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

MICROBIOLOGICAL METHODS FOR COASTAL WATER QUALITY MONITORING

Fourth Report

Published under the joint sponsorship of the
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WORLD HEALTH ORGANIZATION
Regional Office for Europe
Copenhagen, 1985

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Fourth report on a joint WHO/UNEP meeting

Split
15-20 April 1985

WORLD HEALTH ORGANIZATION
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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 marine pollution monitoring in the region, in close cooperation with the international agencies concerned.

The scientific component of the Action Plan, the joint coordinated Mediterranean Pollution Monitoring and Research Programme (MED POL) was aimed at providing a framework and the necessary knowledge for a monitoring system of this nature. During the pilot phase of this programme (MED POL Phase I) carried out between 1976 and 1981, 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 Sea (MED POL Phase II), covering the period 1981-1990, and in accordance with the relevant articles of the Convention and its related protocols, practically all Mediterranean countries have submitted national monitoring programmes, and a number of these are fully operational, some of them since 1983.

For comparison of the results and quality control of the analyses at both the national and the regional level, a series of intercalibration exercises on microbiological methods for coastal water quality monitoring was commenced in 1983 following a preparatory exercise held at the Instituto Superiore di Sanita, Rome, in November 1982. The exercises, conducted alternately in English and in French, are directed to the laboratories of the host country which are participating or intend to participate in the monitoring programme, and to a few laboratories in other countries, with the aim of ensuring continuity of organization and participation. The first exercise within the series was held at the Escola Tecnica Superior d'Enginyers de Camins, Canals i Ports, Barcelona (7-11 November 1983), the second at the Environmental Pollution Control Project, Athens (25-29 June 1984) and the third at the Institut Pasteur, Tunis (12-16 November 1984).

The present intercalibration exercise and consultation meeting was organized by WHO and UNEP in collaboration with the Institute of Oceanography and Fisheries, Split, within the framework of MED POL Phase II. The main purpose of the exercise was to enable the participants to make determinations of bacteriological parameters in identical samples of seawater and shellfish, using the following recommended methods:

- 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);

- determination of faecal coliforms in bivalves by the multiple test tube method (Reference Methods for Marine Pollution Studies No. 5/Rev. 1, UNEP/WHO);

In addition, to obtain further information on the comparability of the membrane filtration culture method and the multiple test tube method, it was intended to have participants make parallel determinations of faecal coliforms on the same seawater samples, using the following method:

- determination of faecal coliforms in seawater by the multiple test tube method (Reference Methods for Marine Pollution Studies No. 22, UNEP/WHO, draft);

The above Reference Methods form part of a comprehensive series formulated by the Regional Seas Programme of UNEP in collaboration with the specialized agencies of the United Nations. This series is designed to eventually cover all the possible parameters set out in the annexes to the protocols adopted in terms of the Convention. They are also intended for use in regions other than the Mediterranean.

The objectives of the consultation meeting included a review of the draft reference method on determination of faecal coliforms in seawater by the multiple test tube method, as well as of the following methods:

- determination of total coliforms in seawater by the multiple test tube method (Reference Methods for marine pollution studies No. 21, UNEP/WHO, draft)

- determination of faecal streptococci in sea water by the multiple test tube method (Reference methods for marine pollution studies No. 23, UNEP/WHO, draft)

- guidelines for monitoring the quality of coastal recreational and shellfish-growing areas (Reference methods for marine pollution studies No. 1, UNEP/WHO, revised draft)

Further objectives of the Consultation meeting were :

- to review the results of previous intercalibration exercises;

- to review the results of the present intercalibration exercise in order to identify technical problems in both methodology and quality control;

- to make appropriate recommendations on future exercises in the series;

- to make appropriate recommendations on the relevant aspects of the long-term monitoring and research programme.

Representatives of the Yugoslav institutions participating in the microbiological aspects of the monitoring component of MED POL Phase II and other national monitoring programmes were invited to take part in the intercalibration exercise and consultation meeting, as well as representatives of institutions in Cyprus, Egypt, Israel, Lebanon, Libya and Turkey also involved in the same programme. In addition, the following international organizations were invited to send representatives : Food and Agricultural Organization (FAO), Intergovernmental Oceanographic Commission (IOC), United National Scientific, Educational and Cultural Organization (UNESCO), World Meteorological Organization (WMO) and International Atomic Energy Agency (IAEA).

1. Opening of the meeting (agenda item 1)

The meeting took place at the Institute of Oceanography and Fisheries, Split, from 15 to 20 April 1985. It was attended by 9 temporary advisers from Yugoslavia and six other Mediterranean countries. There was one representative of WHO/EURO, who also represented the UNEP Coordinating Unit for the Mediterranean Action Plan. A list of participants is given in Annex 3.

Dr L.J. Saliba, Senior Scientist, Mediterranean Action Plan, World Health Organization, Regional Office for Europe, opened the meeting on behalf of the Regional Director, Dr J.E. Asvall. He mentioned the deep involvement of WHO in the Mediterranean Action Plan, within the framework of which this activity was being organized, and stressed the importance of the present series of intercalibration exercises which, together with the standard reference methods, assured comparability of results, thus enabling an overall picture to be drawn on the state of microbial pollution of coastal recreational and shellfish-growing waters in the Mediterranean. He also expressed WHO's appreciation of the work undertaken and the facilities provided by the Institute of Oceanography and Fisheries both for the laboratory exercise and the Consultation meeting. He mentioned the appropriateness of the present venue, as Yugoslavia had been activly involved in the health-related aspects of MED POL from the start of the programme, and the host Intstitute had been an active participant throughout.

Dr Pero Cetinic, Director of the Institute of Oceanography and Fisheries, Split, welcomed participants on behalf of the host institution. He expressed his pleasure that the meeting was being held at the institute which had a long record of fruitful collaboration with the World Health Organization and the United National Environment Programme over many years, especially in the monitoring of coastal recreational and shellfish-growing waters, which was very important. He expressed the hope that such cooperation would continue in the future with the same degree of success. While offering once again the readiness of the institute to provide any possible assistance to participants to enable them to achieve fruitful results in their work, he augured a complete success to the meeting, and the attainment of results which would be of value in future projects of a similar nature.

Dr L.J. Saliba briefly addressed participants on behalf of Mr Aldo Manos, UNEP Coordinator of the Mediterranean Action Plan. Since its original adoption in Barcelona in 1975, the Plan had been actively ongoing. He mentioned the main components of the Action Plan, and stressed that this particular activity should be seen not only in relation to health-related monitoring, but also within the overall framework of the Action Plan as a whole.

Mr A. Pavasovic, Director of the Regional Activity Centre of the Priority Actions Programme (PAP/RAC), addressed participants on behalf of the Centre which was responsible for one of the most important components of the Mediterranean Action Plan. He outlined the role of PAP within the overall framework of the Plan, and the regional projects being undertaken in the fields of human settlements, water resources management, tourism, soil protection, aquaculture and renewable sources of energy. The present meeting had a link with the projects on human settlements and tourism. While also auguring a complete success to the meeting, he invited participants to visit the PAP/RAC in Split to enable them to obtain a better idea of the working of the Centre.

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

Dr L.J. Saliba explained the scope and purpose of the exercise and consultation meeting. During the laboratory exercise, it was important that all participants follow the instructions supplied by the host institution, as comparability of results depended on the observance of the same methodology. It was also important that participants should try to follow the same methodologies in their home laboratories during the sampling and analytical work they performed as part of the monitoring component of MED POL. Otherwise, any overall comparison of results submitted by different laboratories would be difficult.

In addition, during the meeting sessions, participants had to review the drafts of the reference methods for determination of total coliforms, faecal coliforms and faecal streptococci in sea water by the multiple test tube method, and the revised draft of the guidelines for monitoring the quality of coastal recreational and shellfish-growing areas.

3. Election of Officers (agenda item 3)

Dr Nada Krstulovic was elected Chairman, Dr A.F. Boargob Vice-Chairman, and Mr B. Cihangir Rapporteur. Dr L.J. Saliba acted as Secretary to the meeting.

4. Adoption of the agenda (agenda item 4)

The provisional agenda was unanimously adopted.

5. Organization of the meeting and laboratory exercise (agenda item 5

Dr Nada Krstulovic explained the programme and procedures to be followed during the laboratory exercise and consultation meeting. During the former, participants would work in groups of two each. Detailed instructions on laboratory procedures, including the necessary reporting forms, had been provided, and the reference methods would be used only as a back-up. The

provisional programme was agreed upon by the meeting, on the understanding that this might have to be modified depending on progress achieved during the laboratory sessions.

6. Review of previous intercalibration exercises (agenda item 6)

Dr L.J. Saliba briefly outlined the results of previous intercalibration exercises. The exercise held in Rome (22-26 November 1982) was of a preliminary nature, designed to provide the necessary experience on which to base the regular series. The first exercise (Barcelona, 7-11 November 1983) did not produce good correlation between individual results. This was ascribed to two main factors - the heavy floods at the time, which affected the homogeneity of the samples, and variations in individual techniques. During the second exercise (Athens, 25-29 June 1984), good correlation was obtained both between individual results and (within a certain range of values) between the MF and MPN techniques. A similar degree of correlation was obtained during the third exercise (Tunis, 12-16 November 1984). In this last case, correlation between individual results was affected by the fact that some participants were using the MF technique for the first time.

7. Review of revised draft guidelines on monitoring of coastal recreational and shellfish-growing areas (agenda item 7)

Dr Saliba briefly introduced document ICP/CEH 001 mO4/6. The current version of the draft guidelines was based on comments and suggestions made at the consultation meeting held in Athens in June 1984, and on other comments received from a number of Mediterranean institutions thereafter.

The meeting had no comments on the overall lay-out of the document. A number of comments and observations were, however, made on specific points. These mainly dealt with the lists of parameters to be used in minimum and extended monitoring programmes, beach surveillance, and the monitoring of sand on beaches for determination of bacterial and fungal parameters.

The meeting felt that the number of parameters to be used in minimum monitoring (i.e. the mandatory microbiological parameters for sea water - faecal coliforms, faecal streptococci and one pathogenic organism to be selected according the specific requirements determined by the locality) were sufficient. It was stressed, however, that determination of the more important supporting parameters (temperature, salinity, etc.) should also be performed. On account of the fact that in the Mediterranean region (as in others) people spent a considerable time in direct contact with sand on beaches, several participants felt that even in minimum monitoring, one pathogenic organism from sand should also be included. Such an organism could either be bacterial or fungal.

A comprehensive discussion was held regarding sampling of sand on beaches, and the practical problems involved in the interpretation of results. Unlike sea water, which could be considered a relatively homogeneous medium, sand varied in its microbiological content. This variation could be the effect of human activities or of wave action, and no sample of sand could be considered as representative of any beach, unless large quantities were taken. There was also the problem of beaches which were cleaned mechanically at regular intervals (sometimes every morning) and this would seriously affect sampling and interpretation of results.

The meeting agreed that it was not possible at this stage to formulate any specific methodology for the sampling of sand on recreational beaches, as a lot of work had still to be performed to enable sampling to be done in such a way as to provide an accurate picture of the general situation prevailing in any particular beach. It was recommended that such preliminary work be undertaken as part of the research component of MED POL.

The meeting also discussed the interpretation of monitoring results and practical action indicated. In this regard, participants agreed that environmental quality criteria were extremely important, as decisions regarding the use of recreational areas would have to be taken on the basis of such criteria. The meeting noted that within the framework of the research component of MED POL, studies were proceeding on the relationship between the microbiological quality of sea water and the health of swimmers. It was agreed that such epidemiological studies were essential in arriving at firm quality criteria. The difficulties involved in such studies were noted by the meeting. In this regard, the participants were informed that a special consultation meeting to review this problem, and to formulate concrete and realistic proposals for continuation of such studies, was being convened in October 1985.

The meeting agreed that in minimum monitoring programmes, it would not be possible to record all the parameters listed in the Garber classification, and the more important ones identified in the guidelines appeared to be sufficient.

The discussion on the guidelines was conducted throughout on the understanding that the final version of the document would be produced following receipt of further detailed comments from Mediterranean institutes, which would take the remarks passed during the meeting into account.

8. Review of draft reference methods (agenda item 8)

The meeting briefly discussed the contents of the reference methods for determination of total coliforms, faecal coliforms and faecal streptococci in sea water by the multiple test tube method. These documents had been produced by a consultant and reviewed by a leading international expert. It was explained to the meeting that following comments made during the current review, the documents would be circulated to Mediterranean laboratories for further comments prior to the issue of the first substantive versions.

The following comments and suggestions were made regarding the draft reference method for determination of total coliforms in sea water by the multiple test tube method :

- consideration should be given to the possibility of using solid McConkey medium in plates instead of liquid broth in tubes, when performing the confirmatory test;

- consideration should similarly be given to the possibility of substituting sodium thiosulphate by potassium thiosulphate in case of unavailability of the former.

The following comments and suggestions were made regarding the draft reference method for determination of faecal coliforms in sea water by the multiple test tube method :

- As for total coliforms, consideration should be given to the possibility of substituting sodium thiosulphate by potassium thiosulphate in case of unavailability of the former;

- There were doubts as to the need for retaining the indole test. Some participants felt that the McConkey test was sufficient;

- In Section 8.3, (a) the correct incubation temperature as given in the third paragraph should read 44.5°C, not 35 ± 0.5°C, and (b) readings should be taken after 24 hours, not 48 hours.

Regarding the draft reference method for determination of faecal streptococci in sea water by the multiple test tube method, a suggestion was made that use of KF-streptococcus broth as a medium would be advisable.

General remarks and suggestions applicable to all three methods included the following :

- samples should preferably be at the laboratory not later than 12 hours after collection;

- presumptive tests should be checked after 48 hours, not after 24 hours. There appeared to be no scientific reason to retain the 24 hours period;

- the effects of salinity were considered to be an important factor, and it was felt that the reference methods should contain a mention of this.

The meeting agreed that all three methods would have to be checked by Mediterranean laboratories, following which more detailed comments could be made. Similarly, revisions could also be proposed to the other reference methods which utilized the MF technique. In this regard, the following comments were passed with respect to reference method No. 2 rev. 1 (determination of total coliforms in sea water by the membrane filtration culture method) :

- on the basis of personal experience, it was considered that the culture medium, M-endo agar, would not last for the 30-day storage period after preparation. A safer estimate would be 7-10 days;

- in the preparation of the same medium, the components should initially be dissolved in 20 ml ethanol and a little water, and then brought up to the mark.

9. Future action and recommendations (agenda item 9)

As a basis for a discussing future action, the meeting reviewed the results of the present exercise. A detailed report, including results obtained, is given in Annex 1, and the instructions to participants in Annex 2. In general, a reasonably good correlation was obtained between readings of individual participants, and discrepancies were attributed to slight variations in individual techniques. Similarly, differences in experience also contributed to variation between individual results, although in this particular exercise the range of experience between participants was not so pronounced as in the case of previous exercises.

The importance of participants continuing to use the same techniques on their return to their home laboratories was emphasized. If this was not done, it would be difficult to compare results submitted by individual laboratories.

The meeting also made the following specific recommendations :

- The series of intercalibration exercises on microbiological parameters within the monitoring component of MED POL Phase II should be continued, as this was one of the main means of bringing participants from different laboratories together and contributed greatly to harmonization of techniques.

- Apart from intercalibration exercises, consideration should be given to the organization of training courses in microbiological techniques relating to the MED POL monitoring programme. This could be done both on a country basis and on a sub-regional basis.

- In order to facilitate action on the basis of monitoring results, environmental quality criteria for the main microbiological parameters should be adopted on a common regional basis.

- Epidemiological studies on the correlation between coastal water quality and health effects should continue on a wider basis.

- New reference methods should be developed on the determination of other parameters, both bacterial and fungal. In particular, study was required on the monitoring of sand on beaches to find the best way of obtaining reasonably-representative results.

- In future exercises, samples of sea water could possibly be prepared in such a manner as to limit the presence of natural confounding factors, e.g. by having artificial samples with known bacterial concentrations and/or reducing the number of samples and using more dilutions.

Annex 1

RESULTS OF INTERCALIBRATION EXERCISE
Split, 15 - 20 April 1985

Introduction

The purpose of the exercise was to enable participants to carry out determinations of microbial concentrations in the same samples of sea water and shellfish, using uniform methodology, and to compare the results obtained between individual participants for each parameter, in order to identify and attempt to correct any sources of variation.

Organization and methodology

Participants were divided into seven groups of two. Three samples of sea water and one sample of shellfish were analyzed by each group. Sample A was sea water from a heavily-polluted beach, Sample B sea water from a moderately-polluted beach and Sample C sea water from a relatively-clean beach. The shellfish sample was collected from a polluted area.

Sea water samples were analyzed for the following parameters :

- Total coliforms (TC) by the membrane filtration culture (MF) method;
- Faecal coliforms (FC) by the membrane filtration culture (MF) method and by the multiple test tube (MPN) method;
- Faecal streptococci (FS) by the membrane filtration culture (MF) method.

The shellfish sample was analyzed for faecal coliforms (FC) utilizing the multiple test tube (MPN) method.

In all determinations, the methods followed were the reference methods developed by the World Health Organization and the United Nations Environment Programme within the framework of the Mediterranean Action Plan.

Inoculation, filtration and reading of results were performed on a group basis. Throughout the exercise, checks were made to the fullest extent possible on the procedure and readings of each individual participants to note the type of errors and deviations which could influence results beyond the normal variations of the methods themselves.

Participants were supplied with the necessary samples, all equipment, culture media and reagents, and instruction sheets complete with forms for reporting results.

Results and discussions

Readings obtained by each group on membrane filters for each of the three sea water samples, together with a statistical analysis of the results, are attached as an appendix. In sample A, the total coliform count was too high

to enable reading. For faecal coliforms, the range of results obtained was 256 x 10^5 to 163 x 10^6 FC per 100 ml, with one group obtaining a reading of 1 x 10^5 FC/100 ml. The mean concentration of all results was 141 x 10^6 FC/100 ml. For faecal streptococci, the range was 251 x 10^5 to 448 x 10^5 FS per 100 ml, with a mean of 282 x 10^5 FS/100 ml.

In sample B, the range of readings for total coliforms was 154 x 10^4 to 171 x 10^5 TC/100 ml, with a mean of 376 x 10^4; for faecal coliforms, 132 x 10^4 to 127 x 10^5, with one reading of 7 x 10^4 TC/100 ml, with a mean concentration of all results of 311 x 10^4 TC/100 ml; and for faecal streptococci 283 x 10^3 to 820 x 10^3 FS/100 ml, with a mean concentration of 551 x 10^3 FS/100 ml.

In sample C, the range of readings for total coliforms was 4200 to 9800 TC/100 ml, with a mean concentration of 5943 TC/100 ml, for faecal coliforms 2300 to 3575 FC/100 ml, with a mean concentration of 2922 FC/100 ml, and for faecal streptococci 1340 to 2200 FS/100 ml, with a mean concentration of 1844 FS/100 ml.

In all cases, the standard deviation and 95% confidence limits are given in the appendix. For practically all parameters and samples, results obtained were statistically significant.

In the case of determination of faecal coliforms by the multiple test tube (MPN) method, all results obtained for each of samples A, B and C were above 2400 FC per 100 ml. While these results correlate in general to those obtained for the same parameter with the MF method, it was not possible to determine individual differences, or differences between the two methods, statistically. The same results were obtained for the shellfish samples (above 2400 FC/100 ml).

Although the prescribed methodologies were observed faithfully, a certain degree of variation in individual techniques between the different participants was evident. These were minor in nature and not of the type normally covered by standard instructions on methodology. It was concluded, however, that they could very well have contributed to the differences between individual readings.

The samples (both sea water and shellfish) were more highly polluted than anticipated, and the figures obtained even for the cleanest sea water sample (C) was beyond the maximum concentration covered by the MPN table. This precluded a realistic comparison of the MF and MPN results for faecal coliforms.

Conclusions

In general, it can be considered that, taking all factors into account, agreement between the results of individual groups was very good. The actual variation, however, was greater than the statistical analysis would appear to indicate.

There is no doubt that the present exercise served a useful purpose in enhancing the comparability of future results in the bacteriological monitoring of coastal recreational and shellfish-growing waters within the

framework of MED POL. The only problems were : (a) it was not possible to find samples with marginal pollution levels (i.e. where even slight variations in readings could influence legal and/or administrative action) and (b) in a number of cases, the facilities available in the home laboratories of participants were not identical with those provided during the exercise. This last problem accentuates the need for further standardization between laboratories to ensure that at least the minimum common requirements are met.

Appendix

Statistical analysis of microbial concentrations in [illegible] samples

Method: MF

Type of water sample: A

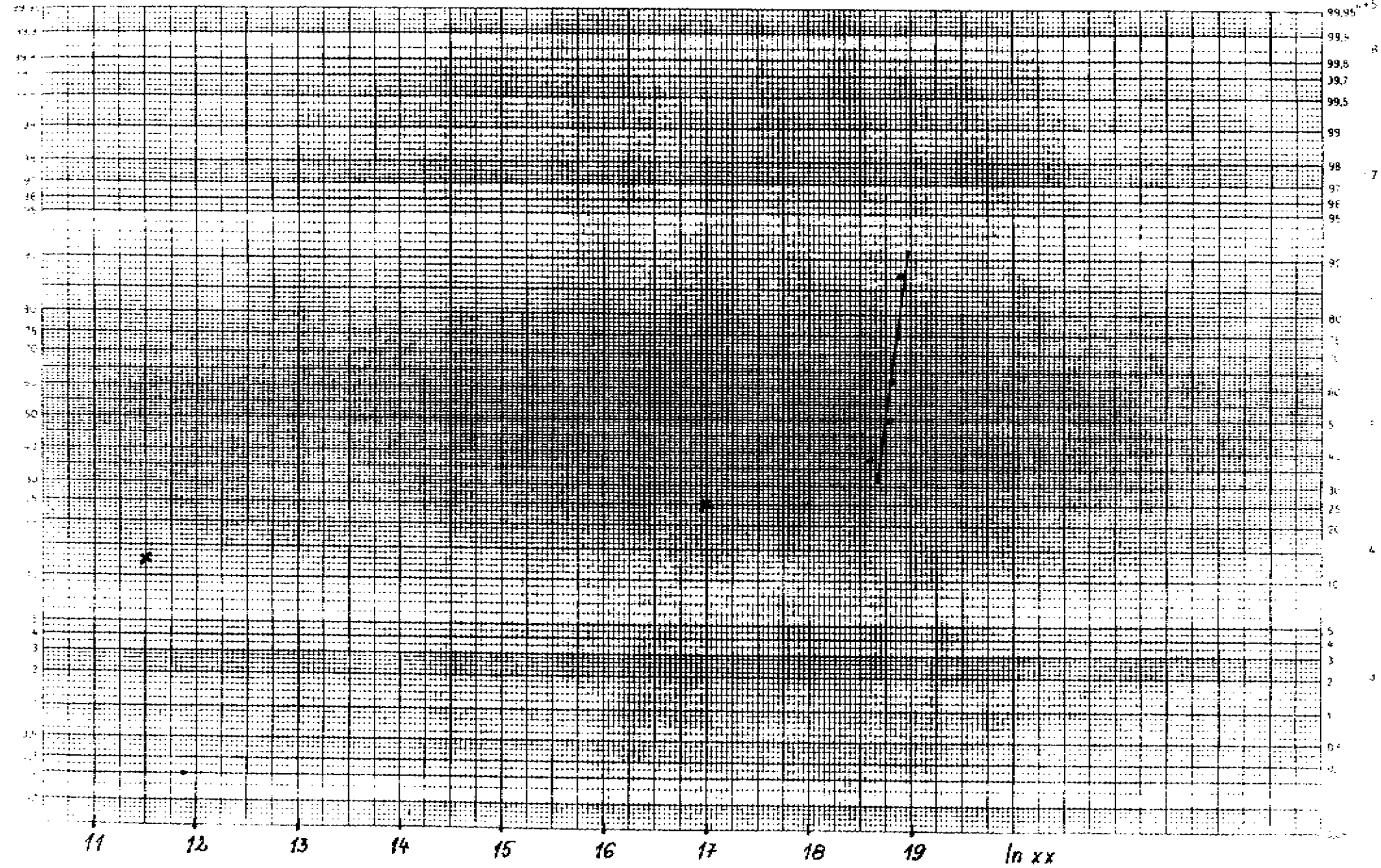
Working group	Microbial concentration per 100 ml		
	Total coliforms	Faecal coliforms	Faecal streptococci
1	-	$256 \cdot 10^5$	$308 \cdot 10^5$
2	-	$1 \cdot 10^5$	$448 \cdot 10^5$
3	-	$156 \cdot 10^6$	$272 \cdot 10^5$
4	-	$163 \cdot 10^6$	$251 \cdot 10^5$
5	-	$131 \cdot 10^6$	$256 \cdot 10^5$
6	-	$143 \cdot 10^6$	$288 \cdot 10^5$
7	-	$148 \cdot 10^6$	$286 \cdot 10^5$
8	-	-	-
9	-	-	-
10	-	-	-

Culture medium (agar)	m-Endo	m-FC	m-Enterococcus
Number of identical samples		7	7
Concentration interval, in colonies per 100 ml		$1 \cdot 10^5$ $163 \cdot 10^6$	$251 \cdot 10^5$ $448 \cdot 10^5$
Mean concentration, in colonies per 100 ml		$141 \cdot 10^6$	$282 \cdot 10^5$
Mean concentration, in natural logarithms		18.765	17.155
Standard deviation, in natural logarithms		0.105	0.110
95% confidence interval of microbial concentrations		$98 \cdot 10^6$ $157 \cdot 10^6$	$226 \cdot 10^5$ $351 \cdot 10^5$
95% confidence interval of median microbial concentrations		$128 \cdot 10^6$ $155 \cdot 10^6$	$255 \cdot 10^5$ $312 \cdot 10^5$

Fig. 1. $FC50 = 141 \cdot 10^6 FC/100\,ml$ x points rejected (See fig. 2.)

$FC90 = 166 \cdot 10^6 FC/100\,ml$

$S = 0.105$



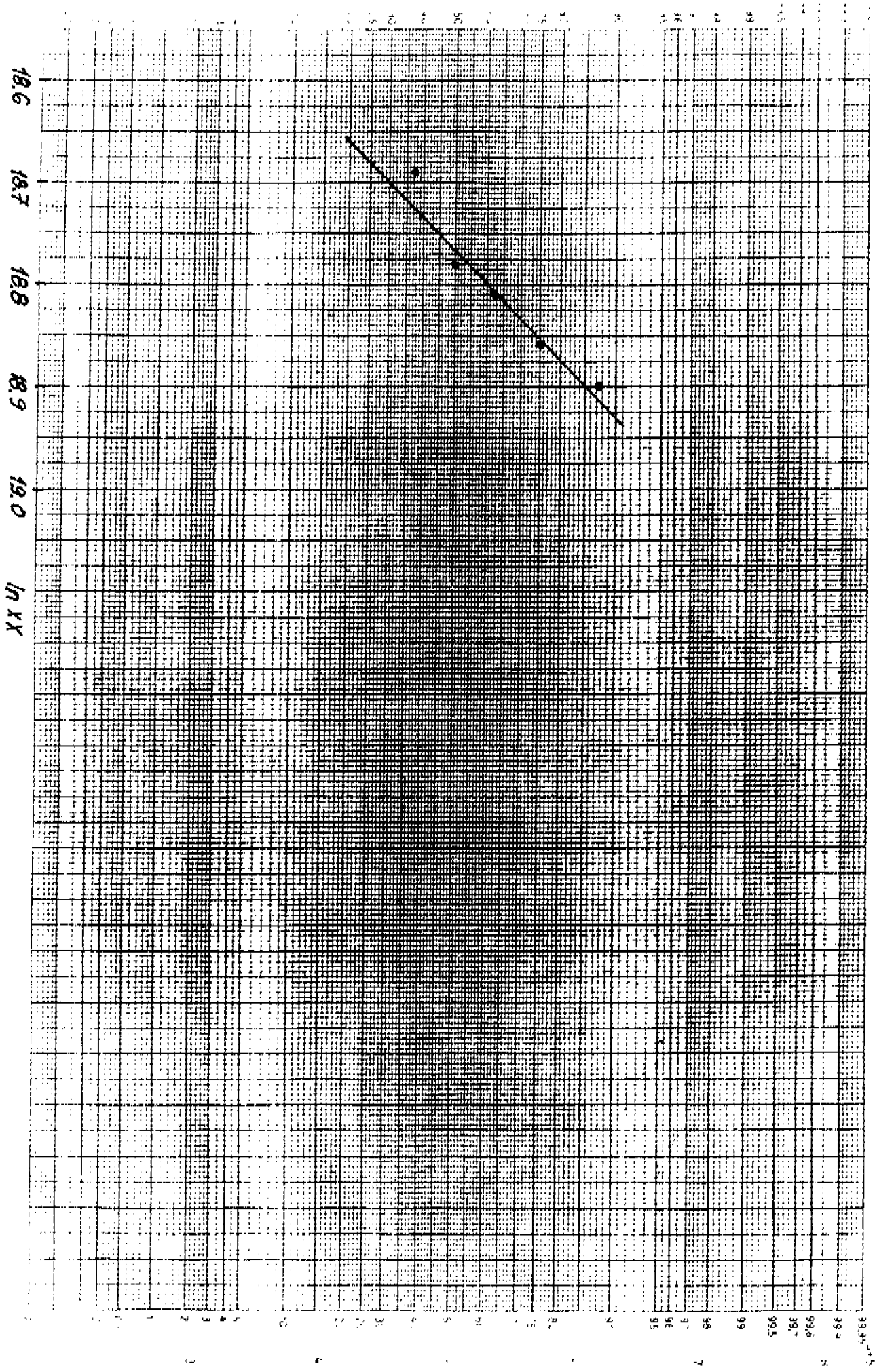


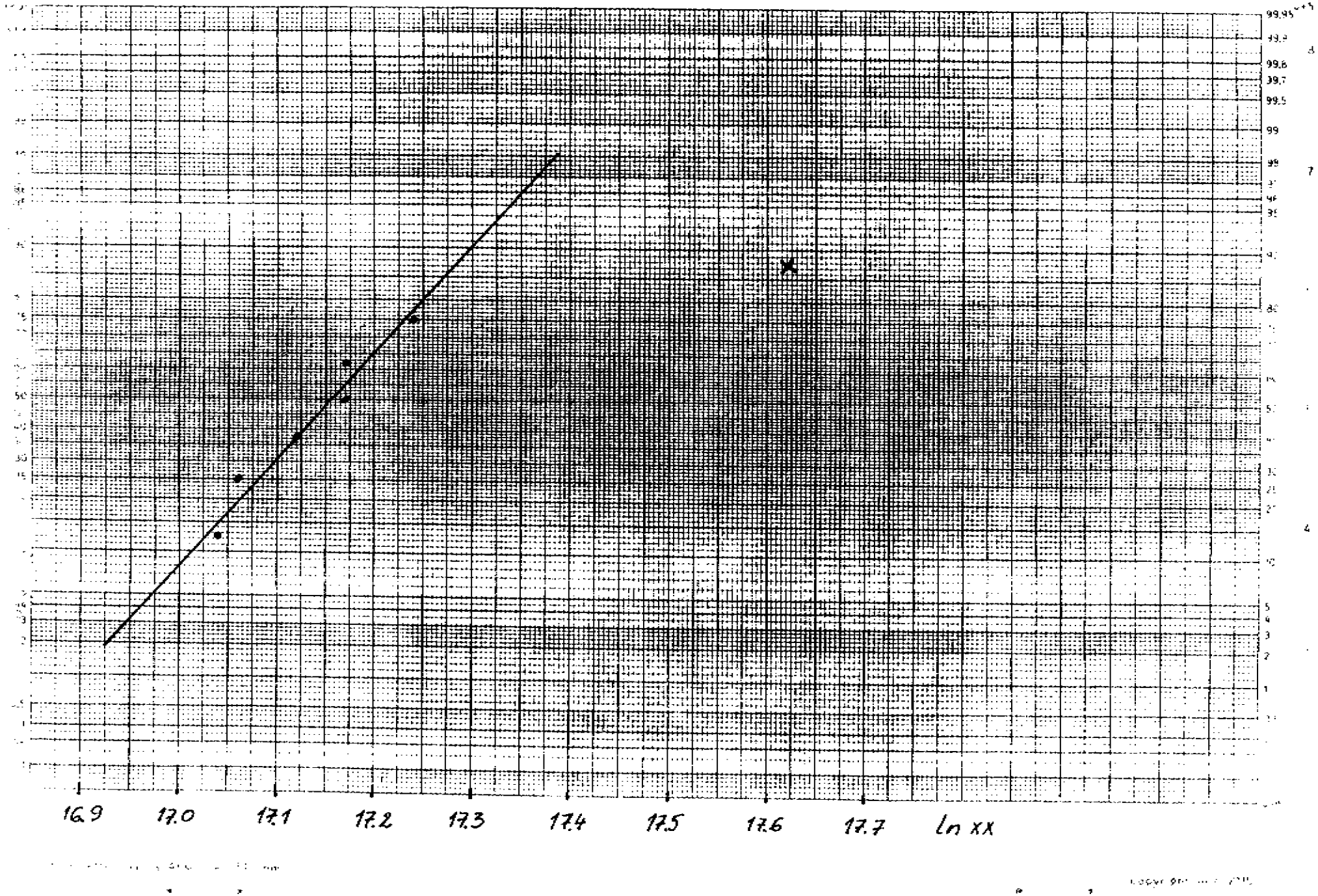
Fig. 2.

FS 50 = 282 · 10^5 FS/100 ml

FS 90 = 326 10^5 FS/100 ml

S = 0.110

x points rejected



Statistical analysis of microbial concentrations in coastal water samples

Method: MF

Type of water sample: B

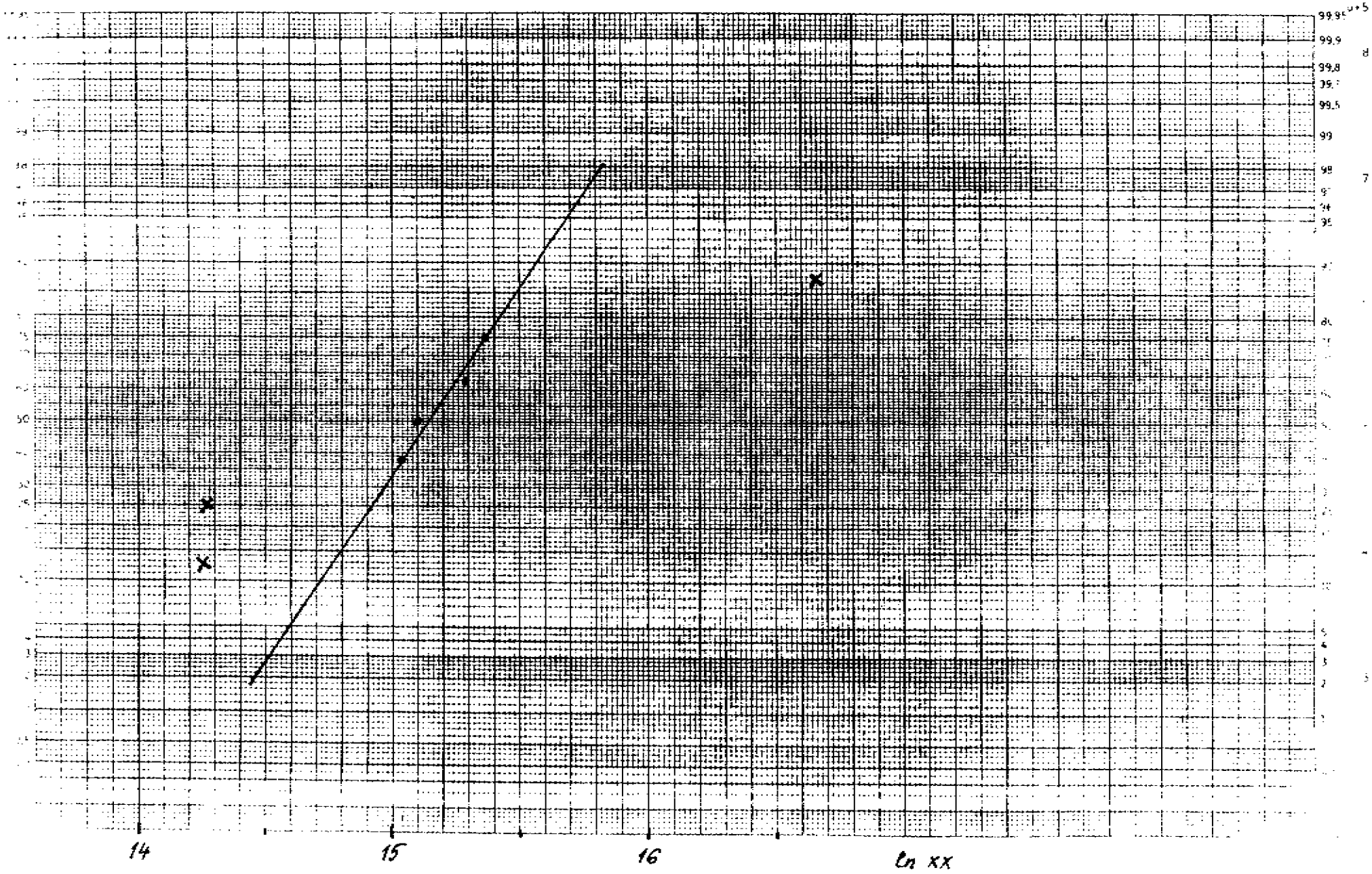
Working group	Microbial concentration per 100 ml		
	Total coliforms	Faecal coliforms	Faecal streptococci
1	$156 \cdot 10^4$	$132 \cdot 10^4$	$283 \cdot 10^3$
2	$360 \cdot 10^4$	$330 \cdot 10^4$	$490 \cdot 10^3$
3	$438 \cdot 10^4$	$338 \cdot 10^4$	$460 \cdot 10^3$
4	$171 \cdot 10^5$	$127 \cdot 10^5$	$820 \cdot 10^3$
5	$154 \cdot 10^4$	$7 \cdot 10^4$	$640 \cdot 10^3$
6	$474 \cdot 10^4$	$456 \cdot 10^4$	$820 \cdot 10^3$
7	$342 \cdot 10^4$	$292 \cdot 10^4$	$560 \cdot 10^3$
8	-	-	-
9	-	-	-
10	-	-	-
Culture medium (agar)	m-Endo	m-FC	m-Enteroccocu
Number of identical samples	7	7	7
Concentration interval, in colonies per 100 ml	$154 \cdot 10^4$ – $171 \cdot 10^5$	$7 \cdot 10^4$ – $127 \cdot 10^5$	$283 \cdot 10^3$ – $820 \cdot 10^3$
Mean concentration, in colonies per 100 ml	$376 \cdot 10^4$	$311 \cdot 10^4$	$551 \cdot 10^3$
Mean concentration, in natural logarithms	15.14	14.95	13.22
Standard deviation, in natural logarithms	0.33	0.85	0.44
95% confidence interval of microbial concentrations	$194 \cdot 10^4$ – $727 \cdot 10^4$	$381 \cdot 10^3$ – $254 \cdot 10^5$	$238 \cdot 10^3$ – $242 \cdot 10^4$
95% confidence interval of median microbial concentrations	$277 \cdot 10^4$ – $510 \cdot 10^4$	$141 \cdot 10^4$ – $682 \cdot 10^4$	$367 \cdot 10^3$ – $828 \cdot 10^3$

$TC\ 50 = 376 \cdot 10^4\ TC/100\ ml$

$TC\ 90 = 578 \cdot 10^4\ TC/100\ ml$

$S = 0.33$

x points rejected

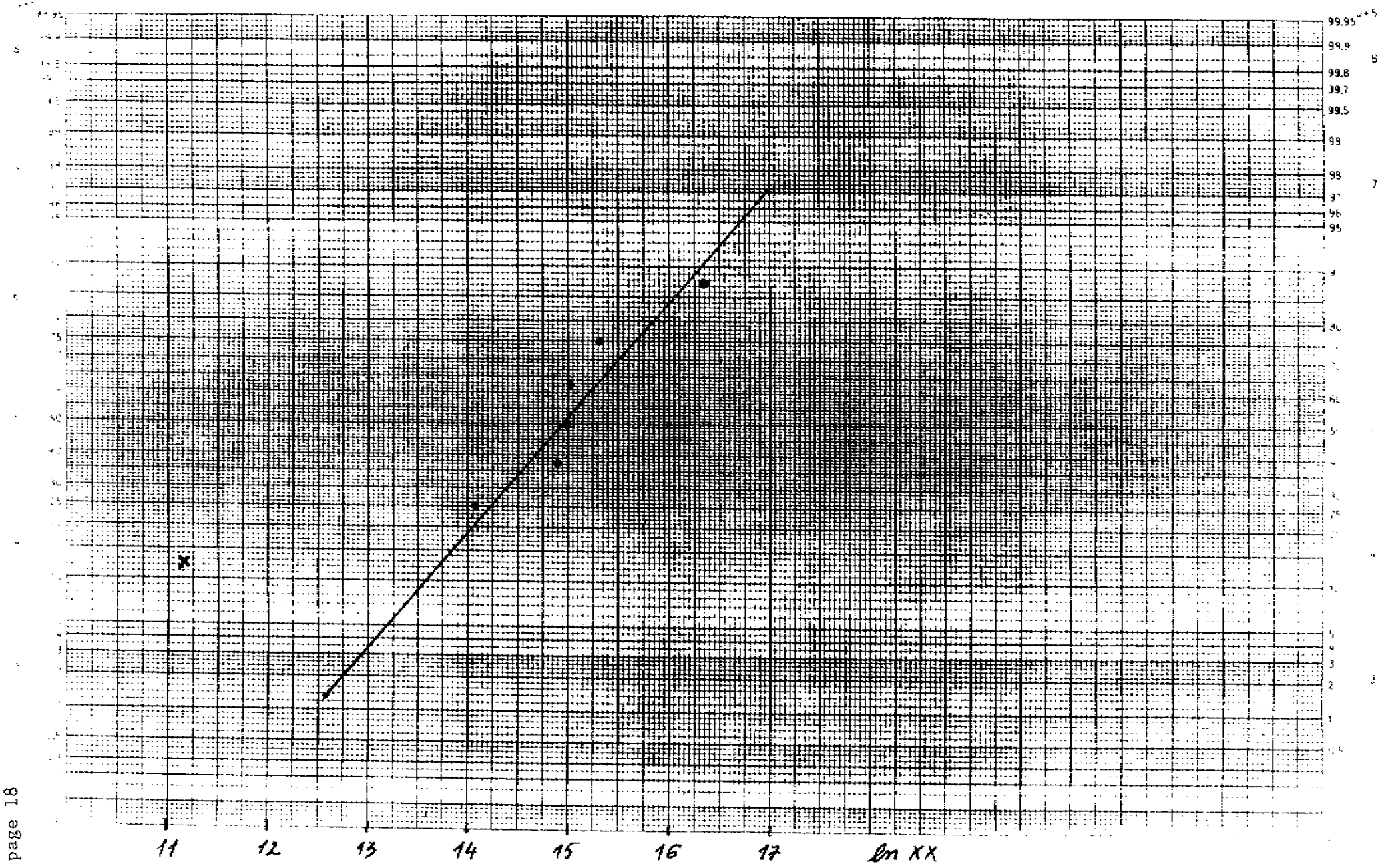


FC 50 = 311 · 10^4 FC/100 ml

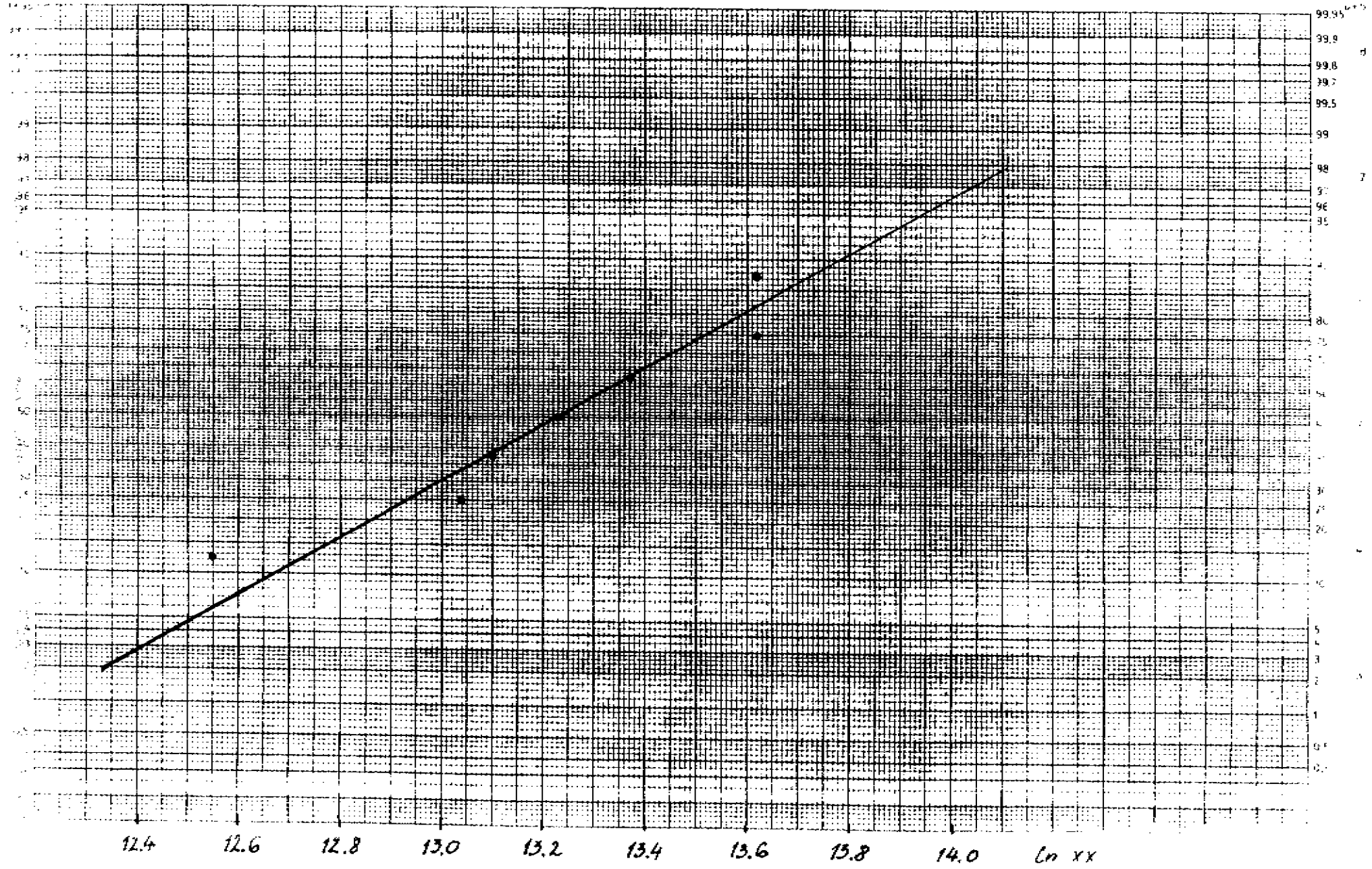
FC 90 = 120 · 10^5 FC/100 ml

S = 0.850

x points rejected



FS50 = 551 · 10^2 FS/100 ml
FS90 = 965 · 10^3 FS 100 ml
S = 0.44



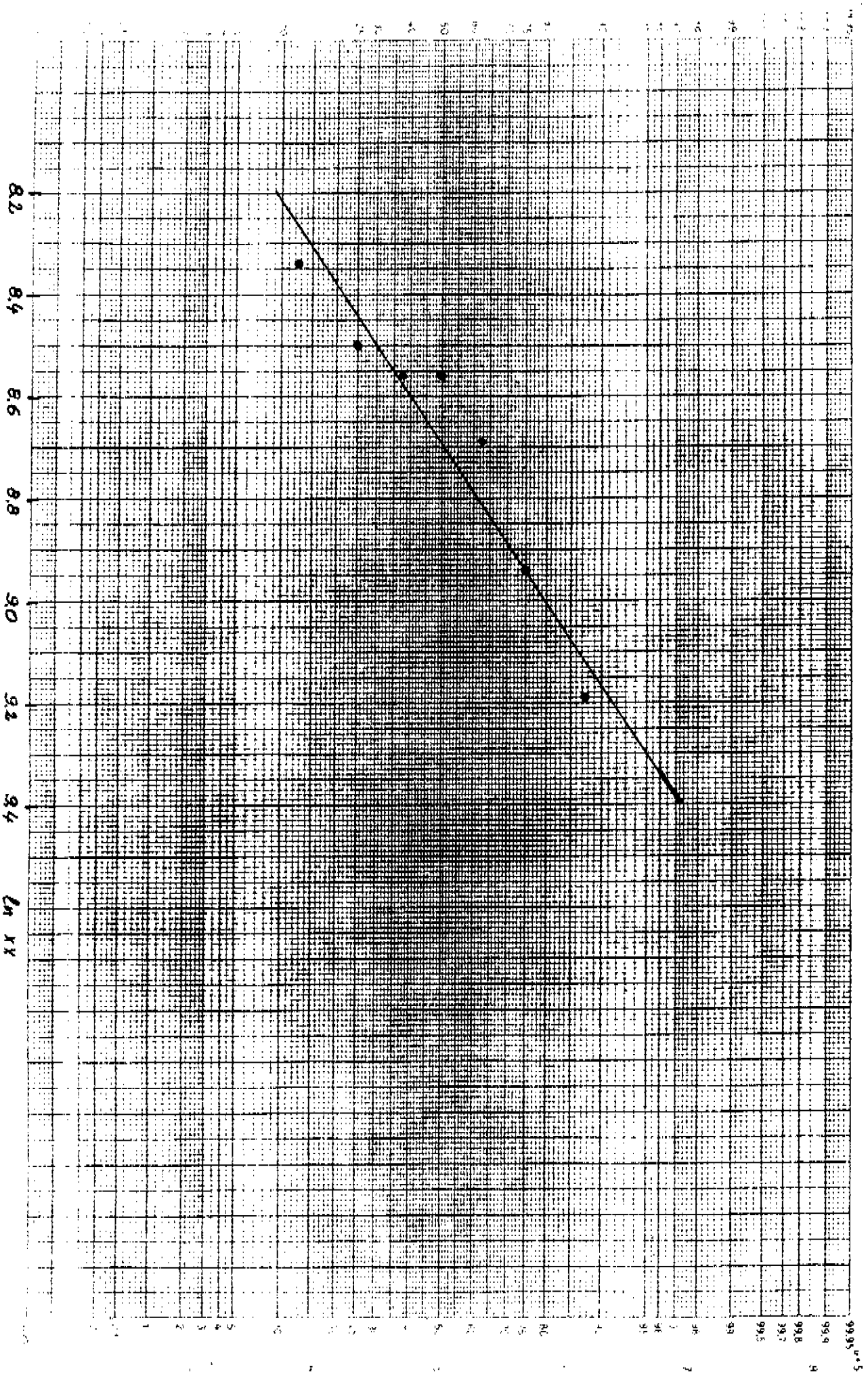
Statistical analysis of microbial concentrations in coastal water samples

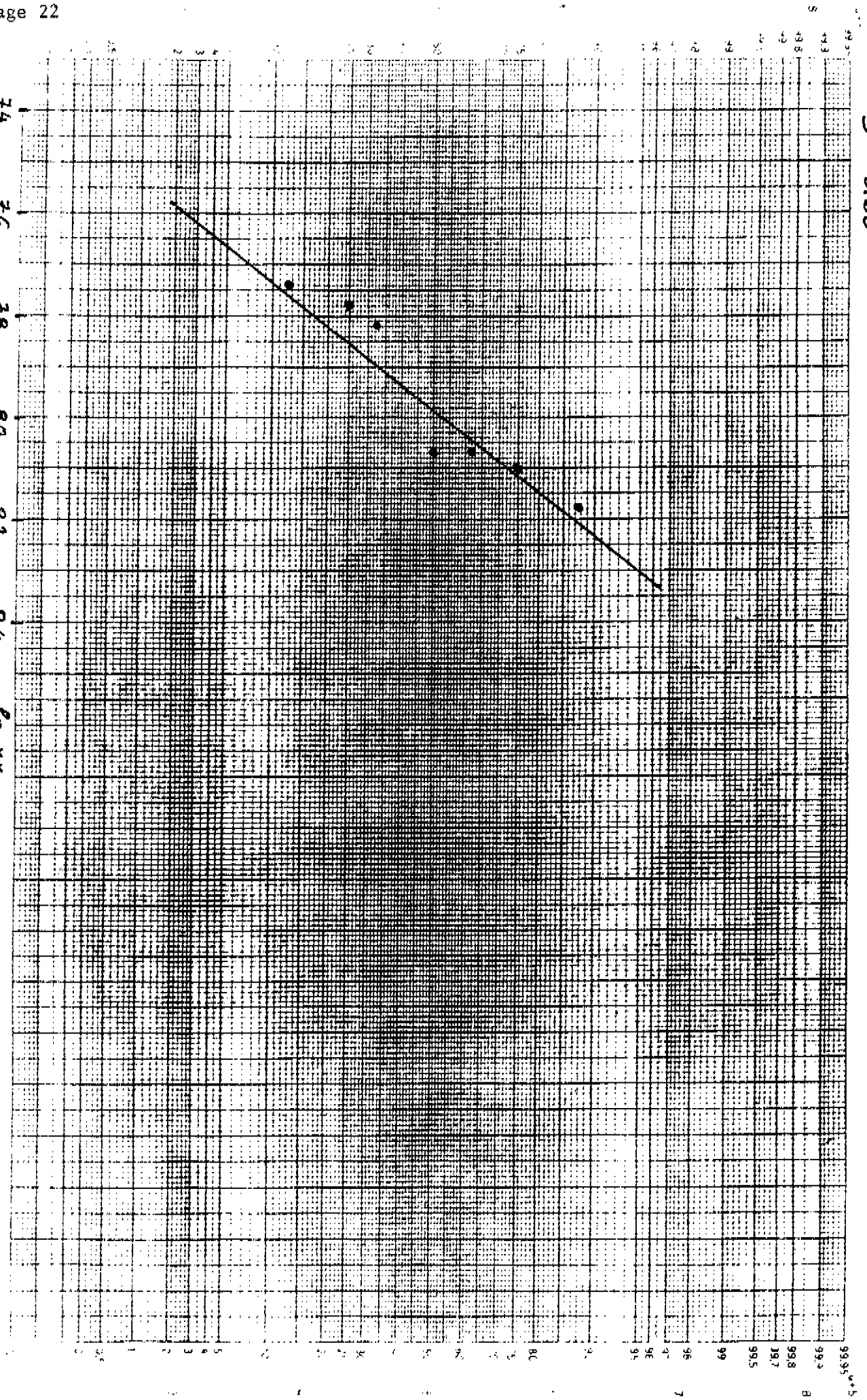
Method: MF

Type of water sample: C

Working group	Microbial concentration per 100 ml		
	Total coliforms	Faecal coliforms	Faecal streptococci
1	5200	3200	2125
2	4900	3575	1600
3	7650	3300	2100
4	4200	3200	1340
5	9800	2500	1600
6	5200	2300	1800
7	5950	2400	2200
8	-	-	-
9	-	-	-
10	-	-	-

Culture medium (agar)	m-Endo	m-FC	m-Enteroccocus
Number of identical samples	7	7	7
Concentration interval, in colonies per 100 ml	4200 9800	2300 3575	1340 2200
Mean concentration, in colonies per 100 ml	5943	2922	1844
Mean concentration, in natural logarithms	8.69	7.98	7.52
Standard deviation, in natural logarithms	0.37	0.20	0.22
95% confidence interval of microbial concentrations	2864 12456	1998 4316	1189 2864
95% confidence interval of median microbial concentrations	4220 8366	2428 3516	1505 2260

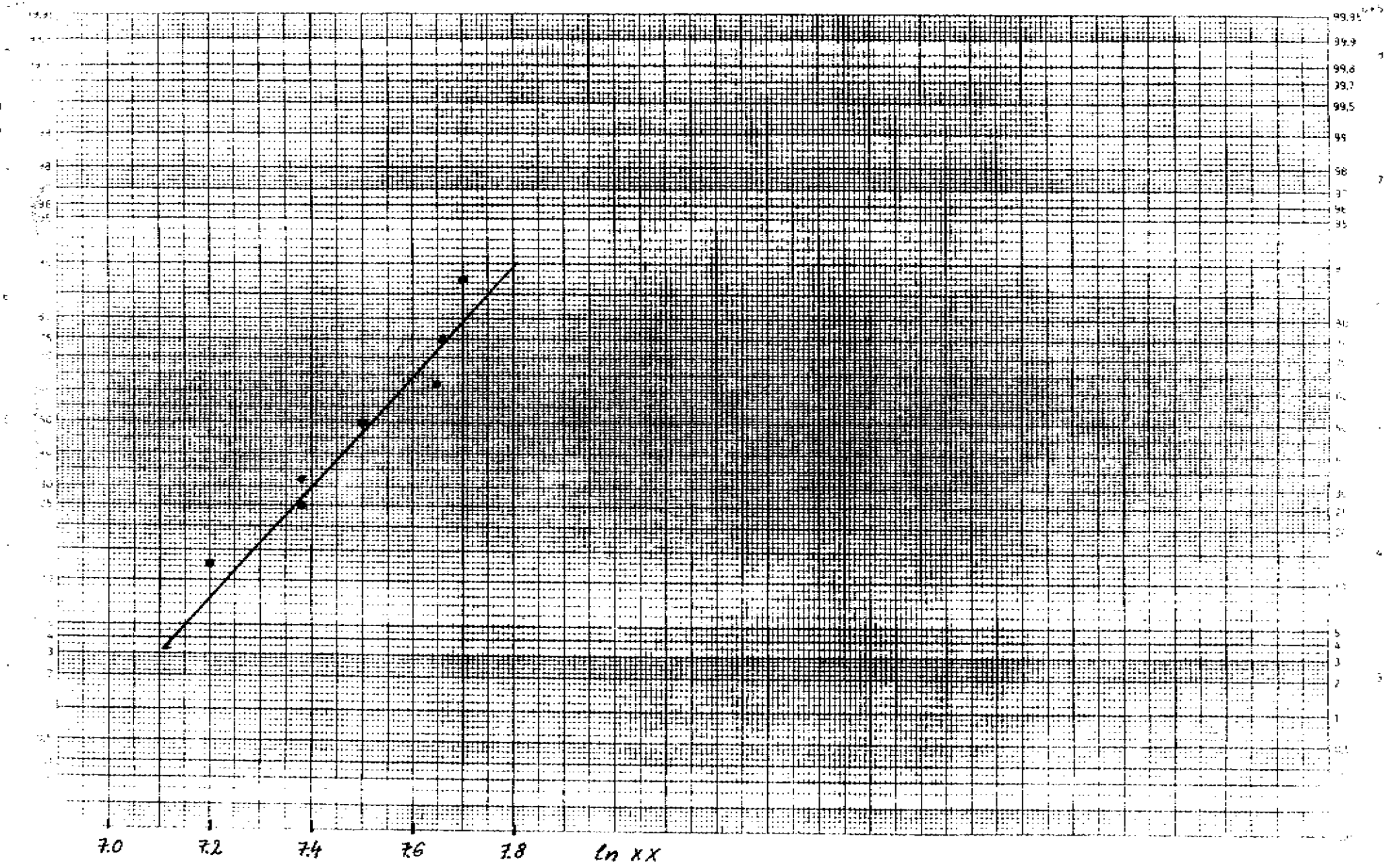

TC 50 = 5943 TC/100 ml
TC 90 = 9604 TC/100 ml
S = 0.37
8.2
8.4
8.6
8.8
9.0
9.2
9.4
ln xx


FC 50 = 1912 FC/100 ml
FC 90 = 3752 FC/100 ml
S = 0.20
7.4
7.6
7.8
8.0
8.2
8.4
Ph xx

FS 50 = 1844 FS/100 ml

FS 90 = 2440 FS/100 ml

S = 0.22



Annex 2

I N T E R C A L I B R A T I O N E X E R C I S E

AND CONSULTATION MEETING ON MICROBIOLOGICAL
METHODS FOR COASTAL WATER QUALITY MONITORING
WHO/UNEP JOINT PROJECT
MED POL Phase II

Instructions to the participants

Institute for Oceanography and Fisheries

Split, 15 - 20 April 1985

I N T E R C A L I B R A T I O N E X E R C I S E

AND CONSULTATION MEETING ON MICROBIOLOGICAL METHODS FOR COASTAL WATER QUALITY MONITORINF

WHO/UNEP JOINT PROJECT

MED POL Phase II

1. Working groups

The participant will be devided into eight groups of two persons each.
Each group will be marked by Roman numerals I through VIII
The participants will be marked by the numbers 1 and 2.
The composition of each group must be unchanged troughout the exercise.

2. Samples

Each working group will receive identical samples of three types of water and shellfish from a restricted area:

- sample A of highly polluted water
- sample B of moderately polluted water
- sample C of slightly polluted water
- sample D shellfish from a restricted area

3. Metodology

seawater - total coliforms (MF)
- faecal coliforms (MF and MPN)
- faecal streptococci (MF)

shellfish- faecal coliforms (MPN)

4. Laboratory materials

Each working group will be issued the following items:

4.1. All necessary materials for membrane filtration
- three sterile funnels
- petri plates of 15 cm diameter 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 (20 and 10 ml)
- membrane filters
- flask for rinsing with sterile buffer solution
- all additional materials required for the analyses

4.2. All necessary materials for the multiple tube method

- tubes with medium
- sterile pipettes (1 and 10 ml)
- flask with Kovac's reagent
- Pasteur's pipettes
- all additional materials required for the analyses

5. Preparation of the dilution series

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

Before taking aliquots from the original sample or from the dilutions these must be vigorously shaken in order to guarantee that representative aliquots are taken.

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 flask should be immedeately identified by type of water, e.g. A, and the degree of dilution, e.g. 10^{-3}.

6. Identification of petri plates and tubes

The petri plates and tubes should be marked:

- with number of group (from I to VIII)
- with number of participant (1 or 2)
- with sumple number and dilution (e.g. A, 2o ml, 10^{-1})

7. Instructions for filtration

Filtration should be started with the higest dilution in order to avoid contamination from samples containing higher concentrations of bacteria.

Different pipette for each sample should be used.
All dilutions of a given sample should be filtered using the same funnel.

8. Presentation of results

The results should be entered in appropriate forms.

Form 1. MF : total coliform
faecal coliform
faecal streptococci

Form 2. MPN: faecal coliforms in seawater

From 3. MPN: faecal coliforms in shellfish

Calculate results using Table 1. (for seawater) and Table 2. (for shellfish),

For further information see UNEP/WHO Reference Methods for Marine Pollution Studies (enclosed to your materials).

FORM 1

MEMBRANE FILTRATION METHOD

Working group No. _____ Date: ___ Aprile 1985

Members of group: _____

_____

Type of water sample: _____

Results of microbiological analyses

Quantity of water filtered ml	Dilution	Number of colonies per membrane		
		Total coliforms	Faecal coliforms	Faecal streptococci
50	1 : 1			
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			

Microbial concentration,
in colonies per 100 ml

Comments:

FORM 2

MULTIPLE TEST TUBE METHOD
FAECAL COLIFORMS

Working group No. _____ Date: ____ April 1985

Members of group _____

_____

Type of water sample: _____

Aliquots transferred ml	Faecal coliform test number of positive reactions					
	Lactose 36°C		MacConkey 44.5°C		Indole test 44.5°C	
	24h	48h	24h	48h	24h	48h
10						
1						
0,1						
0,01						
0,001						

Test results (see table 1)

_____ MPN in MacConkey broth and parallel test for indole

_____ faecal coliforms/100 ml of sea-water

_____ 95 % confidence limits

Comments:

FORM 3

SHELLFISH

MULTIPLE TEST TUBE METHOD

FAECAL COLIFORMS

Working group No. _____ Date: ____ April 1985

Members of group _____

_____

Faecal coliform test

Aliquots transferred ml	number of positive reactions					
	Lactose 36°C		MacConkey 44.5°C		Indole test 44.5°C	
	24h	48h	24h	48h	24h	48h
10						
1						
0,1						
0,01						
0,001						

Test results (see table 2)

_____ MPN in MacConkey broth and parallel test for indole

_____ faecal coliforms/g shellfish flesh

_____ 95 % confidence limits

Comments:

Annex 3

LIST OF PARTICIPANTS

TEMPORARY ADVISERS

Ms Svjetlana Andreis
Institute of Public Health, Zagreb, Yugoslavia

Mr Jean Belian
National Council for Scientific Research, Centre for Marine Research, Jounieh, Lebanon

Dr Abdul Fattah Boargob
National Academy for Scientific Research, Tripoli, Socialist People's Libyan Arab Jamahiriya

Ms V. Buttignoni
Health Protection Institute, Pula, Yugoslavia

Mr B. Cihangir
Dokuz Eylul University, Institute of Marine Sciences and Technology, Izmir, Turkey

Ms Fatma Gungor
Dokuz Eylul University, Institute of Marine Sciences and Technology, Izmir, Turkey

Dr D. Hrsak
Rudjer Boskovic Institute, Centre for Marine Research, Zagreb, Yugoslavia

Dr N. Krstulovic
Institute of Oceanography and Fisheries, Split, Yugoslavia

Ms R. Marusic
Faculty of Civil Engineering, University of Zagreb, Zagreb, Yugoslavia

Mr G. Papageorgiou
State General Laboratory, Nicosia, Cyprus

Dr N. Platzner
Ministry of Health, District Public Health Office, Beer-Sheeva, Israel

Ms K. Margeta
Public Health Institute, Rijeka, Yugoslavia

Mr M. Solic
Institute of Oceanography and Fisheries, Split, Yugoslavia

Dr N. Stambuk
Institute of Public Health, Split, Yugoslavia

Mr B. Stjepcevic
Marine Biology Institute, Kotor, Yugoslavia

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

* Also representing UNEP MED Unit, Athens