and institutions were invited to send representatives: the Food and Agriculture Organization of the United Nations (FAO), the Inter-Governmental Oceanographic Commission (IOC), the United Nations Educational, Scientific and Cultural Organization (UNESCO), the World Meteorological Organization (WMO), and the International Atomic Energy Agency (IARC).

1. Opening of the meeting

The consultation meeting and intercalibration exercise were organized by the Escola Tècnica Superior d'Enginyers de Camins, Canals i Ports, in Barcelona from 7 to 11 November 1983. They were attended by 28 temporary advisers from Spanish institutes, other Mediterranean countries and West Africa. UNEP and the WHO Regional Office for Europe each sent a staff member. The list of participants appears in Annex 6.

Dr L.J. Saliba, WHO Senior Scientist, Mediterranean Action Plan, WHO Regional Office for Europe, opened the meeting on behalf of Dr Leo A. Kaprio, WHO Regional Director for Europe. He briefly reviewed the activities carried out under MED POL which had led to the present exercise, within the context of the Mediterranean Action Plan. He conveyed WHO's appreciation for the work done and premises furnished by the Escola Tècnica Superior d'Enginyers de Camins, Canals i Ports, as well as the assistance of the Universitat Politècnica de Barcelona, the Departament de Sanitat i Seguretat Social de la Generalitat de Catalunya, and the Comissio interdepartemental per le Recerca i Innovacio tecnologica. He considered it particularly appropriate that the first exercise in the series was being held in Barcelona, the very city where the intergovernmental meeting in 1975 had adopted the Mediterranean Action Plan, as well as the Conference of Plenipotentiaries which had adopted the Convention for the Protection of the Mediterranean Sea against Pollution.

Dr G. Ferraté, Rector of the Universitat Politècnica de Barcelona, welcomed the participants and stressed the interest taken by the university in the intercalibration exercise and the consultation meeting. He, too, expressed appreciation of the effort made by the Escola Tècnica Superior d'Enginyers de Camins, Canals i Ports, in arranging the meeting and said that the premises being built for the new school would be available for hosting other meetings convened by WHO and UNEP.

Dr E. Oñate, Director of the Escola Tècnica Superior d'Enginyers de Camins, Canals i Ports, said that the facilities of the school were at the full disposal of the participants and wished them a successful intercalibration exercise and consultation meeting.

Dr F.S. Civili, UNEP Marine Scientist, Coordination Unit of the Mediterranean Action Plan, then addressed the meeting. He recalled that the consultation was taking place within the framework of MED POL II, under the coordination of UNEP and with the collaboration of the international agencies concerned, especially WHO with regard to the health aspects of MED POL. More specifically, the consultation was part of the continuous monitoring component of MED POL. The majority of governments had already submitted national monitoring programmes. Reference methods were now being developed for all the mandatory parameters, and intercalibration exercises would be organized every year to guarantee the quality of data collected by participating laboratories. The presence of investigators from other regions was evidence of the effort UNEP was making to standardize the analytical methods for all those taking part in the UNEP Regional Seas Programme. Thanks were due to the Spanish scientific community for its very active participation in the meeting, as well as the scientific bodies at both university and government level in Barcelona that had made every possible effort to ensure the success of the work.

Dr R. Mujeriego, Director of the Departamento de Ingeneria Sanitaria y Ambiental of the Escola Tècnica Superior d'Enginyers de Camins, Canals i Ports, thanked WHO and UNEP for their confidence in his department for organizing the intercalibration exercise and consultation meeting. Thanks

were also due to the Universitat Politècnica de Barcelona, the Departament de Sanitat i Seguretat Social de la Generalitat de Catalunya and the Comissio interdepartemental per la Recerca i Innovacio tecnologica for their support in the organization of the meeting.

2. Scope and purpose

Dr Saliba described the scope and purpose of the exercise and the meeting. He referred to the role of the WHO Regional Office for Europe in relation to MED POL II and to the collaboration by the WHO Regional Office for the Eastern Mediterranean in the programme. In that respect, the present exercise should not be regarded as an isolated activity but rather as the first in a series forming part of a long-term programme. The purpose of the exercise is not only to compare scientific results, but also to bring investigators together in order to facilitate collaboration and improve contacts for the future. He noted that the WHO/EURO project office in Athens, located in the Coordinating Unit for the Mediterranean Action Plan, was always at the disposal of Mediterranean laboratories who might need assistance or advice in connection with the programme. Moreover, the project office in Athens enjoyed the full support of the WHO Regional Office for Europe, and where applicable the Regional Office for the Eastern Mediterranean in Alexandria, and could serve as an intermediary whenever necessary without detriment to the direct links that already existed between governments and those offices.

Dr Mujeriego then gave details of the organization of the intercalibration exercise which would be held at the laboratory during the week and distributed a summary of instructions for performance of the exercise, based on the relevant UNEP/WHO bacteriological reference methods. These instructions are reproduced in Annex 1.

3. Election of officers

Dr Mujeriego was elected Chairman, Professor S. Jekov Vice-Chairman and Mr S. Grané Terradas and Dr P. Bernard Co-Rapporteurs. Dr Saliba acted as Secretary.

4. Adoption of the agenda

The provisional agenda was adopted unanimously.

5. Review of previous intercalibration results (Rome, November 1982)

The final version of the report of the intercalibration exercise held in Rome from 22 to 26 November 1982 was distributed to the participants.

The data assembled during the exercise had been analysed using the same method as that proposed for interpreting the results of the current exercise in Barcelona. The analysis was presented in the form of a working document (Annex 2). The Chairman drew the meeting's attention to a number of changes agreed upon at the previous consultation meeting, namely:

- the use of two significant digits only in the final presentation of the bacterial counts;
- the use of m-Endo gel in place of pads and m-FC broth for the determination of total coliforms.

He also called for the promotion of:

- studies on indicator microbes of the marine pollution (investigation of their survival in the sea would also be desirable);
- studies on parameters and factors that might invalidate bacteriological results in making counts.

The participants commented on the following points:

- interpretation of results obtained with the membrane filtration culture method;
- the strategy and methodology of sampling;
- selective media recommended by the reference methods for determination of bacterial indicators of marine pollution, proposed under MED POL II.

6. Review of interim guidelines for statistical analysis and evaluation of results of microbiological monitoring

The document entitled "Evaluation and interpretation of data from microbiological monitoring of coastal recreational waters and shellfish-growing areas" was presented (Annex 3). The statistical basis for the method was studied, as well as its advantages and limitations in relation to the current methods.

The participants agreed to study the document in detail and submit their comments to the Secretariat. It was noted that full account would be taken of the comments in developing the document for the publication of an interim reference method under MED POL II.

7. Review of previous intercalibration results (Catalonia, summer 1983)

An intercalibration exercise organized by the Departamento de Ingeneria Sanitaria y Ambiental, in collaboration with the Departament de Sanitat et Securitat Social de la Generalitat de Catalunya, was held in Catalonia in the summer of 1983.

The results of the exercise performed on microbial parameters by 13 laboratories in Catalonia over a period of 10 weeks were statistically analysed and presented to the meeting (Annex 4). Reference was made to a report entitled "La calidad de las aquas litorales", issued in July 1983 by the Departament de Sanitat et Securitat Social, and its contents were briefly summarized.

8. Conduct and results of the intercalibration exercise

The work done during the intercalibration exercise in the laboratory was regularly reviewed. Seven groups of three participants carried out determinations of total coliforms, faecal coliforms and faecal streptococci in nine different seawater samples, in the course of three consecutive sessions.

The bacteriological analyses were performed using the following reference methods for marine pollution studies:

- determination of total coliforms in seawater by the membrane filtration culture method (Reference Methods for Marine Pollution Studies No. 2/Rev. 1, 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/WHO);
- determination of faecal streptococci in seawater by the membrane filtration culture method (Reference Methods for Marine Pollution Studies No. 4/Rev. 1, UNEP/WHO).

The microbial concentrations obtained by the seven groups were analysed by computer, using the methodology proposed in the document attached as Annex 5, as well as the graphical method currently used for quality control.

Review of the results showed a very great dispersion of bacterial concentrations, with fairly large confidence intervals, as compared with the results obtained during the exercises in Rome and in Catalonia the previous summer.

The possible explanations for this variation may include the following.

(1) Differences in the bacteriological training of the participants. Evaluation of results from the first to the third day showed that they were adjusting to the analytical techniques used.

(2) Heavy rainfall in the region during the sampling brought a land-based input to the coastal waters which greatly increased their bacterial load and their organic and mineral concentration. As a result, a large number of dilutions were necessary, bringing a greater risk of error in taking representative quantities of water in the dilution flasks.

(3) The results showed that it is essential to perform the necessary homogenization and dilution operations with great care when carrying out any bacteriological analysis of seawater.

The results demonstrated the effectiveness and simplicity of the proposed methods for interpretation and evaluation of bacterial concentrations obtained in a coastal water quality monitoring programme.

9. Proposals for future action, and recommendations

In addition to specific recommendations concerning the different agenda items, the participants drew up recommendations on the following points.

(1) The need to carry out studies on quality control and the importance of ensuring that results obtained in monitoring bacteriological parameters are comparable. In this respect, particular attention must be paid to the series of intercalibration exercises proposed as part of the monitoring component of MED POL II, which should be carried out on a regular basis.

(2) Reference methods for determination of pathogens in seawater should be developed as soon as possible.

(3) Steps should be taken to produce a handbook on microbiological quality control in the laboratory. For this purpose, account should be taken of the experience gained and the data collected during the previous and current exercises.

(4) As part of the research component of MED POL II, more laboratories should be encouraged to take part in the current activities in respect of (a) the correlation between microbiological quality of coastal waters and health effects and (b) the survival of pathogens. For the first of these activities, consideration should be given to examinations of sand on beaches that are heavily used.

Annex 1

INSTRUCTIONS FOR PERFORMANCE OF THE INTERCALIBRATION EXERCISE

1. Working groups

The participants will divide into eight groups of three persons.

The composition of each group must be unchanged throughout the exercise.

2. Microbiological analyses

Analysis of each of the three microbial indicators (total coliforms, faecal coliforms and faecal streptococci) should be carried out by the same person for all water samples supplied during the exercise.

3. Water samples

Each working group will receive identical samples of three types of water:

- sample A of highly polluted water;
- sample B of moderately polluted water;
- sample C of slightly polluted water.

The three samples will be issued on the three days of exercise: day 1, day 2 and day 3.

4. Laboratory materials

Each working group will be issued the following items:

- all necessary materials for membrane filtration;
- three sterile plastic funnels;
- petri plates of 15 cm diameter for incubation of up to five membranes;
- flasks containing 90 ml of sterile buffer solution allowing successive dilutions by the addition of 10 ml of sample water;
- sterile pipettes (10 ml);
- membrane filters;
- a flask for rinsing with sterile buffer solution;
- all additional materials required for the analyses.

5. Instructions for dilution

The transfers involved in preparing different dilutions from a given water sample should be carried out using the same pipette.

The dilutions should be carried out by one order of magnitude at a time, adding 10 ml of sample water to a flask containing 90 ml of sterile buffer solution. The flasks should be immediately identified by type of water, e.g. A-1, and the degree of dilution, e.g. 10^{-4}, if the dilution corresponds to an initial quantity of water for a total of 10 000 quantities.

All necessary precautions should be taken to ensure that sample water taken from a flask is representative of the content of that flask, on the one hand by adequately stirring the water in the flask and rinsing the pipette several times and on the other by ensuring that all the sample water is transferred to the buffer solution, by repeated rinsing of the pipette in the dilution.

6. Instructions for filtration

Once the dilutions are ready, measurement of the quantities of water to be filtered should be carried out using a different sterile pipette for each sample, starting with the largest dilution.

All dilutions of a given sample should be filtered using the same funnel.

7. Identification of petri plates

The petri plates should be marked:

- on the front, with the number of the working group;
- on the back, with a letter indicating the type of water, followed by a number corresponding to the day in the exercise, e.g. A-3.

In addition, at the location of each membrane, a serial number should be given showing the quantity of water in dilution. These numbers should also be entered in the checklist to enable subsequent recording of the numerations of each membrane.

8. Quantities of water for filtration

In all, five quantities of water for filtration should be taken consecutively from the quantities given on the checklist.

As an approximate indication, the range of quantities of water used to identify faecal coliforms should be raised by one level in relation to that used to determine total coliforms.

Similarly, the range of quantities of water used to identify faecal streptococci should be raised by one level in relation to that used to determine faecal coliforms.

9. Numeration requirements

The recommended limits for numeration of colonies appearing on a membrane are as follows:

- from 20 to 80 colonies, for total coliforms;
- from 20 to 60 colonies, for faecal coliforms
- from 20 to 100 colonies, for faecal streptococci.

Should none of the membranes meet the recommended criteria, the numeration closest to the relevant limits should be taken, and this should be clearly indicated. For more details, see UNEP/WHO Reference Methods for Marine Pollution Studies.

10. Presentation of results

The results of the analysis should be expressed in terms of the number of colonies per 100 ml of water examined. This concentration is determined by dividing the number of colonies enumerated on the membrane by the quantity of water filtered and by the corresponding dilution (negative power of 10) and by multiplying this result by 100. For instance, a numeration of 28 colonies in a membrane on which 20 ml of water of 1:1000 dilution have been filtered corresponds to a concentration of:

$$\frac{28}{20 \times 10^{-3}} \times 100 = 140 \cdot 10^{3} \text{ colonies/100 ml}$$

For further information, see UNEP/WHO Reference Methods for Marine Pollution Studies.

Working group No. _____ Date: ____ November 1983

Members of group: _____

_____

_____

_____

Type of water sample: _____ Temperature of water: _ C

Results of microbiological analyses

Quantity of water filtered ml	Dilution	Number of colonies per membrane		
		Total coliforms	Faecal coliforms	Faecal streptococci
20	1:1			
5	1:1			
20	1:10			
5	1:10			
20	1:100			
5	1:100			
20	1:1 000			
5	1:1 000			
20	1:10 000			
5	1:10 000			
20	1:100 000			
5	1:100 000			
Microbial concentration, in colonies per 100 ml				

Comments:

Annex 2

STATISTICAL ANALYSIS OF PREVIOUS INTERCALIBRATION RESULTS
Rome, November 1982

Table 1. Results of microbiological monitoring of coastal water samples: membrane filtration method

Type of water sample: A-1

Working group	Microbial concentration per 100 ml		
	Total coliforms	Faecal coliforms	Faecal streptococci
1	-	190 000	6 700
2	-	190 000	4 700
3	-	110 000	6 000
4	-	152 000	6 700
5	-	280 000	5 100
6	-	300 000	5 200
7	-	-	-
8	-	-	-
9	-	-	-
10	-	-	-

Table 2. Results of microbiological monitoring of coastal water samples: membrane filtration method

Type of water sample: B-1

Working group	Microbial concentration per 100 ml		
	Total coliforms	Faecal coliforms	Faecal streptococci
1	-	1	7
2	-	5	5
3	-	2	7
4	-	4	5
5	-	5	5
6	-	5	5
7	-	-	-
8	-	-	-
9	-	-	-
10	-	-	-

Table 3. Results of microbiological monitoring of coastal water samples: membrane filtration method

Type of water sample: C-1

Working group	Microbial concentration per 100 ml		
	Total coliforms	Faecal coliforms	Faecal streptococci
1	-	11	10
2	-	11	12
3	-	18	11
4	-	6	11
5	-	10	13
6	-	10	13
7	-	-	-
8	-	-	-
9	-	-	-
10	-	-	-

Table 4. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method

Type of water sample: A-1

Parameter	Microbial indicator		
	Total coliforms	Faecal coliforms	Faecal streptococci
Culture medium (agar)	m-Endo	m-FC	KF-streptococcus
Number of identical samples	-1	6	6
Concentration interval, in colonies per 100 ml	-1 -1	300 000 110 000	6 700 4 700
Mean concentration, in colonies per 100 ml	-1	190 000	4 700
Mean concentration, in natural logarithms	-1.00	12.15	8.63
Standard deviation, in natural logarithms	-1.00	0.47	0.18
95% confidence interval of microbial concentrations	-1 -1	118 148 305 549	4 668 6 717
95% confidence interval of median microbial concentrations	-1 -1	62 161	83 120

Table 5. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method

Type of water sample: B-1

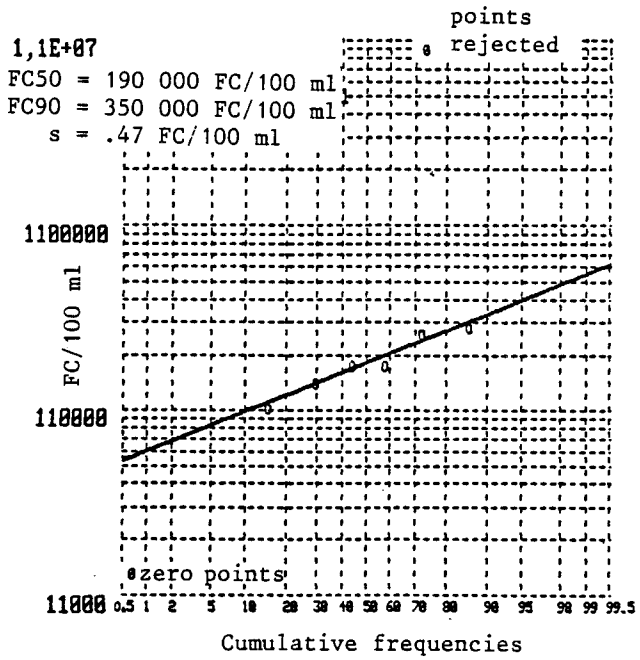
Parameter	Microbial indicator		
	Total coliforms	Faecal coliforms	Faecal streptococci
Culture medium (agar)	m-Endo	m-FC	KF-streptococcus
Number of identical samples	-1	6	6
Concentration interval, in colonies per 100 ml	-1 -1	5 1	7 5
Mean concentration, in colonies per 100 ml	-1	3	5
Mean concentration, in natural logarithms	-1.00	1.10	1.61
Standard deviation, in natural logarithms	-1.00	0.76	0.18
95% confidence interval of microbial concentrations	-1 -1	1 6	4 6
95% confidence interval of median microbial concentrations	-1 -1	46 216	83 120

Table 6. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method

Type of water sample: C-1

Parameter	Microbial indicator		
	Total coliforms	Faecal coliforms	Faecal streptococci
Culture medium (agar)	m-Endo	m-FC	KF-streptococcus
Number of identical samples	-1	6	6
Concentration interval, in colonies per 100 ml	-1 -1	18 6	13 10
Mean concentration, in colonies per 100 ml	-1	10	10
Mean concentration, in natural logarithms	-1.00	2.30	2.40
Standard deviation, in natural logarithms	-1.00	0.41	0.13
95% confidence interval of microbial concentrations	-1 -1	7 15	10 13
95% confidence interval of median microbial concentrations	-1 -1	66 151	88 114

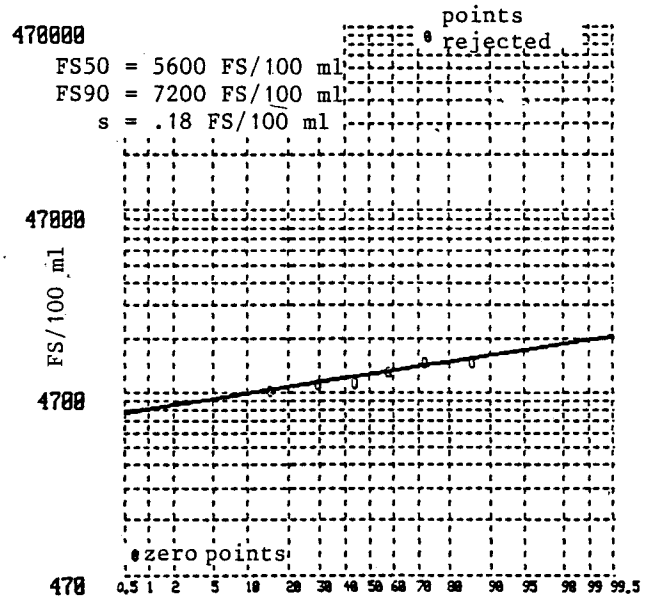
Fig. 1. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: A-1
Microorganism: FC

Culture medium: m-FC agar

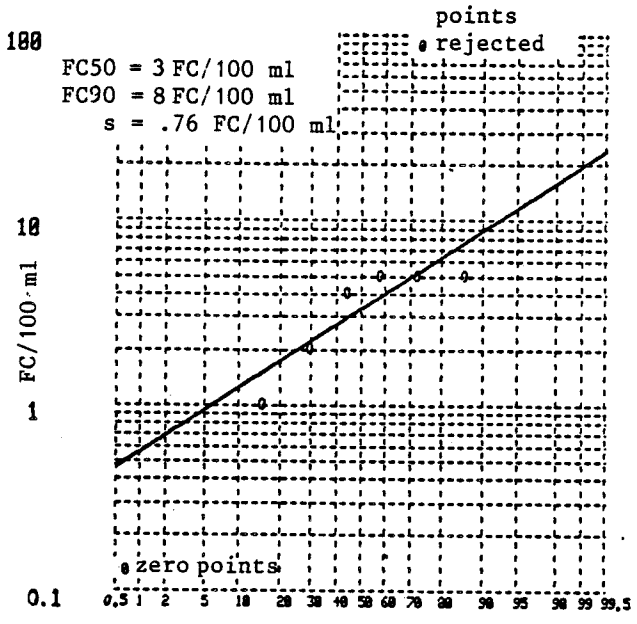
Fig. 2. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: A-1
Microorganism: FS

Culture medium: KF-streptococcus agar

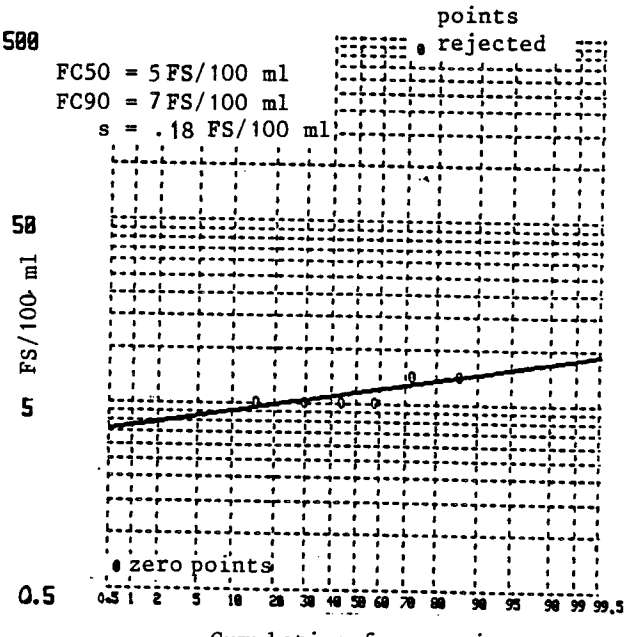
Fig. 3. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: B-1
Microorganism: FC

Culture medium: m-FC agar

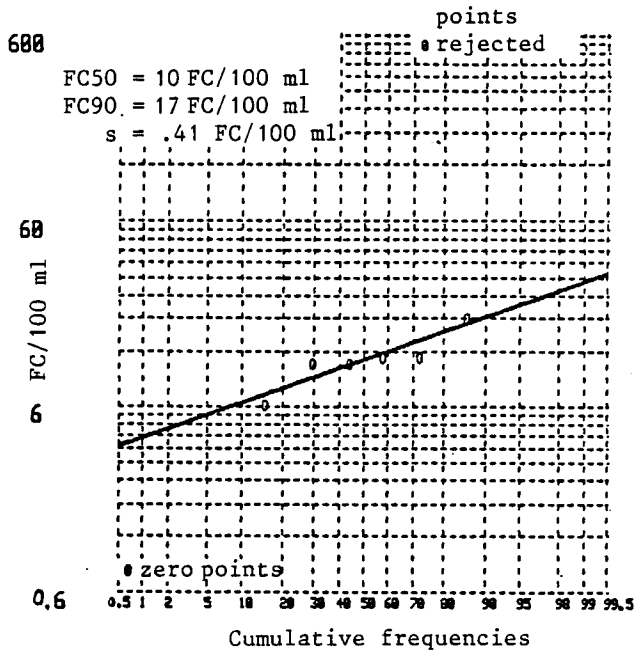
Fig. 4. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: B-1
Microorganism: FS

Culture medium: KF-streptococcus agar

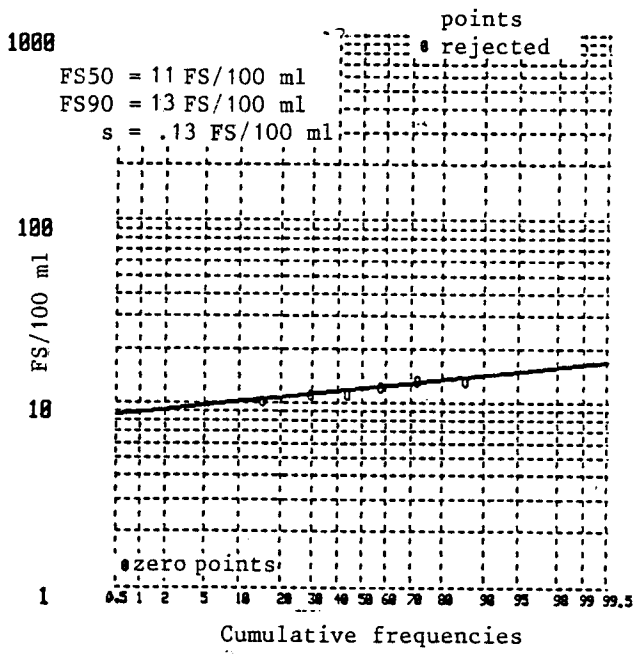
Fig. 5. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: C-1
Microorganism: FC

Culture medium: m-FC agar

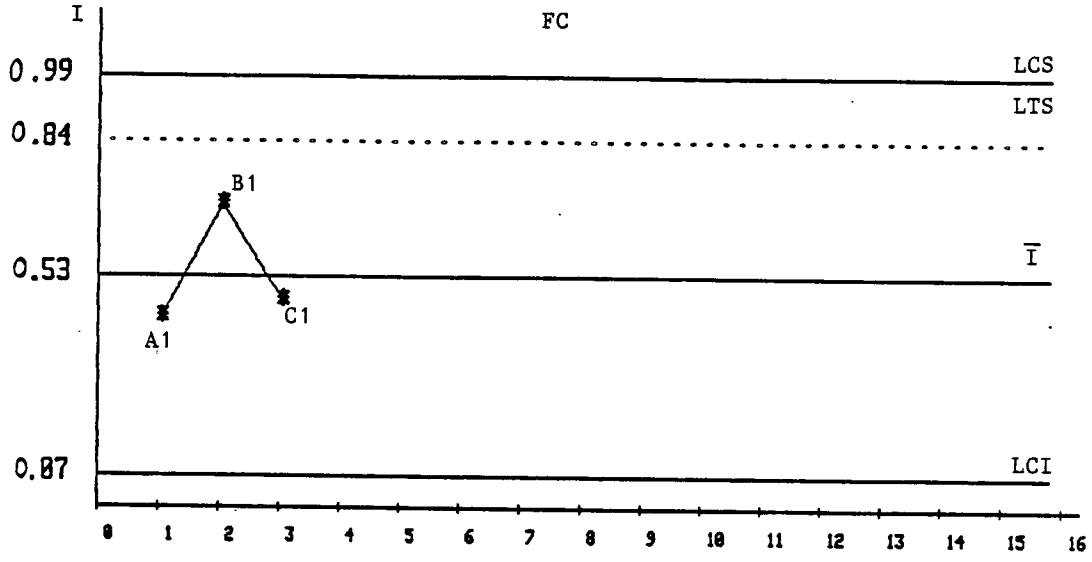
Fig. 6. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: C-1
Microorganism: FS

Culture medium: KF-streptococcus agar

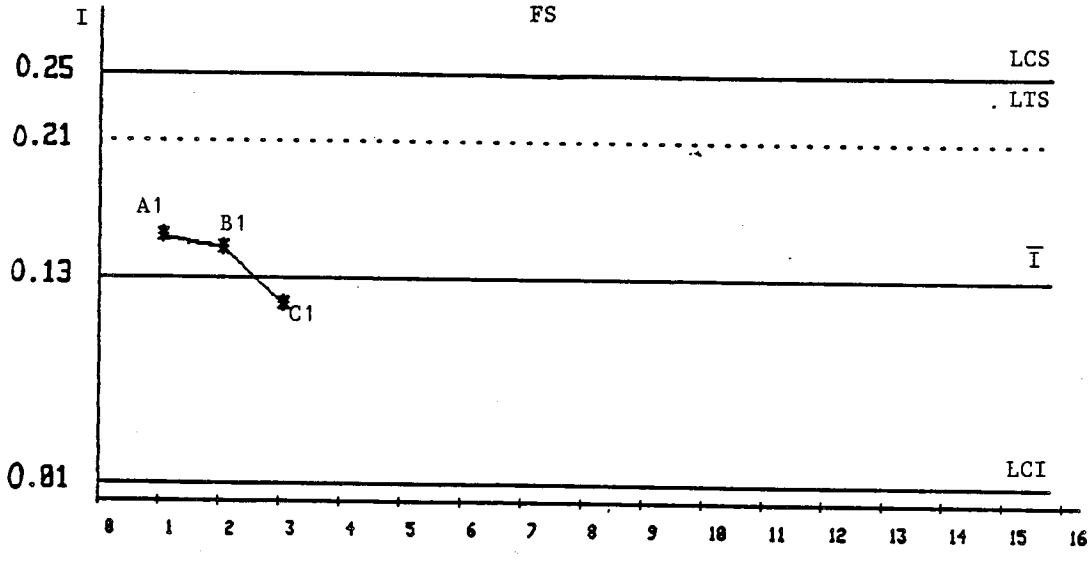
Fig. 7. Graph for checking microbiological analyses of coastal water samples: membrane filtration method



Microorganism: FC

Culture medium: m-FC agar

Fig. 8. Graph for checking microbiological analyses of coastal water samples: membrane filtration method



Microorganism: FS

Culture medium: KF-streptococcus agar

Annex 3

EVALUATION AND INTERPRETATION OF DATA FROM MICROBIOLOGICAL MONITORING OF COASTAL RECREATIONAL WATERS AND SHELLFISH-GROWING AREAS

1. Scope and field of application

The method described is suitable for the evaluation and interpretation of microbiological data from coastal and shellfish-growing waters, in temperate and tropical seas, and is designed to be used in the sanitary surveillance of bathing and shellfish-growing waters.

Microbial indicators exhibit a highly variable concentration at a given sampling station, depending, among other factors, on the sampling time of the day and the day of sampling. Compliance with national and international criteria and standards requires determination of the microbial concentration not exceeded on a certain percentage of the samples collected, and it is therefore of interest to use a systematic method for data evaluation that, in addition to giving the microbial concentrations associated with the established percentages, helps in understanding the temporal variation of the microbiological quality of a coastal water, and possibly provides some insight into the type and relative importance of the factors affecting it.

2. References

Benjamin, J.R. & Cornell, C.A. Probability, statistics and decision for civil engineers. New York, NY, McGraw-Hill, 1970.

Hahn, G.J. & Shapiro, S.S. Statistical models in engineering. New York, NY, John Wiley & Sons, 1967.

IAWPRC. Progress in water technology. Oxford, Pergamon Press, 1980, Vol. 12.

ICSEM/UNEP. Pollution of the Mediterranean: report on a workshop (Cagliary, 1980). ICSEM, Monaco, 1981.

Guidelines for health-related monitoring of coastal water quality: report on a group of experts jointly convened by WHO and UNEP (Rovinj, 1977). Copenhagen, WHO Regional Office for Europe, 1977a.

Health criteria and epidemiological studies related to coastal water pollution: report on a group of experts jointly convened by WHO and UNEP (Athens, 1977). Copenhagen, WHO Regional Office for Europe, 1977b.

Coastal quality monitoring of recreational and shellfish areas (MED VII): report on a meeting of principal investigators jointly convened by WHO and UNEP (Rome, 1979). Copenhagen, WHO Regional Office for Europe, 1979.

Coastal water quality control in the Mediterranean: final report on the joint WHO/UNEP coordinated pilot project (MED VII) (1976-1980). Copenhagen, WHO Regional Office for Europe, 1981.

3. Principles

The evaluation and interpretation method described is based on a statistical model and has to be applied to a homogeneous series of experimental concentrations, obtained at a sampling station, over a continuous period of time, and expressed in terms of a specified microbial indicator.

The concentration of microbial indicators present in the water samples collected at a sampling station, over a continous period of time, has been shown to follow quite closely a lognormal probability distribution. In other words, the natural logarithm of the microbial concentrations appears to follow a normal probability distribution quite closely.

The lognormal probability distribution that most closely fits an experimental set of microbial concentrations can be obtained through a graphical interpolation technique, which allows the direct estimation of the microbial concentrations not exceeded in any given percentage of the samples, as well as providing several statistical parameters that are useful in understanding the factors affecting the microbiological quality of the coastal waters studied.

This statistical method for evaluating and interpreting microbiological data can also be applied numerically, using appropriate computer programmes.

4. Methodological comparisons

Compliance with national and international criteria and standards of microbiological quality usually requires the determination of the microbial concentration not exceeded in a given percentage of the water samples analysed. A subsequent comparison between the resulting microbial concentrations and those established by the criteria or standards provides the basis for assessing the microbiological quality of the water with respect to the criteria or standards considered.

Although most of the existing criteria and standards for microbiological water quality are expressed in terms of two concentrations of a specified microbial indicator, which should not be exceeded in two corresponding percentages of the samples, very few of the criteria or standards give explicit indications on how to derive the appropriate microbial concentrations from the set of experimental data.

As an illustration, the WHO/UNEP interim criteria on the recreational waters (WHO, 1979) specify that 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.

The ranking method

The method most frequently used for deriving the microbial concentrations required for water quality evaluation involves the ranking of the experimental concentrations, in increasing order, and the subsequent selection of the microbial concentration having an order number equal to that resulting from the product of the total number of samples considered and the percentage specified by the criteria or standards. Assuming the number of concentrations available was n = 20, the microbial concentrations of concern when applying the WHO/UNEP interim criteria would be those with order numbers n50 = 20 x 0.50 = 10 and n90 = 20 x 0.90 = 18 respectively.

This ranking method has the following features.

(1) It is very simple to perform, as it involves simple ordering and multiplication operations, making unnecessary the use of any complex formula or laborious graphical analyses.

(2) It frequently leads to the practical difficulty of having to interpret order numbers which are not integers. Unless the number of experimental results available "n" is not appropriate, determination of its product by the corresponding percentage of compliance will result in a real number, with the subsequent difficulty of having to associate it with one of the integers representing the order number of the experimental set. As an example, assuming the number of concentrations available was n = 12, the order numbers of interest when applying the WHO/UNEP criteria concentration would be n50 = 12 x 0.50 = 6 and n90 = 12 x 0.90 = 10.8 respectively. While the former number is an integer, the latter is a real number and does not correspond with any of the 12 integers representing the same number of ranking positions of the experimental concentrations.

This difficulty is usually solved by the use of a rounding-off criterion that converts the real number into an integer, which can then be used for identifying the desired microbial concentration. The most commonly used rounding-off criterion consists in adding 0.5 units to the real number and then dropping the fraction part of the resulting number. According to this criterion, the previously obtained real number n90 = 10.8 would be converted into the integer n90 = 11.

(3) The precision of the microbial concentration thus selected is quite variable and fairly low, being mainly determined by its relative ranking position within the ordered series of available resuults. Any concentration included within the range defined by the concentrations immediately above and below that associated with a specified percentage could have been chosen as corresponding to the same order number of the concentration actually selected.

(4) The method does not take into account the absolute values of any of the experimental results, other than those associated with the percentages specified by the criteria or standards.

(5) As the method concentrates on selecting one or two specific microbial concentrations out of a set of experimental values, it does not provide any insight into the temporal variation of the microbiological quality of the water at the sampling station considered.

Appendix 1 illustrates the process of evaluation of the microbiological quality of a coastal water in the Mediterranean according to the WHO/UNEP interim criteria, using the ranking method previously discussed.

Lognormal distribution method

The statistical method proposed for the evaluation and interpretation of microbiological results is based on the observed property of the microbial concentrations, measured at a sampling station, to follow a lognormal probability distribution. The method involves determination of the normal distribution that most closely fits the natural logarithms of the experimental results. The adjustment procedure may be performed either graphically or numerically, both alternatives being capable of producing identical results, provided the calculation steps are adequately specified.

The following characteristics of the lognormal probability distribution method should be noted.

(1) The procedure is slightly more elaborate than the ranking method. Although it does not involve complex formulae, it demands some knowledge of geometry and certain skills in graphical treatment of data. Strict adherence to the procedure and a minimum of practical training will ensure its successful performance by any skilled technician.

(2) There are no practical difficulties concerning the total number of results available. Any set of experimental results can be evaluated, although the benefits of this technique become more evident with higher numbers of microbial concentrations. Data sets containing more than 10 experimental results provide the best interpretation conditions.

(3) The precision of the method can be statistically ascertained and is generally superior to that of the ranking method.

(4) The method takes into account the absolute values of all the microbial concentrations considered, which results in a more precise estimation of the concentration not exceeded in any percentage of the samples.

(5) The method entails determination of the lognormal probability distribution that most closely fits the experimental results and thus provides very helpful insight concerning the temporal variation of the microbiological quality at the sampling station considered, as well as the relative variation among two or more stations.

5. Technical materials

The preparation of the graphical plot from a set of microbial concentrations, as required by the lognormal distribution method, involves the use of the following technical means.

5.1 A calculator capable of furnishing natural logarithm values. As an alternative, either a logarithmic table or a graphical logarithmic scale can be used.

5.2 A sheet of either normal probability paper or lognormal probability paper. The sheets of normal and lognormal probability paper give two coordinate axes, one of them having a non-linear scale, corresponding to the normal probability distribution, and the other having either an arithmetic scale or a logarithmic scale respectively.

Figs 1 and 2 show these two types of probability paper.

The specific property of normal probability paper is that a set of experimental values belonging to a normally distributed population will fit a straight line when plotted.

As the microbial concentrations obtained from a sampling station are considered to follow a lognormal probability distribution, their graphical analysis makes it necessary either to calculate the natural logarithms of the data and then plot them as normal probability paper or, more simply, to plot the data directly on lognormal probability paper.

Where no type of probability paper is available, the probability scales shown in Figs 1 and 2 may be used.

5.3 A transparent drawing rule, approximately 30 cm long.

5.4 Auxiliary drawing tools, such as pencils and eraser.

Fig. 1. Normal probability paper for evaluation of microbiological data

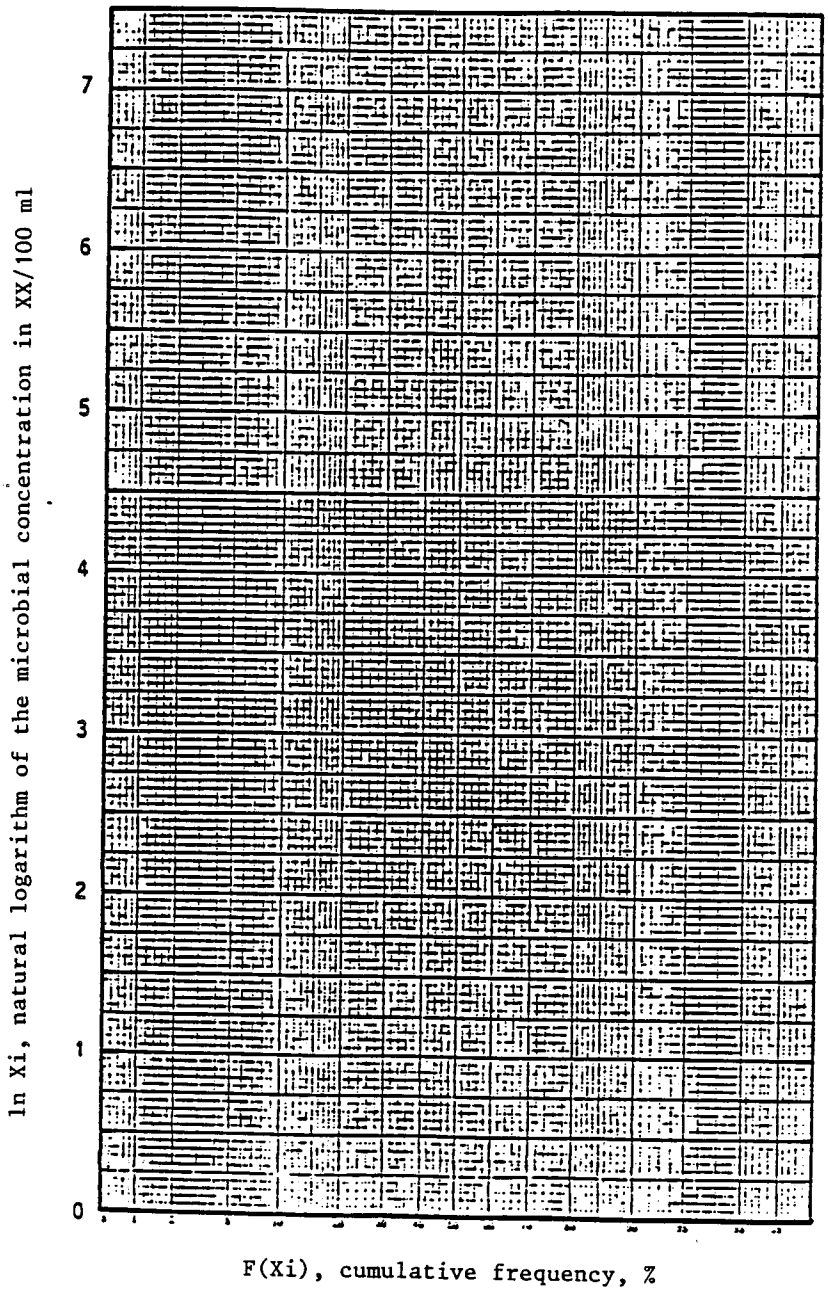
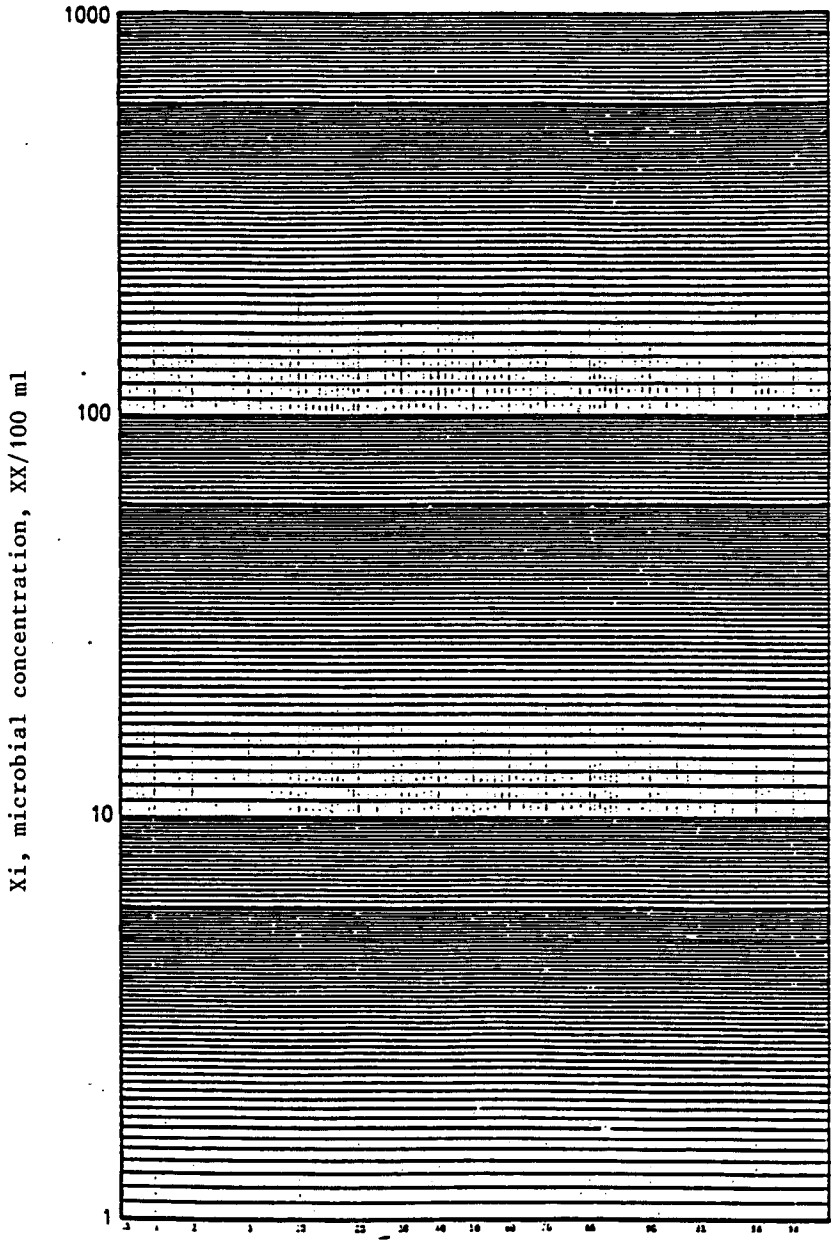


Fig. 2. Lognormal probability paper for evaluation of microbiological data



5.5 The appropriate data recording forms, from which to obtain the experimental data and on to which to record the statistical parameters derived from the evaluation process.

6. Operating procedures

The procedure for application of the lognormal distribution method is described below and illustrated in Appendix 2.

The following steps are necessary when a lognormal probability plot from a set of microbial concentrations.

6.1 From the laboratory recording forms, obtain the set of consecutive microbial concentrations that, corresponding to a specified microbial indicator, covers the time period of interest.

6.2 Rank the experimental results in increasing order of magnitude and obtain a new set of microbial concentrations in which every value is smaller or equal to that following it.

6.3 Prepare a sheet of probability paper. When normal probability paper is available, the previously ordered set of microbial concentrations has to be converted to natural logarithms. This transformation can be performed either numerically, using a calculator or a logarithmic table, or graphicaly, using a logarithmic scale taken from the ordinates axis of Fig. 2.

When lognormal probability paper is available, there is no need for transformations, as the microbial concentrations will be plotted directly in a coordinate system such as that appearing in Fig. 2.

6.4 Calculate the expected cumulative frequency, F(Xi), associated with each of the previously ordered microbial concentrations, using the expression:

$$F(Xi) = \frac{i}{n + 1} \times 100$$

where:

- Xi = microbial concentration in the i-th position;
- F(Xi) = cumulative frequency associated with the data value in the i-th position;
- i = order number of each microbial concentration;
- n = total number of microbial concentrations in the set.

6.5 When normal probability paper is available, plot the log-transformed values, ln Xi, versus the corresponding cumulative frequencies, F(Xi).

When lognormal probability paper is available, the microbial concentrations, Xi, should be plotted directly in relation to the corresponding cumulative frequencies, F(Xi).

The coordinate axis used for plotting the log-transformed data is usually referred to as the observational scale and the coordinate axis for plotting the F(Xi) as the cumulative axis. Whether the observational or cumulative axis appears on the abscissa is irrelevant and depends on the way the plotting paper has been prepared.

6.6 The lognormal probability distribution that best fits the experimental results will now be obtained by graphical interpolation of a straight line to the previously plotted points. A detailed discussion of the interpolation methods appears in section 7.

This straight line represents the cumulative probability distribution of the microbial concentrations not exceeded in a certain percentage of the cases.

6.7 The microbial concentration not exceeded in a given percentage of the set of concentrations considered can be obtained graphically by finding the concentration associated (through the previously drawn straight line) with the percentage of interest.

A convenient notation for the microbial concentration not exceeded in, for example, 50% of the samples would be XX50, where XX designates the two initials of the microbial indicator considered.

As an illustration, TC50 = 2480 TC/100 ml would mean that 50% of the total coliform concentrations considered are smaller than or equal to 2480 total coliforms per 100 ml, at the selected water sampling station, and during the time period specified.

6.8 Calculate the standard deviation of the lognormal probability distribution. The standard deviation is a direct measure of the scattering of the experimental results from their mean value, and thus gives a clear indication of the variation, within the time period considered, of the microbiological quality of the water at the sampling station surveyed.

The standard deviation of a lognormal probability distribution is defined by the expression:

$$s = \ln XX84 - \ln XX50 = \ln XX50 - \ln XX16$$

where:

- s = standard deviation of the lognormal probability distribution;
- XX84, XX50, XX16 = microbial concentrations derived from the interpolated probability distribution, which are not exceeded in 84%, 50% and 16% of the samples respectively

The above definition of the standard deviation must always be borne in mind, when using either normal or lognormal probability paper, to prevent any confusion arising from the type of scale used for plotting microbial concentrations.

The standard deviation of the probability distribution is directly related to the geometrical slope of the straight line representing the lognormal probability distribution. The higher the standard deviation of the probability distribution, the closer the straight line to the vertical position.

6.9 The confidence interval of the set of microbial concentrations can be obtained directly from the probability distribution previously drawn.

The confidence interval of the $(1-\alpha) \times 100$ percentage is defined by the following limits:

$$(XX\ (\alpha/2);\ XX\ (1-\alpha/2))$$

where:

- XX = initials of the microbial indicator;
- α = level of significance.

As an illustration, the 90% confidence interval of the concentrations measured at a sampling station would be defined by the two concentrations:

$$(XX05;\ XX95)$$

where XX05 and XX95 are the concentrations of the microbial indicator denoted by XX, which were not exceeded in 5% and 95% of the samples, as estimated from the graphically interpolated lognormal distribution.

6.10 The confidence interval of the median microbial concentration, XX50, at a given sampling station is defined by the following two limits:

$$\left(\exp\left(\ln XX50 - \frac{s}{\sqrt{n}} t_{1-\alpha/2,\ n-1}\right);\ \exp\left(\ln XX50 + \frac{s}{\sqrt{n}} t_{1-\alpha/2,\ n-1}\right)\right)$$

where:

- XX50 = median microbial concentration estimated from the lognormal probability distribution, XX/100 ml;
- s = standard deviation of the lognormal probability distribution;
- $t_{1-\alpha/2,n-1}$ = value of the cumulative Student's t distribution with n-1 degrees of freedom;
- α = level of significance;
- $(1-\alpha)100$ = confidence interval, %;
- n = number of microbial concentrations included in the data set.

Table 18 of the Guidelines for health related monitoring of coastal water quality (WHO/UNEP, 1977a) gives a summary of the most frequently used values of the Student's t distribution.

6.11 The lognormal probability distribution previously obtained defines all the statistical parameters necessary for further hypothesis testing, both for the distribution itself and for comparisons of this distribution with others obtained at the same or different sampling stations.

7. Interpolation techniques

The recommended criterion for interpolating a straight line to a set of plotted points involves visually drawing a straight line such that the areas defined on each side, by the virtually polygonal line connecting concsecutive points, are approximately equal.

Although more exact interpolation techniques can be used, such as the least squares method, practical experience from interpolation of numerous sets of microbial concentrations shows that an experienced analyst can produce straight-line interpolations of comparable precision to those achieved with more elaborated numerical methods.

Practical difficulties encountered when trying to interpolate a straight line, through a scattered cloud of data points, should be considered as an indication of the lack of adjustment to the proposed lognormal distribution model - a condition which would not be improved by the precise interpolation that can be performed by complex numerical methods.

8. Practical considerations

Visual examination of the experimental data points appearing in a lognormal probability plot is a practical and direct method for testing whether or not the results follow a lognormal distribution. The closer the plotted points fit a straight line, the better the experimental data follow a lognormal probability distribution.

Data points located at both tails of the distribution frequently diverge from the overall linear tendency of the other points. Practical experience shows that a close fit of the majority (from 70% to 90%) of the central points can be considered a strong indication of the validity of the proposed statistical model.

Although samples with zero microbial concentration cannot be logarithmically transformed, and thus cannot be plotted on probability paper, they should be taken into account for all practical purposes during the data-ordering process. Only when applying a statistical test for goodness-of-fit should they be considered, being located at the far bottom of the cumulative frequency axis, under their corresponding F(Xi) value.

When the plotted points cannot be adjusted to a straight line, it is most likely that the variation among the microbial concentrations cannot be interpreted by the lognormal probability distribution model proposed in this document.

However, a visual inspection of the pattern followed by the plotted points may give some clues with regard to what other models could be used for interpreting the data or to the appropriateness of adopting a certain subdivision of the data set considered, as they may appear to follow two distinct lognormal distributions.

A detailed analysis of the data sets which do not follow a lognormal distribution model can provide very valuable insight to the data analyst, as well as helping to improve interpretation skills.

9. Interpretation

From the straight line that most closely fits the set of experimental points drawn in the probability paper, the following parameters can be directly obtained:

- XX50 = microbial concentration not exceeded in 50% of the samples;
- XX84 = microbial concentration not exceeded in 84% of the samples;
- XX90 = microbial concentration not exceeded in 90% of the samples.

Similarly, any other microbial concentration not exceeded in a given percentage of the samples can be read from the probability plot.

The standard deviation of the lognormal distribution can be obtained by the expression:

$$s = \ln XX84 - \ln XX50 = \ln XX50 - \ln XX16$$

which requires calculation of the natural logarithms of the concentrations previously obtained.

The confidence interval of the set of microbial concentrations and the confidence interval of the median concentration can be obtained by the expressions appearing in sections 6.9 and 6.10 respectively.

Appendix 2 illustrates the calculation procedure for processing microbiological data from a water sampling station on the Mediterranean coast.

10. Compliance with standards

To determine whether or not the microbial concentrations measured at a given sampling station comply with the applicable criterion or standard, it is necessary only to compare the microbial concentrations specified in the criterion or standard with the correponding microbial concentrations derived from the lognormal distribution model.

When the criterion or standard applicable contains two concentration limits for a given microbial indicator, the proposed model provides further insight into the degree of compliance at the sampling station considered, through visual comparison of the probability distribution derived from the experimental data, and that defined by the criterion or standard itself.

11. Evaluation report

The reporting form for the evaluation and interpretation of the microbiological quality at a sampling station should include information on the following items.

11.1 The identification code of the sampling station.

11.2 The microbial indicator considered.

11.3 The microbiological method used.

11.4 The time period covered.

11.5 The total number of data available.

11.6 The number of samples with zero microbial concentration.

11.7 The criterion or standard of microbiological quality considered.

11.8 The high or low degree of adjustment of the experimental data to the lognormal distribution model.

Only when the adjustment to the proposed model is adequate should information on the following items be determined from the probability distribution derived from graphical interpolation of the data points.

11.9 The microbial concentrations not exceeded in the percentages of the samples specified by the criterion or standard.

11.10 The standard deviation of the distribution, s.

11.11 The 95% confidence interval of the microbial concentrations.

11.12 The 95% confidence interval of the median microbial concentration.

11.13 The evaluation of the microbiological quality according to each of the concentration limits specified by the criterion or standard considered.

11.14 The overall evaluation of the microbiological quality of the sampling station according to the criterion or standard considered.

11.15 The lack of adjustment of the data to the lognormal distribution model, as well as the relative microbial concentrations defined by the experimental lognormal distribution and the values imposed by the criterion or standard.

Table 2.2 in Appendix 2 gives an example of a report on an evaluation of the microbiological quality of a Mediterranean coastal water according to the WHO/UNEP interim criteria, using the lognormal probability distribution method.

Appendix 1

EVALUATION OF THE MICROBIOLOGICAL QUALITY OF A MEDITERRANEAN COASTAL WATER BY THE RANKING METHOD

Table 1.1. Faecal coliform concentrations at a water sampling station on the Mediterranean coast, summer 1982

Date	FC/100 ml
16 June 1982	92
23 June 1982	1600
30 June 1982	36
7 July 1982	0
14 July 1982	140
21 July 1982	4
28 July 1982	0
4 August 1982	36
11 August 1982	4
18 August 1982	8
25 August 1982	0
14 September 1982	32

Table 1.2. Microbiological quality of a coastal water: arrangement of experimental data when using the ranking method

Order number	Microbial concentration FC/100 ml
1	0
2	0
3	0
4	4
5	4
6	8
7	32
8	36
9	36
10	92
11	140
12	1600

The evaluation of the microbiological quality of this coastal water, according to the WHO/UNEP interim criteria for recreational waters, requires the selection of the concentrations not exceeded in 50% and 90% of the samples.

The order numbers associated with those percentages are:

$$n50 = 12 \times 0.50 = 6$$
$$n90 = 12 \times 0.90 = 10.8$$

respectively.

Considering that the order numbers appearing in Table 1.2 are integers, the previous value n90 = 10.8 has to be rounded off and transformed into an integer. The commonly used criterion for rounding off numbers transforms the n90 = 10.8 value into n90 = 11, which can then be identified in Table 1.2.

The faecal coliform concentrations not exceeded in 50% and 90% of the samples can be obtained from Table 1.2, i.e. those associated with the order numbers n50 = 6 and n90 = 11, and are:

FC50 = 8 FC/100 ml
FC90 = 140 FC/100 ml

From the results shown in Table 1.3, it can be concluded that the microbiological quality of the coastal waters surveyed does not exceed either of the two microbial limits, and consequently can be considered satisfactory according to the WHO/UNEP interim criteria.

Table 1.3. Microbiological quality of a recreational coastal water in the Mediterranean: comparison of limits specified by the WHO/UNEP interim criteria and observed values

Water quality parameter	Microbial concentrations FC/100 ml	
	Specified by criteria	Observed
FC50	100	8
FC90	1000	140

As discussed in the description of the ranking method, any experimental concentration included within the range from 92 to 1600 FC/100 ml would have been ranked in the eleventh position, assuming the other results were unchanged, thus illustrating the low precision of this method and underlining the strong implications that it has for the final outcome of the evaluation procedure.

If instead of the 140 FC/100 ml concentration a value above 1000 FC/100 ml had been observed, the water sampling station would have been classified as unsatisfactory according to the WHO/UNEP interim criteria.

Appendix 2

EVALUATION OF THE MICROBIOLOGICAL QUALITY OF A MEDITERRANEAN COASTAL WATER BY THE LOGNORMAL PROBABILITY METHOD

The following table illustrates the ordering and calculation procedures necessary for applying the lognormal probability model to the set of faecal coliform concentrations appearing in Table 1.1 of Appendix 1.

Table 2.1. Microbiological quality of a coastal water: evaluation by the lognormal probability method

Order number i	Cumulative frequency % F(Xi)	Microbial concentration FC/100 ml Xi	Log-transformed microbial concentration ln Xi
1	8	0	-
2	15	0	-
3	23	0	-
4	31	4	1.39
5	38	4	1.39
6	46	8	2.08
7	54	32	3.47
8	62	36	3.58
9	69	36	3.58
10	77	92	4.52
11	85	140	4.94
12	92	1600	7.38

When normal probability paper is available, the values ln Xi must be plotted in relation to the cumulative frequency, F(Xi). However, in view of the convenience of using lognormal probability paper, the microbial concentrations, Xi, have been plotted in relation to the cumulative frequencies, F(Xi), and they appear in Fig. 2.1.

The data points shown in Fig. 2.1 have been interpolated with a straight line, following the criterion recommended in section 7.

A visual examination of Fig. 2.1 indicates satisfactory agreement between the data points and the probability model proposed. From the probability distribution thus obtained, the faecal coliform concentrations not exceeded in 50% and 90% of the samples can be estimated, and they appear in Table 2.2.

The standard deviation of the probability distribution has been obtained using the expression:

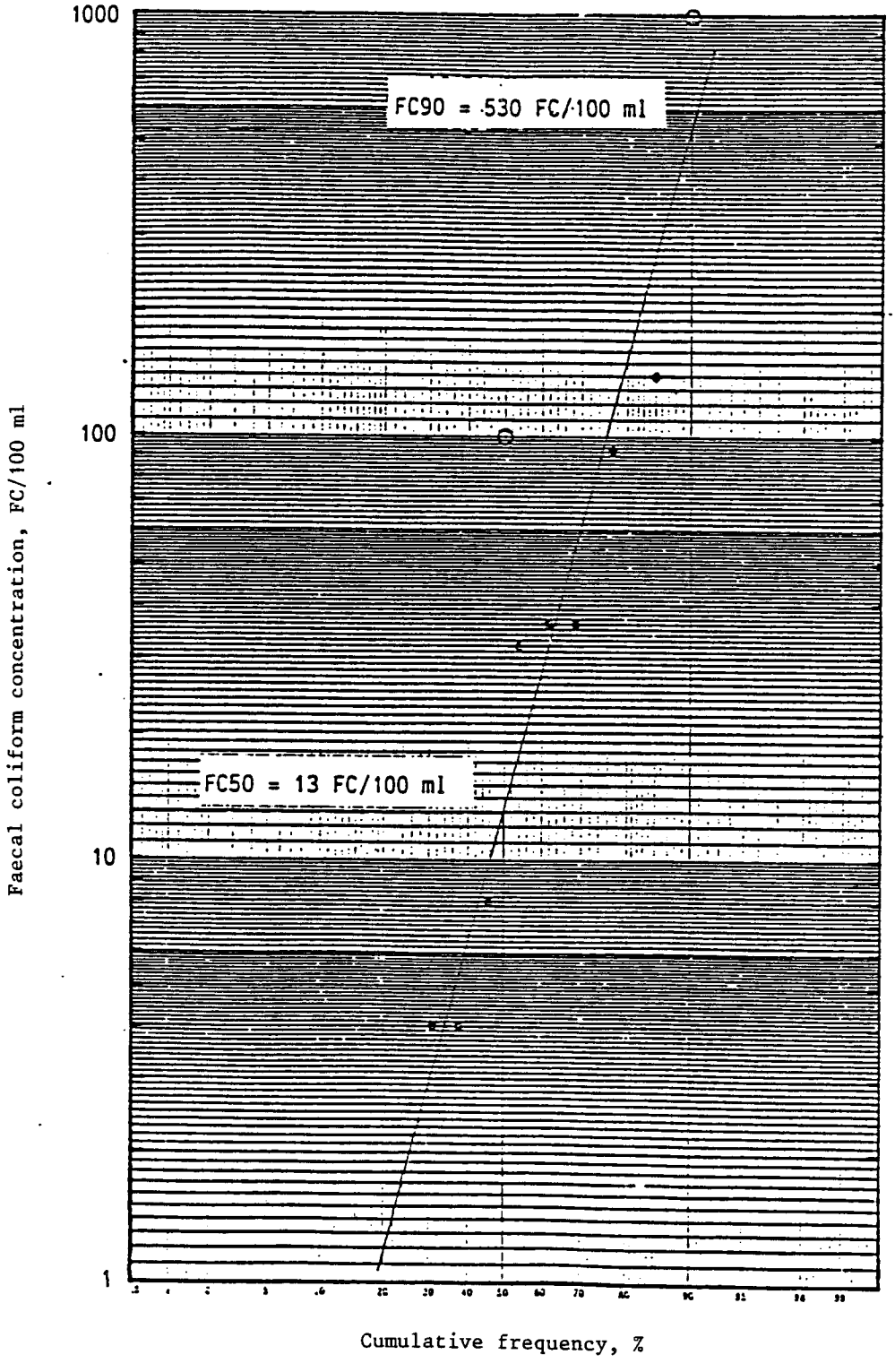
$$s = \ln XX84 - \ln XX50 = \ln 240 - \ln 13 = 5.48 - 2.56 = 2.92$$

The 95% confidence interval of the set of faecal coliform concentrations is defined by the concentrations associated with the 2.5% and 97.5% cumulative frequencies, which are:

(1 CF/100 ml, 3700 CF/100 ml)

The 95% confidence interval of the median faecal coliform concentration can be calculated by the expression appearing in section 6.10. In this case, considering that:

Fig. 2.1. Interpretation of the microbiological quality of a coastal water in the Mediterranean, by the lognormal probability model, and evaluation according to the WHO/UNEP interim criteria (o)



$$\ln FC50 = \ln 13 = 2.56$$
$$s = 2.92$$
$$n = 12$$
$$t_{0.975.11} = 2.201$$

the 95% confidence interval of the median concentration is defined by

(2 FC/100 ml; 83 FC/100 ml)

Table 2.2 summarizes a report on an evaluation of the microbiological quality of the Mediterranean coastal water considered, using the lognormal probability method and taking the WHO/UNEP interim quality criteria as a reference.

Table 2.2. Evaluation of the microbiological quality of water at a sampling station on the Mediterranean coast according to the WHO/UNEP interim criteria, using the lognormal probability method

Item	Value
Station code	-
Microbial indicator	Faecal coliform
Analytical method	Membrane filtration
Period covered	June - September 1982
Number of samples	12
Number of zeros	1
Model agreement	Satisfactory
WHO/UNEP interim quality criteria for experimental concentrations	FC50 = 100 FC/100 ml FC90 = 1000 FC/100 ml
	FC50 = 13 FC/100 ml FC90 = 530 FC/100 ml
Standard deviation	s = 2.92
95% confidence interval of sample	(1 FC/100 ml, 3700 FC/100 ml)
95% confidence interval of median concentration	(2 FC/100 ml, 83 FC/100 ml)
Quality evaluation	Satisfies FC50 limit Satisfies FC90 limit
Overall quality evaluation	Satisfactory

As can be seen in Fig. 2.1, the combined influence of all the microbial concentrations observed results in an estimate for the concentration not exceeded in 90% of the samples of FC90 = 530 FC/100 ml, which is much higher than that shown in Table 1.3.

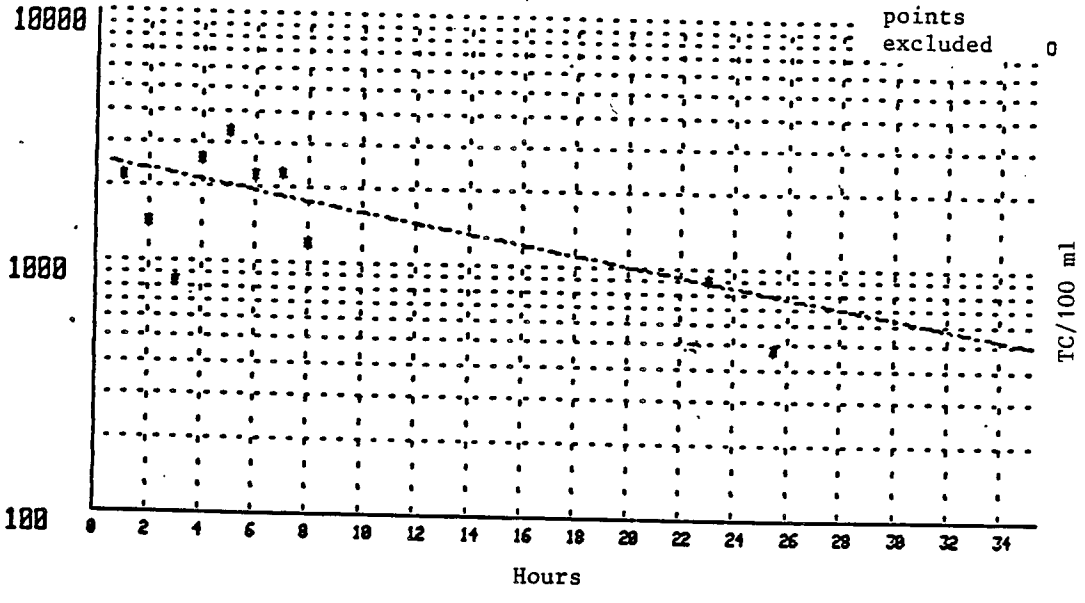
The agreement between the experimental data and the proposed method is satisfactory and reveals a temporal variation of the microbiological quality of the water that is slightly higher than that defined by the WHO/UNEP interim criteria.

Any general deterioration of the microbiological quality at the sampling station studied would move the probability distribution upwards and presumably parallel to itself, causing the upper limit of the criteria to be exceeded and involving a reclassification of the water at the sampling station according to the WHO/UNEP interim criteria.

Annex 4

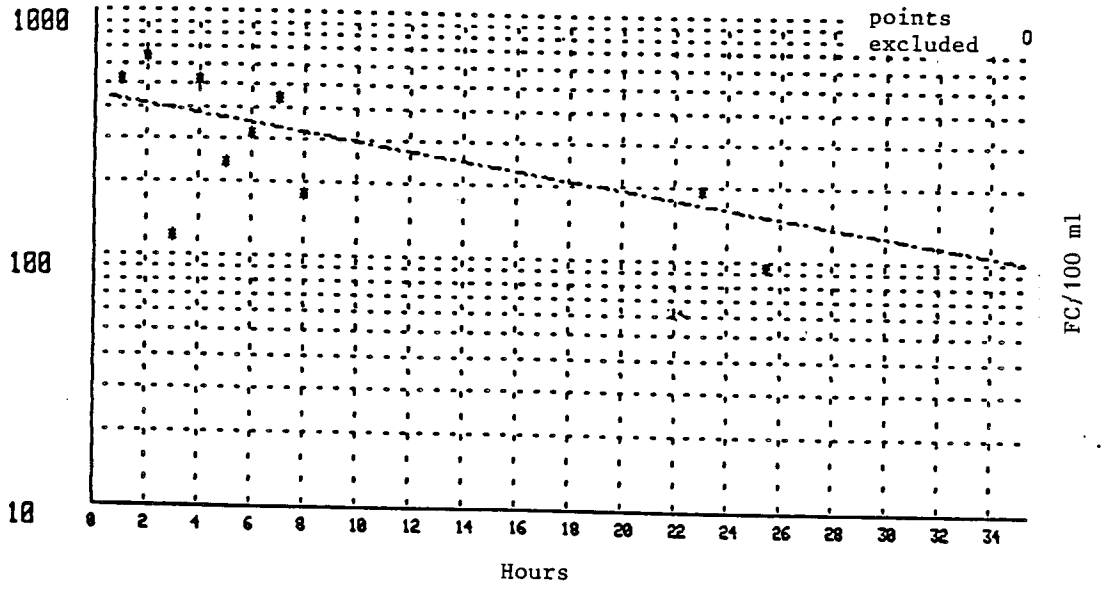
STATISTICAL ANALYSIS OF PREVIOUS INTERCALIBRATION RESULTS
Catalonia, summer 1983

Fig. 1. Intercalibration exercise for laboratories participating in coastal water monitoring



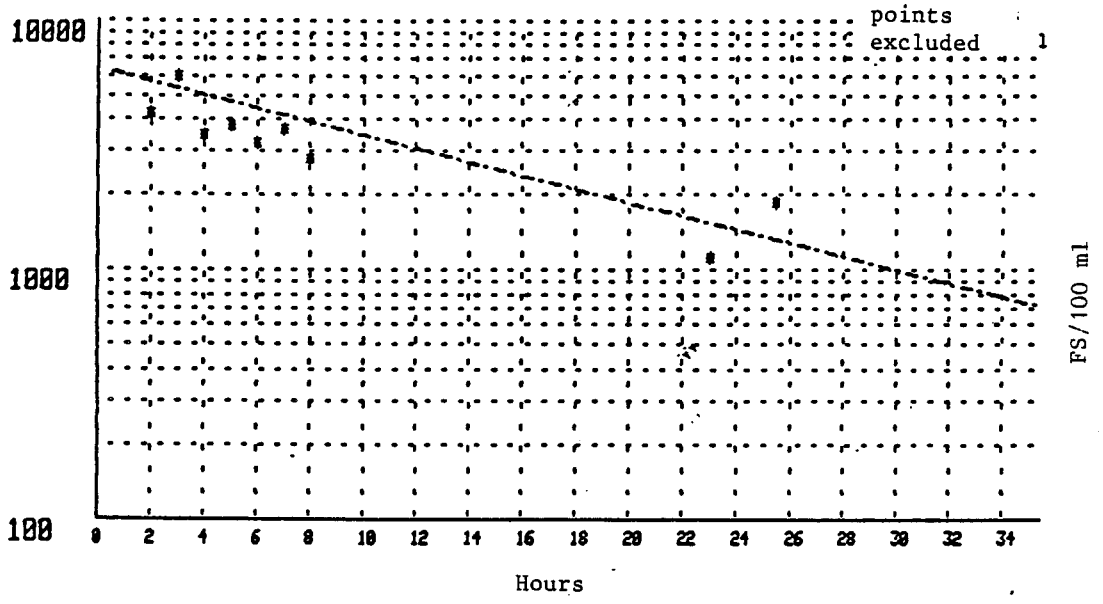
Changes in the concentration of total coliforms in samples taken at the ETSICCP station, 18 July 1983.

Fig. 2. Intercalibration exercise for laboratories participating in coastal water monitoring



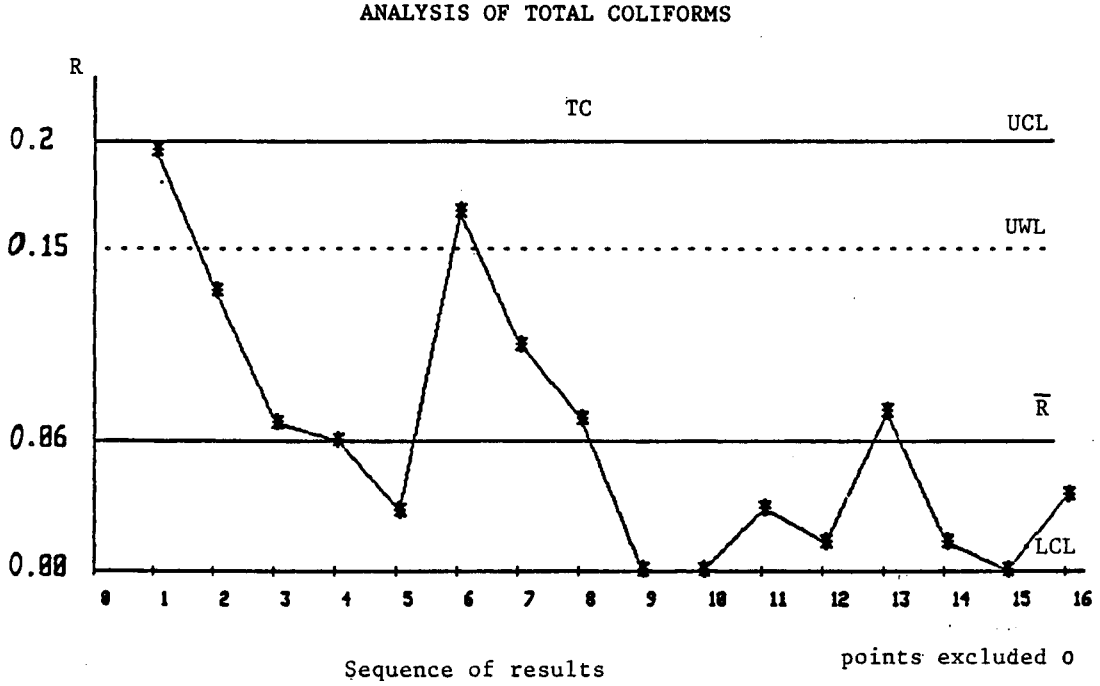
Changes in the concentration of faecal coliforms in samples taken at the ETSICCP station, 18 July 1983.

Fig. 3. Intercalibration exercise for laboratories participating in coastal water monitoring



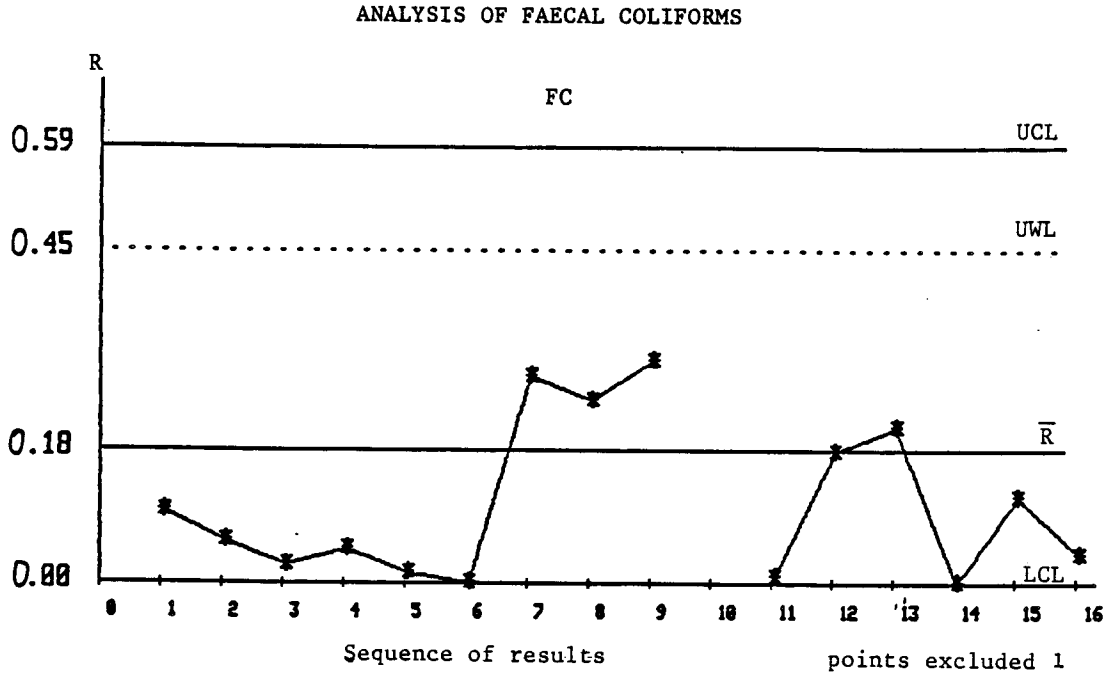
Changes in the concentration of faecal streptococci in samples taken at the ETSICCP station, 18 July 1983.

Fig. 4. Intercalibration exercise for laboratories participating in coastal water monitoring



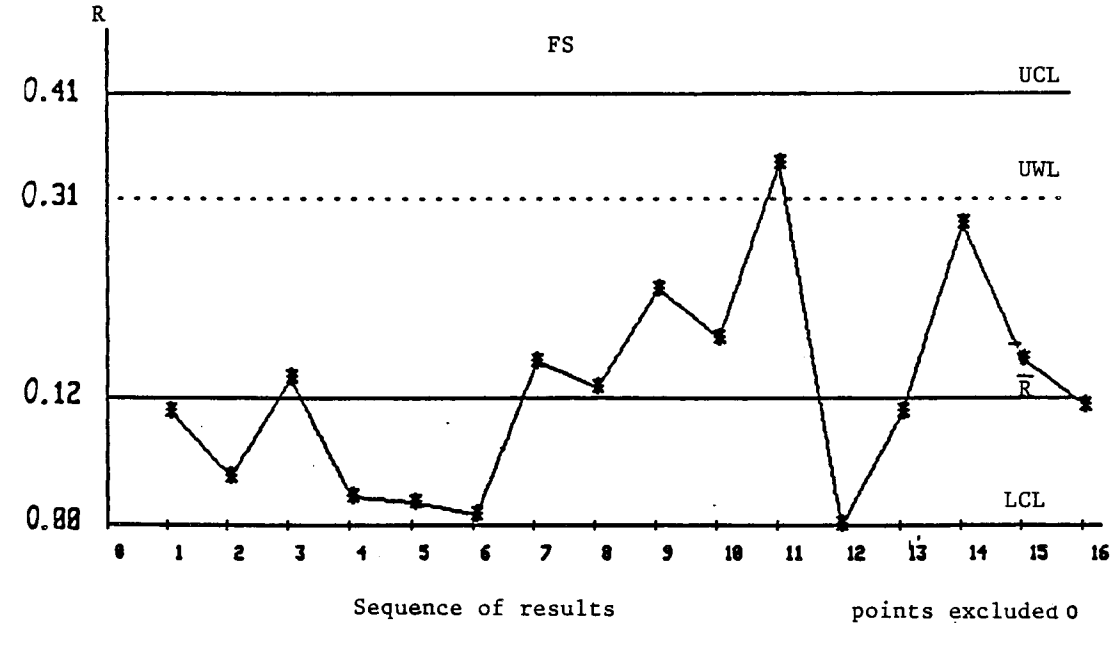
R: degrees of concentration expressed in decimal logarithms

Fig. 5. Intercalibration exercise for laboratories participating in coastal water monitoring



R: degrees of concentration expressed in decimal logarithms

Fig. 6. Intercalibration exercise for laboratories participating in coastal water monitoring



R: degrees of concentration expressed in decimal logarithms

Annex 5

PRESENTATION AND STATISTICAL ANALYSIS OF RESULTS OF THE INTERCALIBRATION EXERCISE

Table 1. Results of microbiological analyses of samples of coastal water: membrane filtration method

Type of water sample: A-1 Date: 7 November 1983

Working group	Microbial concentration per 100 ml		
	Total coliforms	Faecal coliforms	Faecal streptococci
1	75 000 000	4 000 000	1 730 000
2	45 000	264 000	57 400
3	22 000 000	312 000	211 000
4	11 900 000	700 000	210 000
5	3 400 000	12 900	166 000
6	2 600 000	20 000	57 000
7	5 090 000	225 000	64 800
8	-	-	-
9	-	-	-
10	-	-	-

Table 2. Results of microbiological analyses of samples of coastal water: membrane filtration method

Type of water sample: B-1 Date: 7 November 1983

Working group	Microbial concentration per 100 ml		
	Total coliforms	Faecal coliforms	Faecal streptococci
1	1 780 000	162 000	213 000
2	356 000	126 000	76 000
3	1 610 000	149 000	141 000
4	2 240 000	160 000	156 000
5	925 000	92 000	236 000
6	843 000	-	31 500
7	2 700 000	135 000	194 000
8	-	-	-
9	-	-	-
10	-	-	-

Table 3. Results of microbiological analyses of samples of coastal water: membrane filtration method

Type of water sample: C-1 Date: 7 November 1983

Working group	Microbial concentration per 100 ml		
	Total coliforms	Faecal coliforms	Faecal streptococci
1	184 000	50 000	57 000
2	27 000	48 000	48 000
3	404 000	44 000	72 900
4	1 040 000	60 000	44 800
5	260 000	300	57 500
6	41 000	-	16 000
7	120 000	17 500	30 400
8	-	-	-
9	-	-	-
10	-	-	-

Table 4. Results of microbiological analyses of samples of coastal water: membrane filtration method

Type of water sample: A-2 Date: 8 November 1983

Working group	Microbial concentration per 100 ml		
	Total coliforms	Faecal coliforms	Faecal streptococci
1	21 300 000	1 130 000	117 000
2	10 800 000	620 000	80 000
3	10 400 000	340 000	110 000
4	8 200 000	360 000	168 000
5	3 750 000	900 000	115 000
6	9 400 000	1 170 000	32 400
7	6 500 000	195 000	40 000
8	-	-	-
9	-	-	-
10	-	-	-

Table 5. Results of microbiological analyses of samples of coastal water: membrane filtration method

Type of water sample: B-2 Date: 8 November 1983

Working group	Microbial concentration per 100 ml		
	Total coliforms	Faecal coliforms	Faecal streptococci
1	1 580 000	365 000	88 500
2	2 060 000	400 000	92 000
3	1 720 000	165 000	72 000
4	16 400	5 200	3 460
5	620 000	27 500	96 000
6	1 480 000	124 000	39 200
7	1 020 000	127 000	52 000
8	-	-	-
9	-	-	-
10	-	-	-

Table 6. Results of microbiological analyses of samples of coastal water: membrane filtration method

Type of water sample: C-2 Date: 8 November 1983

Working group	Microbial concentration per 100 ml		
	Total coliforms	Faecal coliforms	Faecal streptococci
1	173 000	29 500	36 000
2	144 000	32 800	25 500
3	424 000	27 000	35 600
4	268 000	80 000	124 000
5	80 000	64 000	35 500
6	200 000	-	18 300
7	71 400	26 000	20 700
8	-	-	-
9	-	-	-
10	-	-	-

Table 7. Results of microbiological analyses of samples of coastal water: membrane filtration method

Type of water sample: A-3

Date: 9 November 1983

Working group	Microbial concentration per 100 ml		
	Total coliforms	Faecal coliforms	Faecal streptococci
1	320 000	67 100	10 000
2	480 000	91 400	16 600
3	771 000	42 000	33 500
4	500 000	104 000	19 100
5	202 000	-	27 000
6	80 000	37 500	33 500
7	210 000	-	36 000
8	-	-	-
9	-	-	-
10	-	-	-

Table 8. Results of microbiological analyses of samples of coastal water: membrane filtration method

Type of water sample: B-3

Date: 9 November 1983

Working group	Microbial concentration per 100 ml		
	Total coliforms	Faecal coliforms	Faecal streptococci
1	171 000	32 000	27 500
2	230 000	27 000	11 600
3	210 000	15 000	30 000
4	169 000	32 000	5 000
5	114 000	13 400	13 900
6	235 000	60 000	24 000
7	14 600	200	15 200
8	-	-	-
9	-	-	-
10	-	-	-

Table 9. Results of microbiological analyses of samples of coastal water: membrane filtration method

Type of water sample: C-3 Date: 9 November 1983

Working group	Microbial concentration per 100 ml		
	Total coliforms	Faecal coliforms	Faecal streptococci
1	205 000	36 400	6 800
2	348 000	34 400	8 700
3	348 000	7 000	19 000
4	275 000	-	5 800
5	255 000	14 400	6 200
6	255 000	25 000	60 000
7	145 000	-	2 400
8	-	-	-
9	-	-	-
10	-	-	-

Table 10. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method

Type of water sample: A-1 Date: 7 November 1983

Parameter	Microbial indicator		
	Total coliforms	Faecal coliforms	Faecal streptococci
Culture medium (agar)	m-Endo	m-FC	KF-streptococcus
Number of identical samples	7	7	7
Concentration interval, in colonies per 100 ml	75 000 000 45 000	4 000 000 12 900	1 730 000 57 000
Mean concentration, in colonies per 100 ml	6 800 000	200 000	57 000
Mean concentration, in natural logarithms	15.73	12.21	11.78
Standard deviation, in natural logarithms	1.88	2.42	0.89
95% confidence interval of microbial concentrations	1 300 000 0	23 000 1 700 000	59 000 290 000
95% confidence interval of median microbial concentrations	18 540	11 870	45 220

Table 11. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method

Type of water sample: B-1 Date: 7 November 1983

Parameter	Microbial indicator		
	Total coliforms	Faecal coliforms	Faecal streptococci
Culture medium (agar)	m-Endo	m-FC	KF-streptococcus
Number of identical samples	7	6	7
Concentration interval, in colonies per 100 ml	2 700 000 356 000	162 000 92 000	236 000 31 500
Mean concentration, in colonies per 100 ml	1 300 000	130 000	31 500
Mean concentration, in natural logarithms	14.08	11.78	11.78
Standard deviation, in natural logarithms	0.85	0.25	0.82
95% confidence interval of microbial concentrations	610 000 2 800 000	100 000 170 000	62 000 270 000
95% confidence interval of median microbial concentrations	46 210	77 130	48 210

Table 12. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method

Type of water sample: C-1 Date: 7 November 1983

Parameter	Microbial indicator		
	Total coliforms	Faecal coliforms	Faecal streptococci
Culture medium (agar)	m-Endo	m-FC	KF-streptococcus
Number of identical samples	7	6	7
Concentration interval, in colonies per 100 ml	1 040 000 27 000	60 000 300	72 900 16 000
Mean concentration, in colonies per 100 ml	160 000	35 000	16 000
Mean concentration, in natural logarithms	11.98	10.46	10.65
Standard deviation, in natural logarithms	1.59	0.63	0.59
95% confidence interval of microbial concentrations	39 000 660 000	19 000 66 000	25 000 71 000
95% confidence interval of median microbial concentrations	24 410	52 190	59 170

Table 13. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method

Type of water sample: A-2 Date: 8 November 1983

Parameter	Microbial indicator		
	Total coliforms	Faecal coliforms	Faecal streptococci
Culture medium (agar)	m-Endo	m-FC	KF-streptococcus
Number of identical samples	7	7	7
Concentration interval, in colonies per 100 ml	21 300 000 3 750 000	1 170 000 195 000	168 000 32 400
Mean concentration, in colonies per 100 ml	8 900 000	560 000	32 400
Mean concentration, in natural logarithms	16.00	13.24	11.31
Standard deviation, in natural logarithms	0.64	0.84	0.72
95% confidence interval of microbial concentrations	5 000 000 0	260 000 1 200 000	43 000 160 000
95% confidence interval of median microbial concentrations	56 180	47 210	52 190

Table 14. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method

Type of water sample: B-2 Date: 8 November 1983

Parameter	Microbial indicator		
	Total coliforms	Faecal coliforms	Faecal streptococci
Culture medium (agar)	m-Endo	m-FC	KF-streptococcus
Number of identical samples	7	7	7
Concentration interval, in colonies per 100 ml	2 060 000 16 400	400 000 5 200	96 000 3 460
Mean concentration, in colonies per 100 ml	1 200 000	110 000	3 460
Mean concentration, in natural logarithms	14.00	11.61	11.05
Standard deviation, in natural logarithms	0.60	1.34	0.50
95% confidence interval of microbial concentrations	700 000 2 100 000	33 000 360 000	40 000 99 000
95% confidence interval of median microbial concentrations	58 170	30 330	63 160

Table 15. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method

Type of water sample: C-2 Date: 8 November 1983

Parameter	Microbial indicator		
	Total coliforms	Faecal coliforms	Faecal streptococci
Culture medium (agar)	m-Endo	m-FC	KF-streptococcus
Number of identical samples	7	6	7
Concentration interval, in colonies per 100 ml	424 000 71 400	80 000 26 000	124 000 18 300
Mean concentration, in colonies per 100 ml	160 000	39 000	18 300
Mean concentration, in natural logarithms	11.98	10.57	10.43
Standard deviation, in natural logarithms	0.78	0.57	0.71
95% confidence interval of microbial concentrations	80 000 320 000	22 000 69 000	18 000 64 000
95% confidence interval of median microbial concentrations	49 200	56 180	53 190

Table 16. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method

Type of water sample: A-3 Date: 9 November 1983

Parameter	Microbial indicator		
	Total coliforms	Faecal coliforms	Faecal streptococci
Culture medium (agar)	m-Endo	m-FC	KF-streptococcus
Number of identical samples	7	5	7
Concentration interval, in colonies per 100 ml	771 000 80 000	104 000 37 500	36 000 10 000
Mean concentration, in colonies per 100 ml	300 000	63 000	10 000
Mean concentration, in natural logarithms	12.61	11.05	10.04
Standard deviation, in natural logarithms	0.92	0.58	0.56
95% confidence interval of microbial concentrations	130 000 680 000	32 000 120 000	14 000 38 000
95% confidence interval of median microbial concentrations	43 230	51 190	60 160

Table 17. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method

Type of water sample: B-3 Date: 9 November 1983

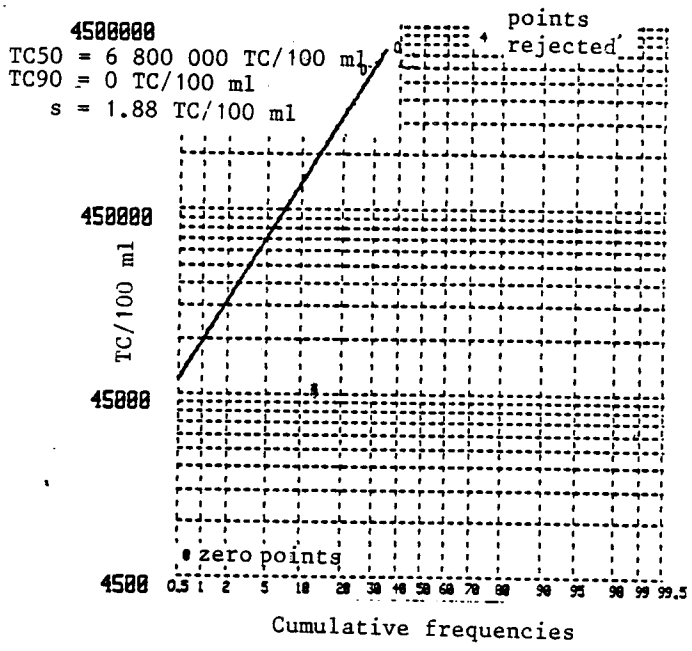
Parameter	Microbial indicator		
	Total coliforms	Faecal coliforms	Faecal streptococci
Culture medium (agar)	m-Endo	m-FC	KF-streptococcus
Number of identical samples	7	7	7
Concentration interval, in colonies per 100 ml	235 000 14 600	60 000 200	30 000 5 000
Mean concentration, in colonies per 100 ml	170 000	23 000	5 000
Mean concentration, in natural logarithms	12.04	10.04	9.68
Standard deviation, in natural logarithms	0.37	0.80	0.74
95% confidence interval of microbial concentrations	120 000 240 000	11 000 47 000	8 300 31 000
95% confidence interval of median microbial concentrations	71 140	48 200	51 190

Table 18. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method

Type of water sample: C-3 Date: 9 November 1983

Parameter	Microbial indicator		
	Total coliforms	Faecal coliforms	Faecal streptococci
Culture medium (agar)	m-Endo	m-FC	KF-streptococcus
Number of identical samples	7	5	7
Concentration interval, in colonies per 100 ml	348 000 145 000	36 400 7 000	60 000 2 400
Mean concentration, in colonies per 100 ml	250 000	20 000	2 400
Mean concentration, in natural logarithms	12.43	9.90	9.14
Standard deviation, in natural logarithms	0.36	0.87	1.22
95% confidence interval of microbial concentrations	180 000 340 000	7 400 54 000	3 100 28 000
95% confidence interval of median microbial concentrations	72 140	36 270	33 300

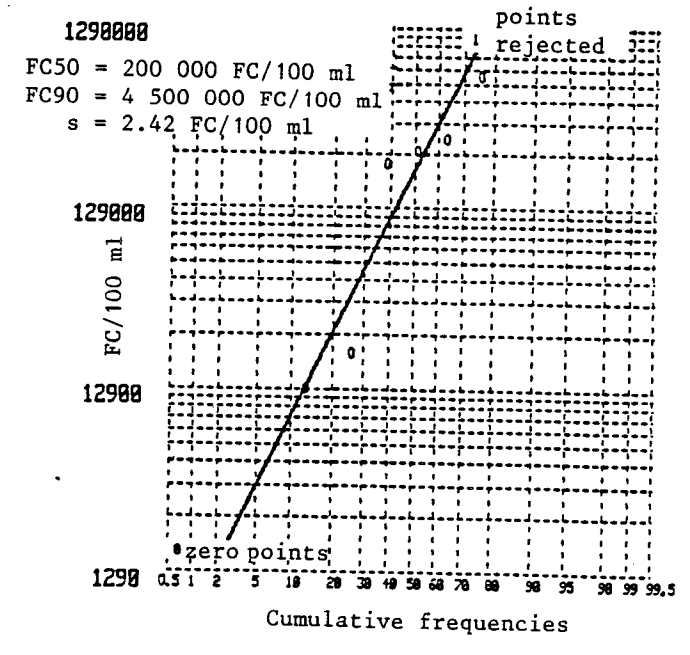
Fig. 1. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: A-1
Microorganism: TC

Date: 7 November 1983
Culture medium: m-Endo agar

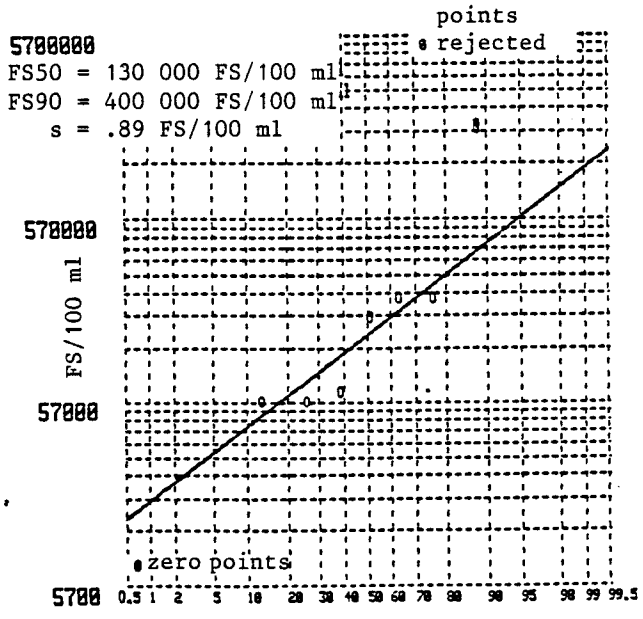
Fig. 2. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: A-1
Microorganism: FC

Date: 7 November 1983
Culture medium: m-FC agar

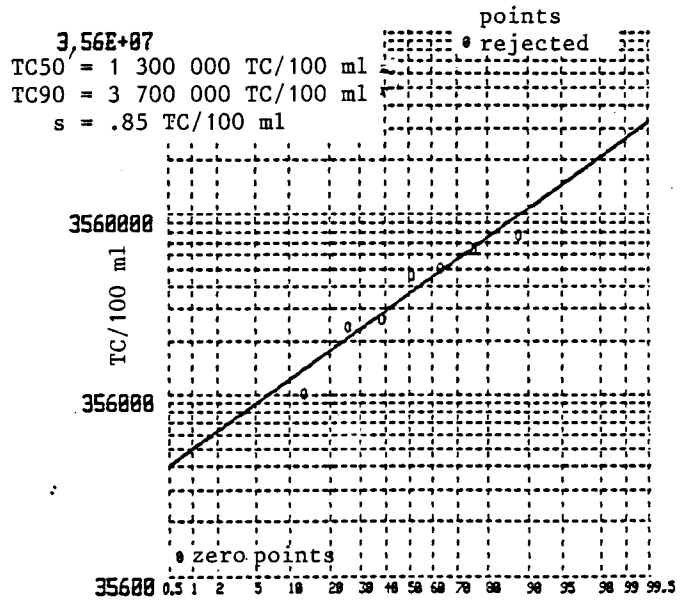
Fig. 3. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: A-1
Microorganism: FS

Date: 7 November 1983
Culture medium: KF-streptococcus agar

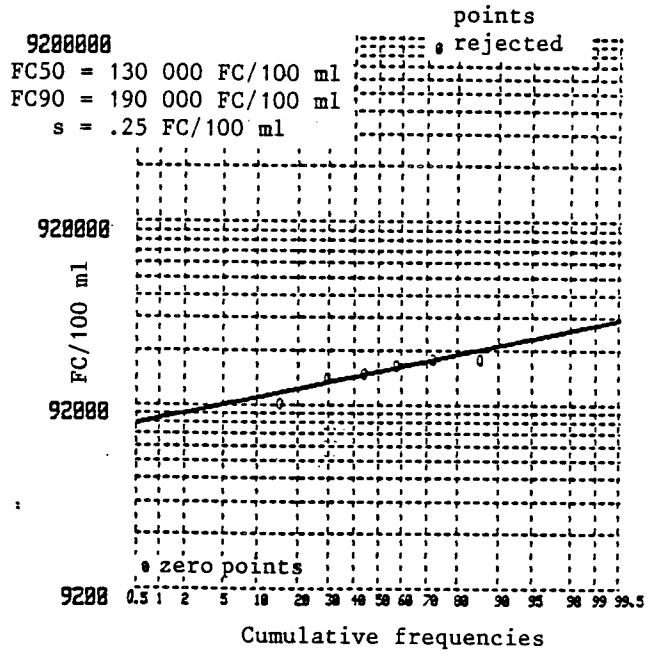
Fig. 4. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: B-1
Microorganism: TC

Date: 7 November 1983
Culture medium: m-Endo agar

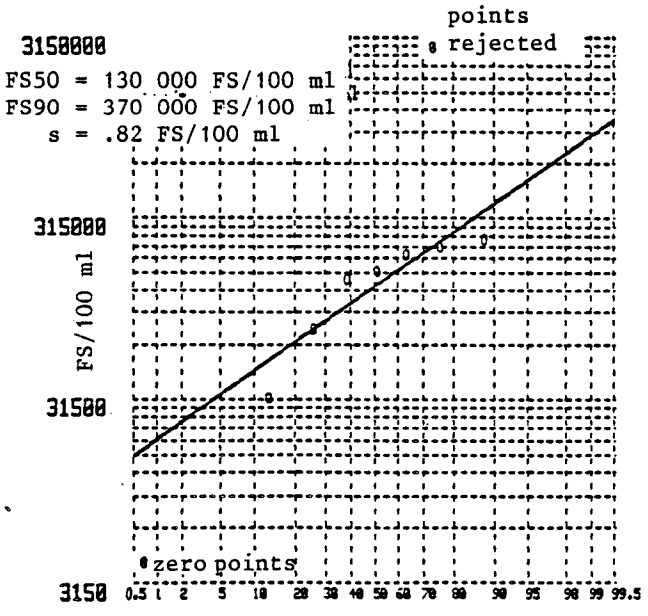
Fig. 5. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: B-1
Microorganism: FC

Date: 7 November 1983
Culture medium: m-FC agar

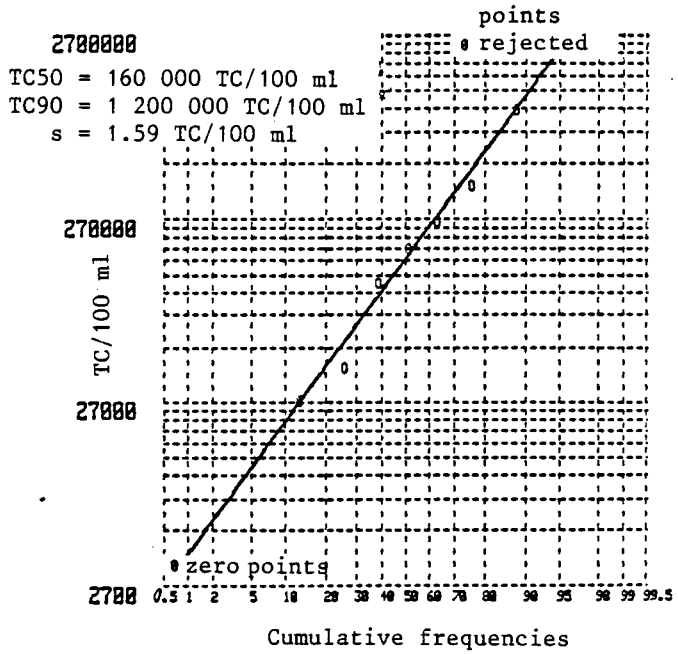
Fig. 6. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: B-1
Microorganism: FS

Date: 7 November 1983
Culture medium: KF-streptococcus agar

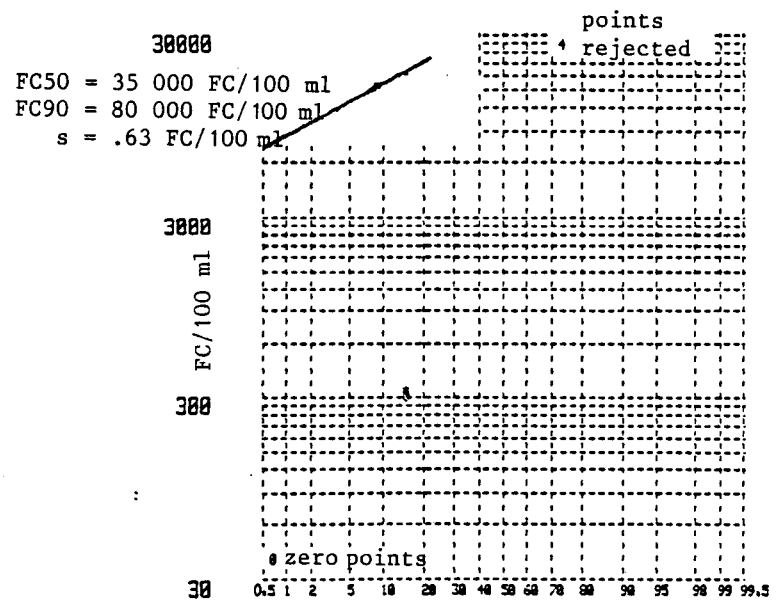
Fig. 7. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: C-1
Microorganism: TC

Date: 7 November 1983
Culture medium: m-Endo agar

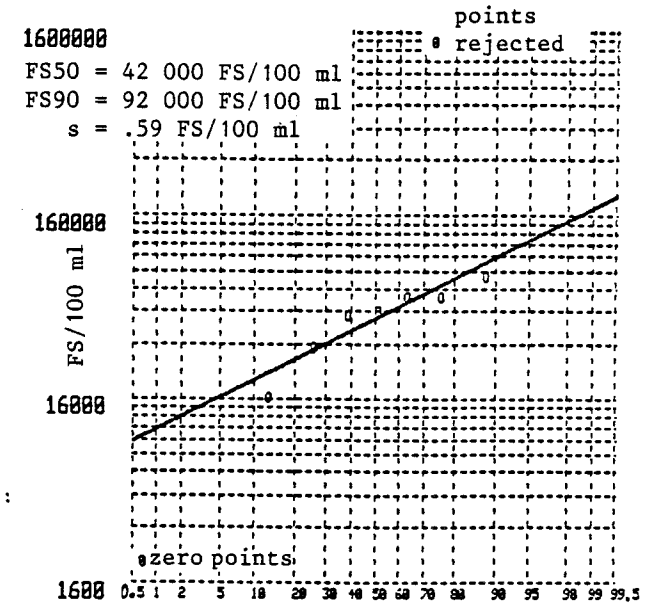
Fig. 8. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: C-1
Microorganism: FC

Date: 7 November 1983
Culture medium: m-FC agar

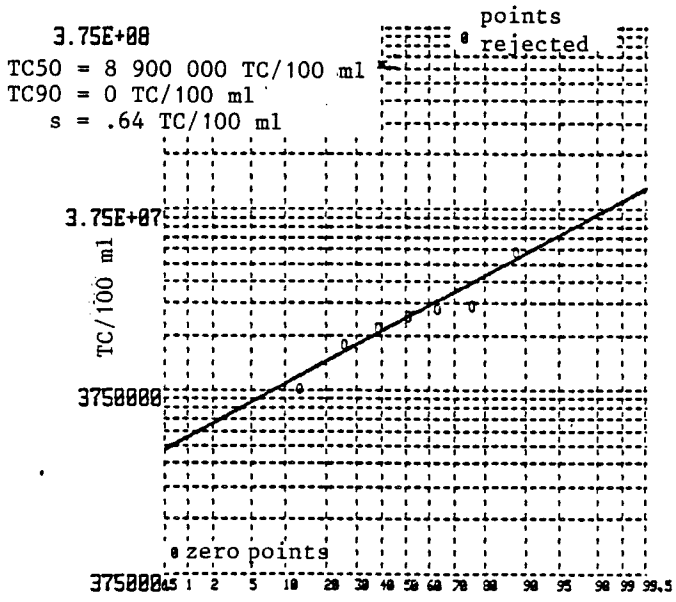
Fig. 9. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: C-1
Microorganism: FS

Date: 7 November 1983
Culture medium: KF-streptococcus agar

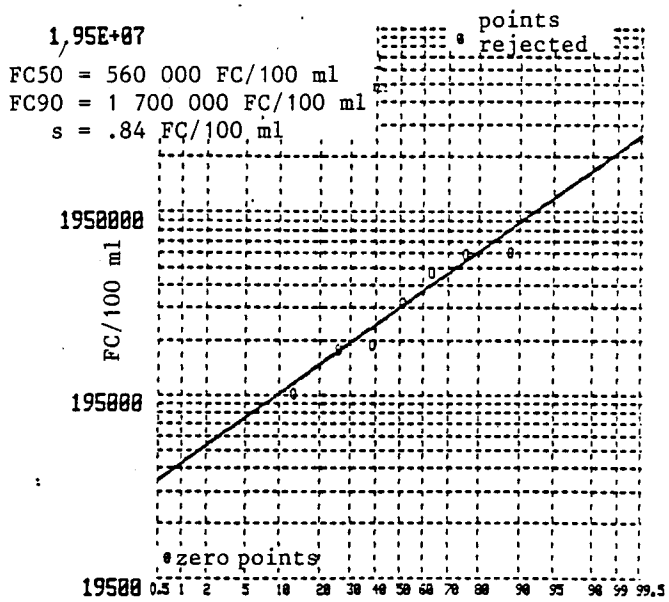
Fig. 10. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: A-2
Microorganism: TC

Date: 8 November 1983
Culture medium: m-Endo agar

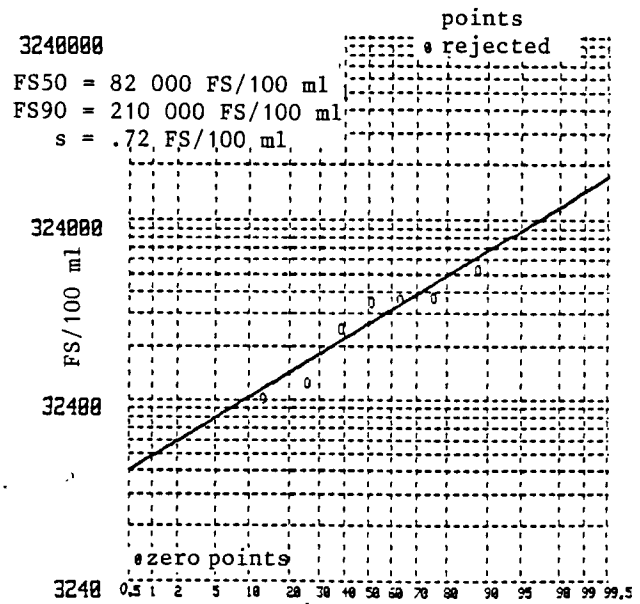
Fig. 11. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: A-2
Microorganism: FC

Date: 8 November 1983
Culture medium: m-FC agar

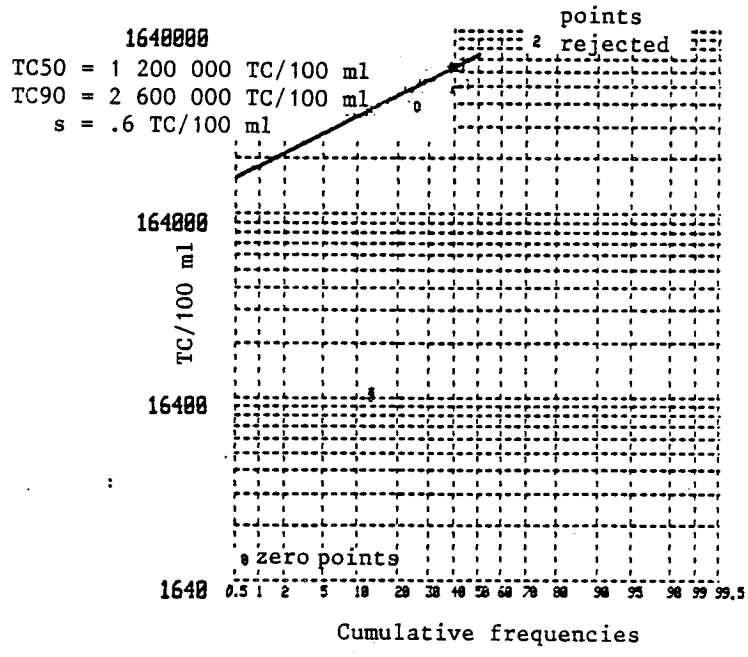
Fig. 12. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: A-2
Microorganism: FS

Date: 8 November 1983
Culture medium: KF-streptococcus agar

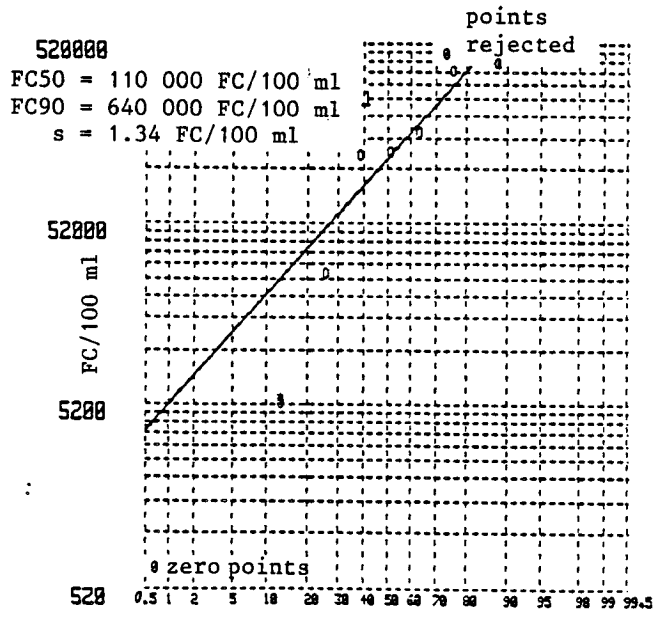
Fig. 13. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: B-2
Microorganism: TC

Date: 8 November 1983
Culture medium: m-Endo agar

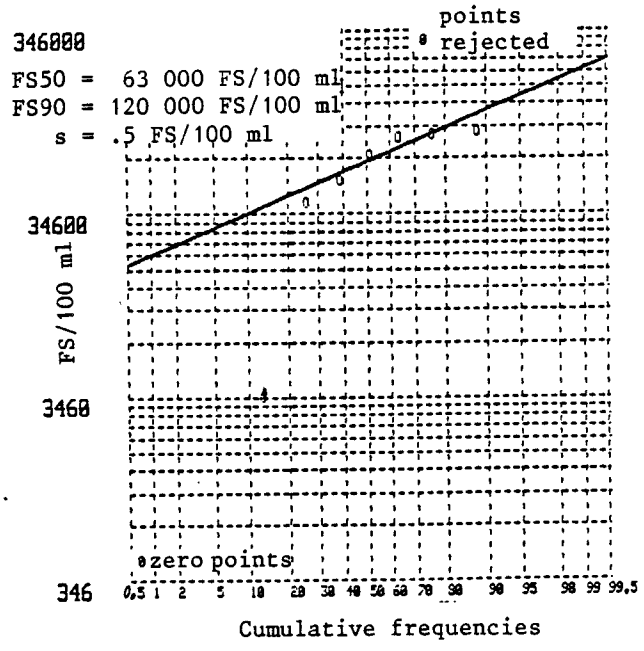
Fig. 14. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: B-2
Microorganism: FC

Date: 8 November 1983
Culture medium: m-FC agar

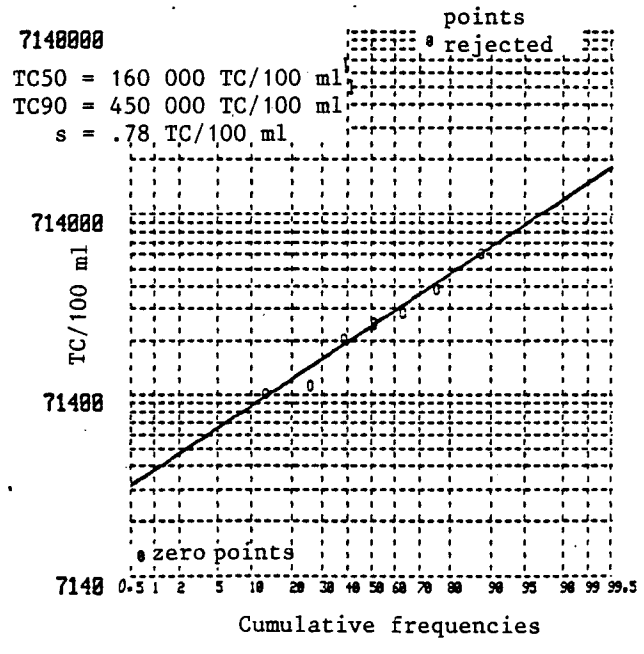
Fig. 15. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: B-2
Microorganism: FS

Date: 8 November 1983
Culture medium: KF-streptococcus agar

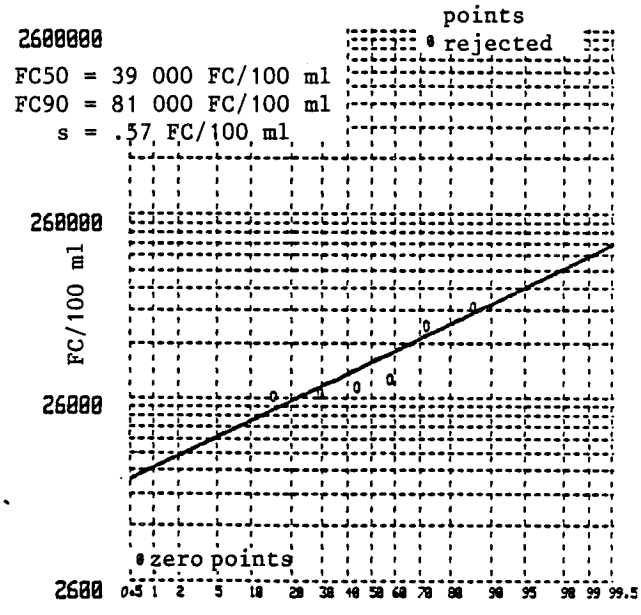
Fig. 16. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: C-2
Microorganism: TC

Date: 8 November 1983
Culture medium: m-Endo agar

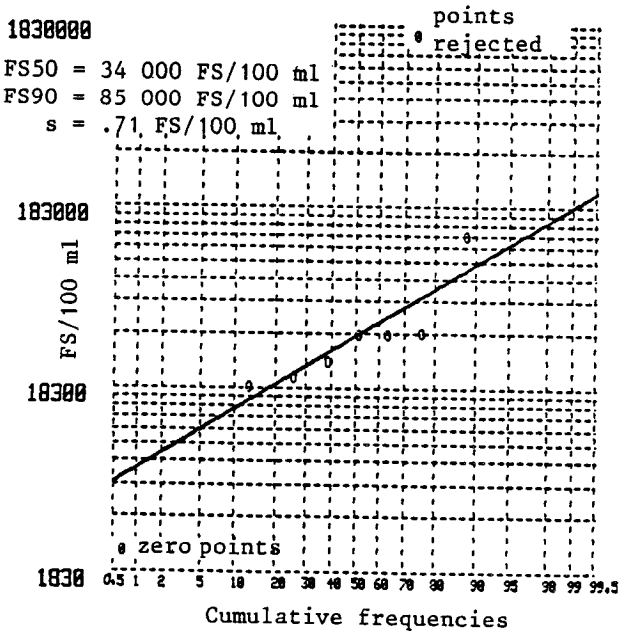
Fig. 17. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: C-2
Microorganism: FC

Date: 8 November 1983
Culture medium: m-FC agar

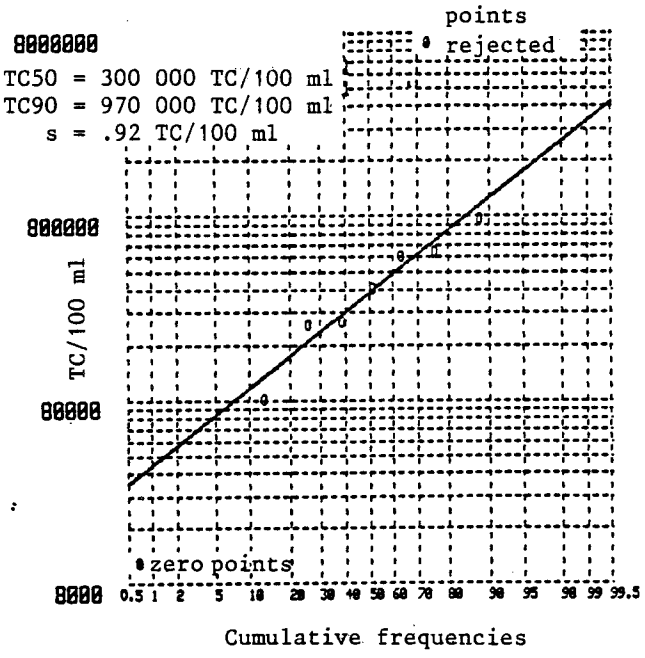
Fig. 18. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: C-2
Microorganism: FS

Date: 8 November 1983
Culture medium: KF-streptococcus agar

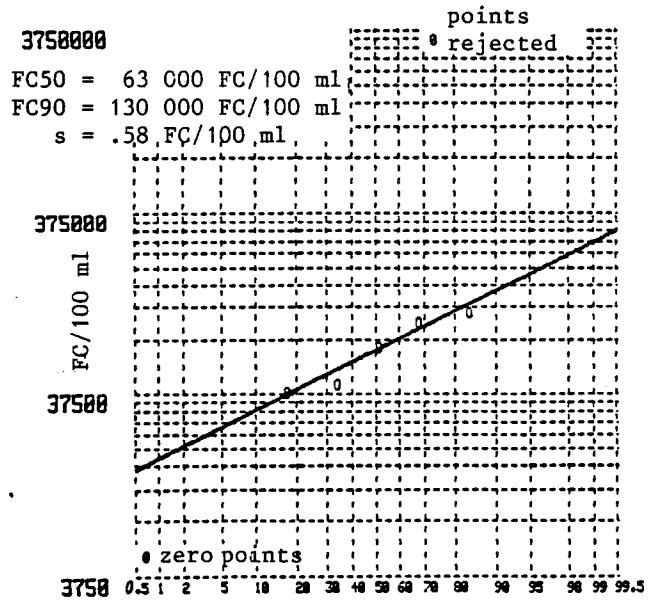
Fig. 19. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: A-3
Microorganism: TC

Date: 9 November 1983
Culture medium: m-Endo agar

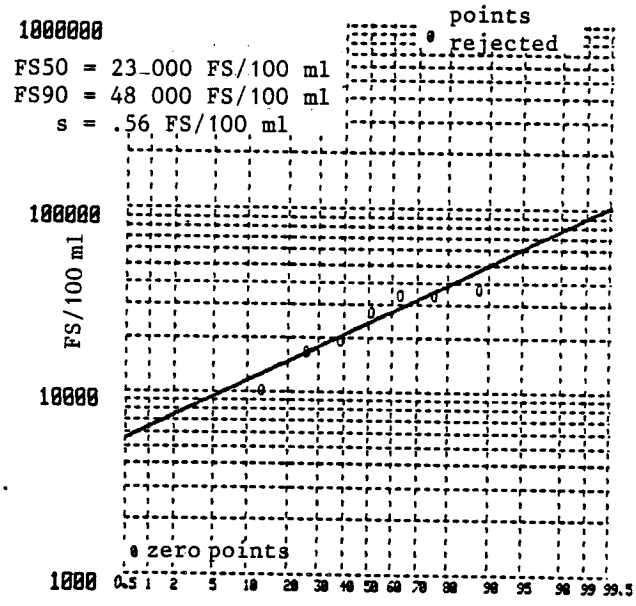
Fig. 20. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: A-3
Microorganism: FC

Date: 9 November 1983
Culture medium: m-FC agar

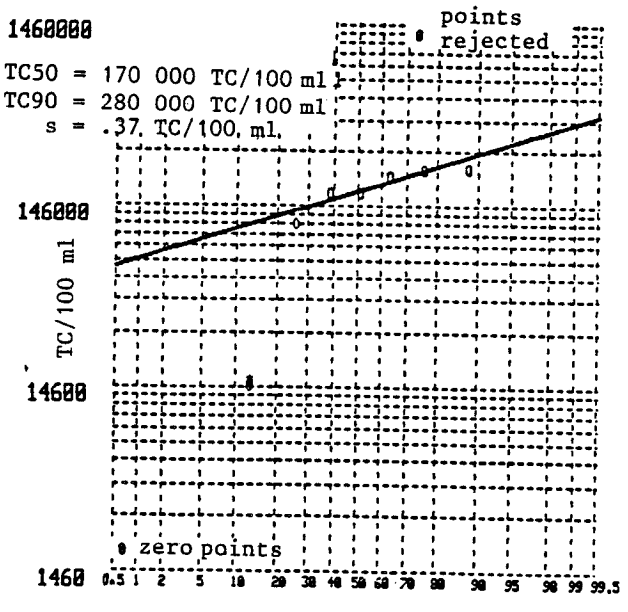
Fig. 21. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: A-3
Microorganism: FS

Date: 9 November 1983
Culture medium: KF-streptococcus agar

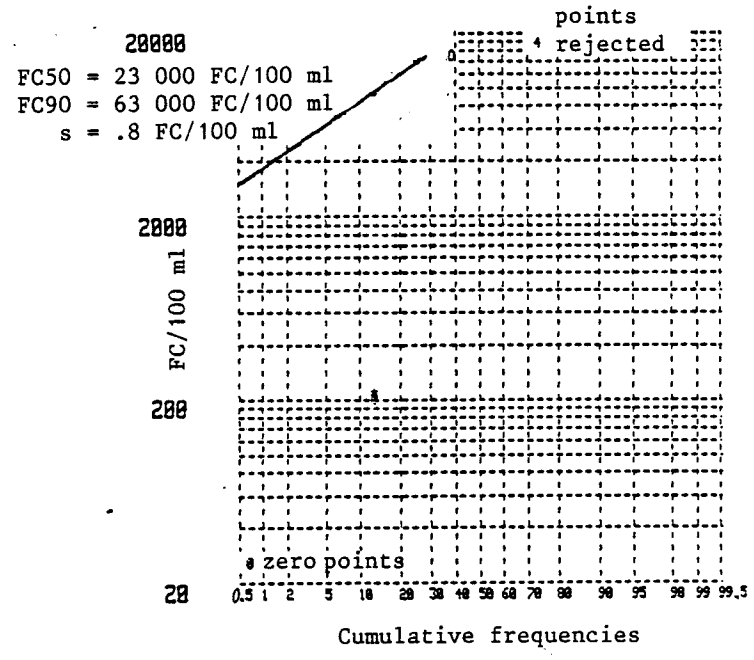
Fig. 22. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: B-3
Microorganism: TC

Date: 9 November 1983
Culture medium: m-Endo agar

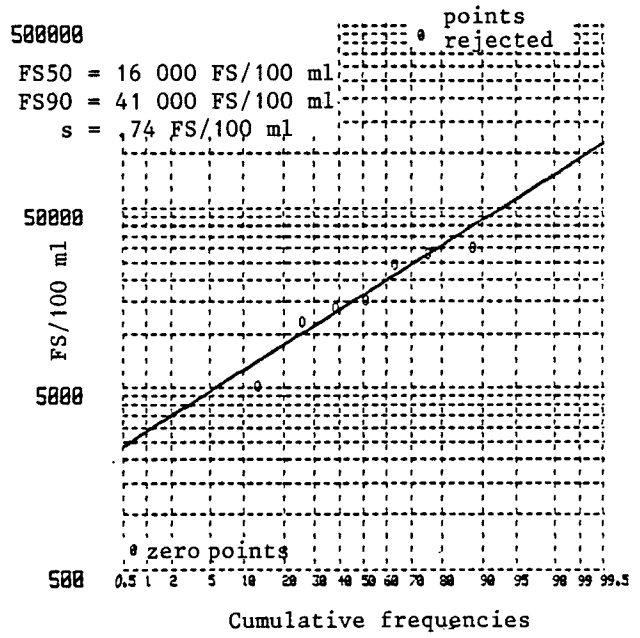
Fig. 23. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: B-3
Microorganism: FC

Date: 9 November 1983
Culture medium: m-FC agar

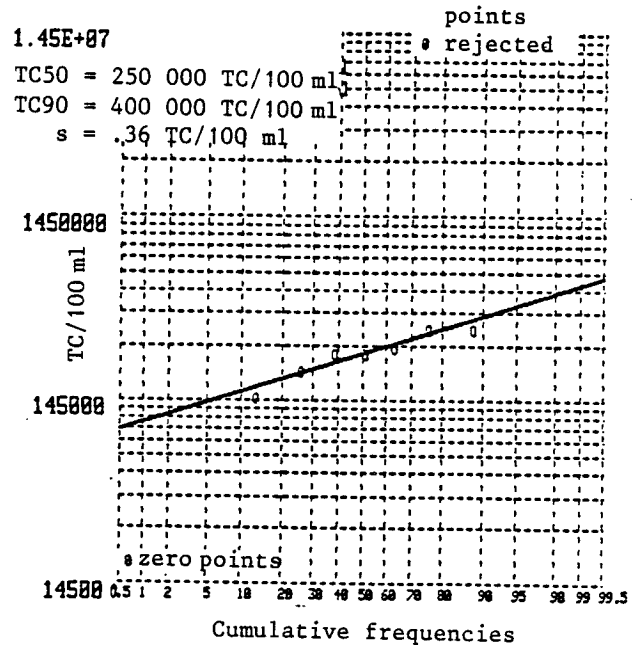
Fig. 24. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: B-3
Microorganism: FS

Date: 9 November 1983
Culture medium: KF-streptococcus agar

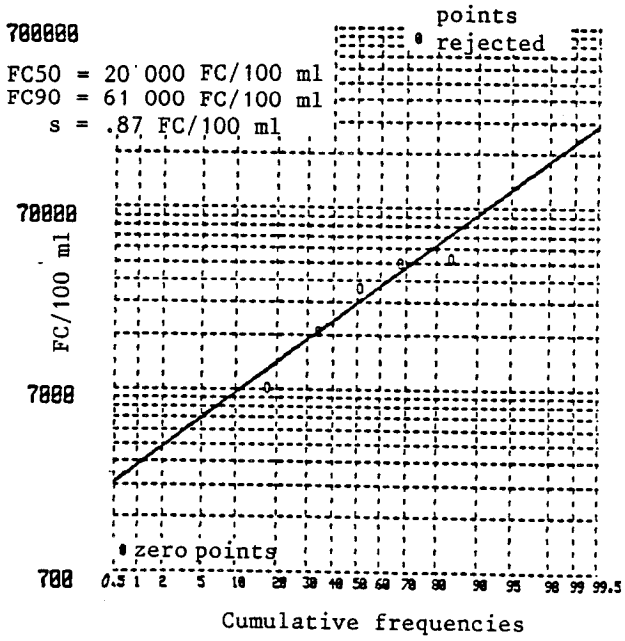
Fig. 25. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: C-3
Microorganism: TC

Date: 9 November 1983
Culture medium: m-Endo agar

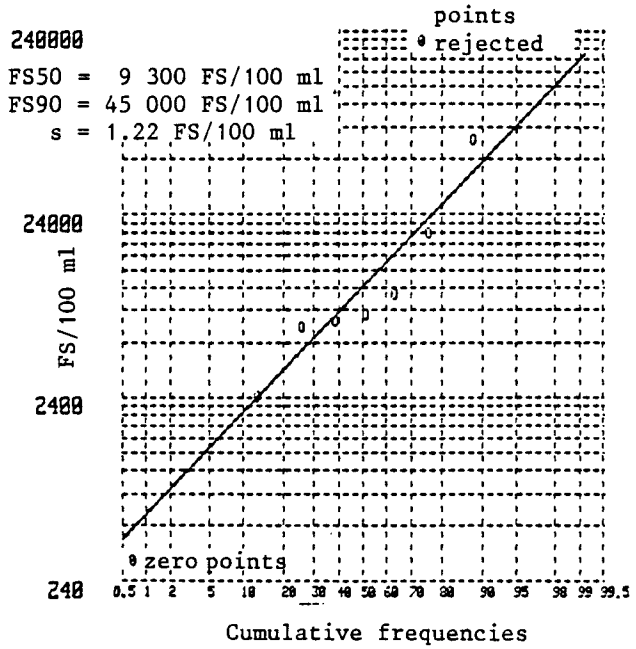
Fig. 26. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: C-3
Microorganism: FC

Date: 9 November 1983
Culture medium: m-FC agar

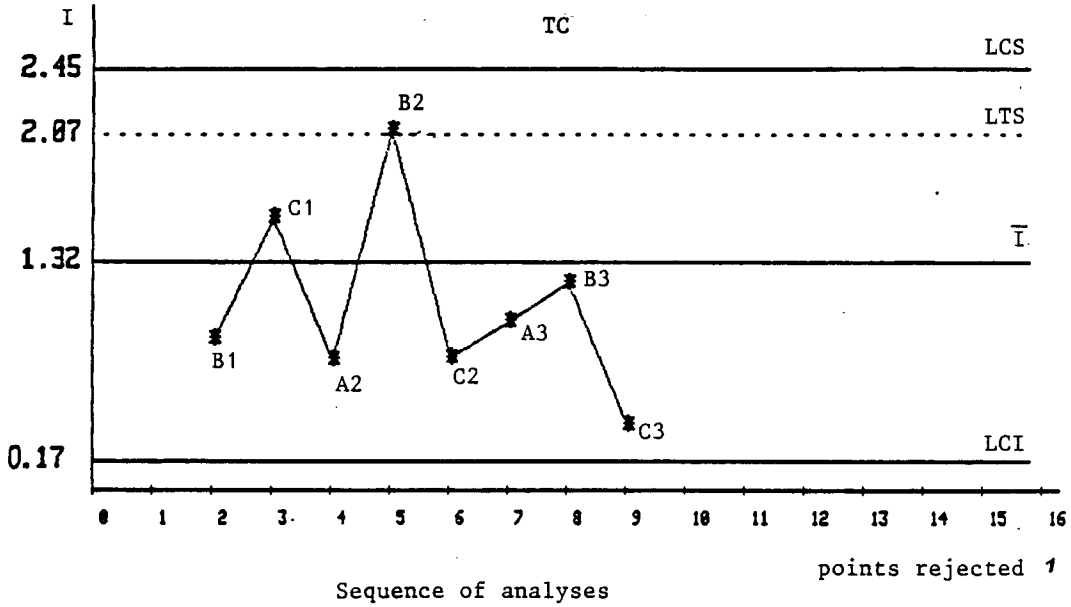
Fig. 27. Statistical analysis of microbial concentrations in coastal water samples: membrane filtration method



Type of water sample: C-3
Microorganism: FS

Date: 9 November 1983
Culture medium: KF-streptococcus agar

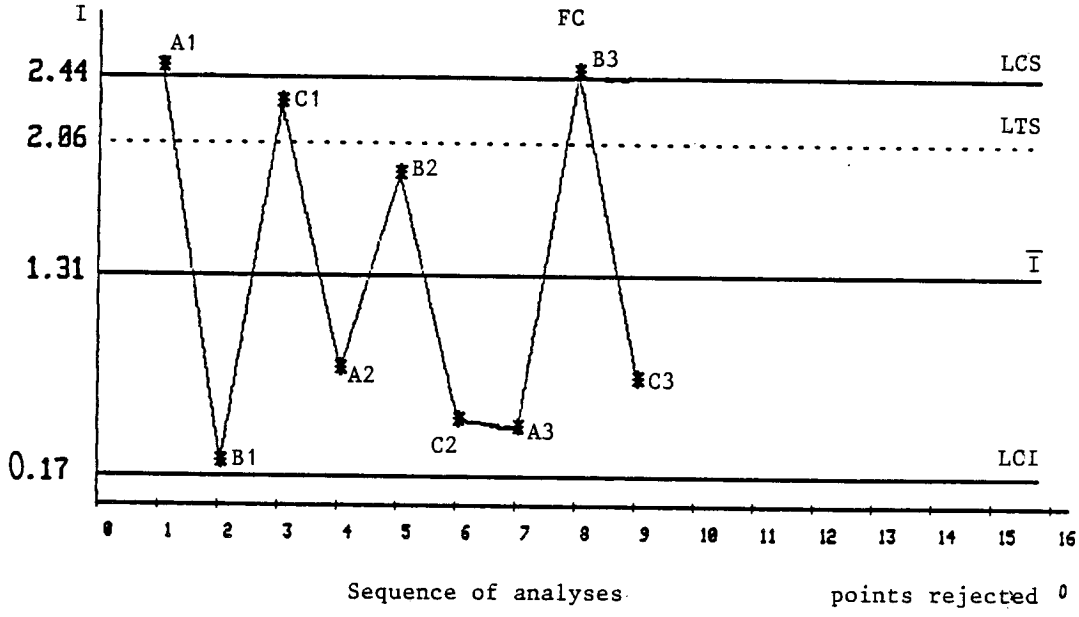
Fig. 28. Graph for checking microbiological analyses of coastal water samples: membrane filtration method



Microorganism: TC

Culture medium: m-Endo agar

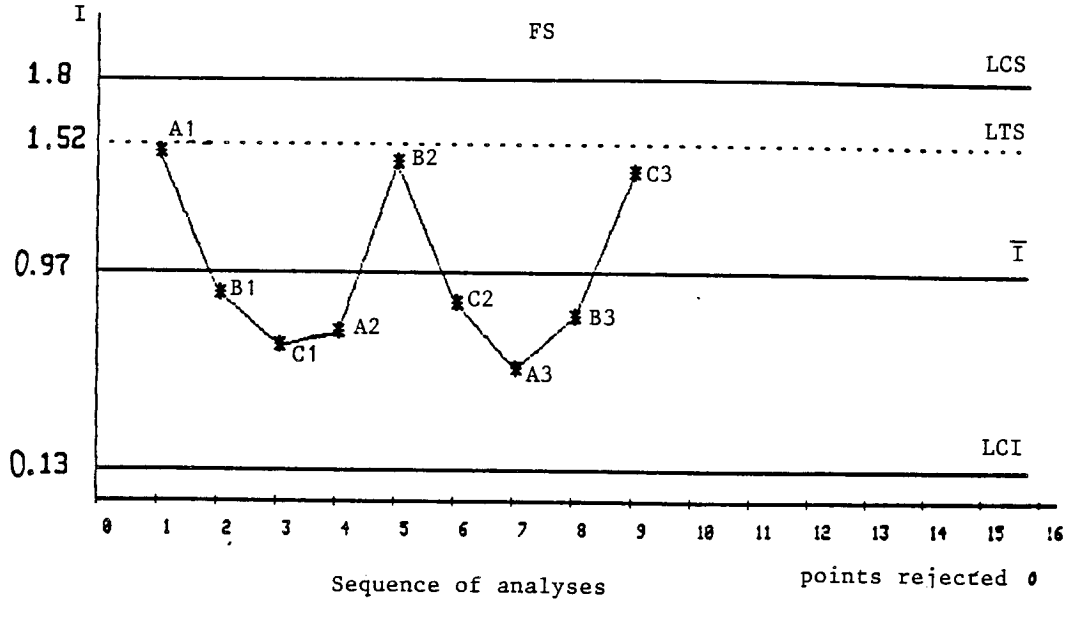
Fig. 29. Graph for checking microbiological analyses of coastal water samples: membrane filtration method



Microorganism: FC

Culture medium: m-FC agar

Fig. 30. Graph for checking microbiological analyses of coastal water samples: membrane filtration method



Microorganism: FS

Culture medium: KF-streptococcus agar

Annex 6

LIST OF PARTICIPANTS

Representatives of laboratories participating in the exercise

Miss F.B. Bâ
Société nationale d'exploitation des eaux du Sénégal (SONEES), Dakar, Senegal

Dr P. Bernard
Institut national de la santé et de la recherche médicale, Contrôle et information de la pollution marine, Nice, France

Dr J.J. Borrego Garcia
Departamento de Microbiología, Facultad de Ciencias, Universidad de Malaga, Spain

Mrs O. Cadahia Mariz
Dirección de Salud de Lugo, Montevideo, Spain

Dr J. Castellvi
Instituto de Investigaciones Pesqueras, Barcelona, Spain

Dr E. Chamekh
Laboratoire d'hygiène municipal de la Ville d'Alger, Algeria

Miss M. Colldecarrera i Compte
Universitat Politécnica de Girona, Spain

Dr K. Djambara
Centre médical des gens de mer, Abidjan, Ivory Coast

Mrs M.-D. Ferrer Escobar
Laboratorio Municipal de Barcelona, Spain

Dr F. Gonzalez Porcel
Dirección de Salud, Consejeria de Sanidad y Seguridad Social de Baleares, Palma de Mallorca, Spain

Mr S. Grané Terradas
Servei Territorial de Promocio de la Salut de Tarragona, Generalitat de Catalunya, Tarragona, Spain

Mrs C. Hosta Robés
Escuela Universitaria Politécnica de Girona, Laboratorio Aguas, Girona, Spain

Professor S. Jekov
Institut Pasteur de Tunis, Tunisia

Miss L. Lodeiro Saénz
Dirección de Salud de la Coruña, Xunta de Galicia, La Coruña, Spain

Professor F. Lucena
Departamento de Microbiología, Facultad de Biología, Universidad de Barcelona, Spain

Mr M. Martos Padilla
Junta de Andalucia, Consejeria de Sanidad, Dirección Provincial de la Salud, Granada, Spain

Mr L. Matia Ribot
Servei de Sanejament Ambiental, Direccio General de Promocio de la Salut, Departament de Sanitat i Seguritat Social, Generalitat de Catalunya, Barcelona, Spain

Mrs I. Moro Gonzalez
Servicio Territorial de Promoción de la Salud, Barcelona, Spain

Professor R. Mujeriego
Escola Tecnica Superior d'Enginyers de Camins, Canals i Ports, Universitat Politécnica de Barcelona, Spain

Mrs M.-V. Navazo Dolz del Castellar
Escuela Nacional de Sanidad, Ciudad Universitaria, Madrid, Spain

Mr L. O'Shanahan
Departamento de Bacteriología, Centro de Tecnología Pesquera, Cabildo Insular de Gran Canaria, Las Palmas, Spain

Mrs J. Pardos i Bosch
Servei Territorial de Promocio de la Salut, Girona, Spain

Mr I. Perales Palacios
Dirección de Salud de Vizcaya, Bilbao, Spain

Mr E. Quesada Fernandez de la Puente
Consejeria de Sanitat, Generalitat de Valencia, Spain

Mr J. Ricart Pifarre
Servicio Territorial de Promoción de la Salud, Lerida, Spain

Mr J. Semeria
Centre scientifique de Monaco, Monte Carlo, Principality of Monaco

Professor E. Vicente
Dto Microbiología, Facultad de Ciencias Biológicas, Universidad de Valencia, Campus Universitario de Burjasot, Valencia, Spain

Miss C. Zigorraga Arrien
Dirección de Salud de Guipuzcoa, San Sebastian, Spain

REPRESENTATIVES OF OTHER ORGANIZATIONS

United Nations Environment Programme (UNEP)

Dr F.S. Civili
Coordinating Unit for the Mediterranean Action Plan, Athens, Greece

WORLD HEALTH ORGANIZATION

Regional Office for Europe

Dr L.J. Saliba
Senior Scientist, WHO EURO Project Office, Coordinating Unit for the Mediterranean Action Plan, Athens, Greece