WORLD HEALTH ORGANIZATION
REGIONAL OFFICE FOR EUROPE
COPENHAGEN

EUR/ICP/EHAZ 94.01/WS01(S)
03255
Original: English
EUR/HFA target 20
21 March 1995

MICROBIOLOGICAL METHODOLOGY FOR MARINE POLLUTION MONITORING

(WHO/UNEP Joint Project, MED POL Phase II)

Summary Report on First National Training Course and Intercalibration Exercise

Athens, 21–26 November 1994

ABSTRACT

Under the sponsorship of WHO and the United Nations Environment Programme, a course on microbiological methodology was organized by the Department of Microbiology of the National School of Public Health in Athens. Eleven microbiologists from nine laboratories in Greece attended, as well as two participants from Croatia and Morocco who will be organizing similar courses in their own countries in 1995. Participants carried out microbiological tests for the detection of bacterial indicators of faecal pollution and some pathogens. The results indicated that standard errors were much lower for MF (membrane filtration) estimates than for MPN (most probable number), but there was no reason to suppose that results differed between methods. The results also showed significantly higher "between-observer" than "within-observer" variation only for *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

TARGET 20

WATER QUALITY

By the year 2000, all people should have access to adequate supplies of safe drinking-water, and the pollution of groundwater sources, rivers, lakes and seas should no longer pose a threat to health.

Keywords

MICROBIOLOGICAL TECHNICS
SEAWATER – standards
WATER POLLUTION – analysis
EDUCATION

Introduction

Under the terms of the 1976 Convention for the Protection of the Mediterranean Sea against Pollution and the 1980 Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources, the contracting parties have undertaken to establish a marine pollution monitoring system for the Mediterranean Sea area and to systematically assess, as far as possible, the levels of pollution along their coasts.

To facilitate the achievement of these objectives, the Long-Term Programme of Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase II) has been a decisive factor in the development of national pollution monitoring programmes, which include the determination of bacterial concentrations in coastal recreational areas and, where applicable, shellfish growing areas, in Mediterranean countries. As a result, several existing microbiological laboratories have been upgraded and new ones established. This has resulted in the need for more trained personnel, as well as for harmonization of methodologies between the different institutions in the various countries to ensure comparable results and to enable regular region-wide assessments to be performed.

From 1994 the organization of training courses in microbiological methods for marine pollution monitoring at overall regional level was discontinued; instead, it was decided to give emphasis to national training courses. Three courses have been planned for the 1994–1995 biennium.

This course and exercise, the first in the series, was organized by the Greek National School of Public Health, in collaboration with WHO and the United Nations Environment Programme (UNEP). The objectives were:

- to further train microbiological laboratory personnel at national level from Mediterranean institutions participating in the MED POL Phase II programme, by familiarizing them with jointly agreed methods for determination of the main bacterial parameters in seawater and shellfish;
- to promote contacts between scientists from different laboratories through discussion of mutual problems in the application of the relevant microbiological techniques;
- to improve comparability of results obtained in the microbiological component of the MED POL programme through intercalibration of data;
- to make recommendations for future training courses.

Methods

The laboratory section of the course included microbiological tests for the detection of bacterial indicators of faecal pollution and some pathogens. The samples were prepared with natural seawater sprinkled with standard bacterial strains. The most probable number (MPN) method and the membrane filtration (MF) method were applied as follows:

(a) MPN method

- total coliforms, faecal coliforms, *E.coli*: two culture broths (minerals modified glutamate medium, lauryl tryptose broth);
- *Staphylococcus aureus:* (m-*Staphylococcus* broth (modified), VJ agar);

(b) MF method

- total coliforms, faecal coliforms, *E.coli*: (m-lauryl sulfate agar, m-FC agar);
- enterococci: (Slanetz and Bartley agar);
- *Pseudomonas aeruginosa: (Pseudomonas* agar);
- *Staphylococcus aureus:* (4-S agar);
- *Campylobacter jejuni:* (Preston broth, Skirrow agar);
- *Salmonella:* (standard preenrichment-enrichment-isolation procedure).

Filtrations for the isolation of total coliforms, faecal coliforms, *Pseudomonas aeruginosa* and enterococci were performed in duplicate. A statistical analysis of data of the quantitative measurements (coliforms, *Pseudomonas aeruginosa, Staphylococcus aureus* and enterococci) was carried out.

Results

Aspects of the data examined in detail include:

- comparison between MPN results employing different data;
- comparison between MF results using different filter;
- comparison between MPN and MF result;
- estimation of within-observer and between-observer variation.

Summary statistics on measurements of microbial densities are shown in Table 1, which described the bacterial densities in the samples provided for the laboratory exercise (see Annex 1 for tables). It can be observed that standard errors were much lower for MF estimates than for MPN. This reflects the fact that MF determinations were carried out in duplicate. The results of comparisons between media, filters and methods are shown in Table 2. In all cases it was clear that there was no reason to suppose that results differed between methods. Table 3 presents dispersion between duplicate measurements per observer. It appears that the discrepancy was unacceptably large in only two cases. Table 4 shows the results of the ANOVAs, indicating that the results of the present

laboratory exercise showed significantly higher between-observer than within-observer variation only for *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The same table shows the repeatability of the counts.

Recommendations

On the last day of the exercise, the participants filled out a questionnaire with comments and recommendations. They were all highly satisfied with both the level of organization and the technical and scientific knowledge they had acquired during the week.

For future trials they recommended other parameters, such as bacteriophages and enteric viruses. They pointed out the lack of a Greek national methodology for the microbiological analysis of seawater and asked the Department to make an effort to rectify this situation.

Annex 1

RESULTS OF LABORATORY WORK

Table 1. Summary statistics on measurements of microbial densities: geometric mean, 95% confidence interval for mean and range of individual measurements

Method and medium/Filter	No.	Geometric mean	95% confidence interval	Range
Total coliforms				
MPN/GL	11	1442	785 – 2651	286 – 4622
MPN/LTB	11	1388	710 – 2715	427 – 10 990
MF	11	1396	1234 – 1579	1000 – 2050
Faecal coliforms				
MPN/GL	11	955	460 – 1982	147 – 4622
MPN/LTB	11	1150	542 – 2437	147 – 4622
MF/FC	11	1186	967 – 1454	600 – 1850
MF/LSA	11	1255	1103 – 1429	840 – 1800
E. coli				
MPN/GL	11	955	460 – 1982	147 – 4622
MPN/LTB	11	1013	446 – 2297	147 – 4622
MF/FC	9	1256	1103 – 1429	1100 – 1850
MF/LSA	10	1280	1142 – 1439	1000 – 1800
Enterococci				
MF	11	800	663 – 967	500 – 1400
P. aeruginosa				
MF	10	3000	2379 – 3783	1650 – 4400
S. aureus				
MPN	11	21	8.5 – 50	0 – 240
MF	9	38	10 – 143	3 – 550

Table 2. Results of comparisons between media, filters and methods

Microorganism	Comparison between	Test statistic	Level of significance
Total coliforms	MPN media	Wilcoxon	0.89
	MPN and MF	Friedman	0.98
Faecal coliforms	MPN media	Wilcoxon	0.24
	MF filters	Wilcoxon	0.48
	MPN and MF	Friedman	0.66
E. coli	MPN media	Wilcoxon	0.74
	MF filters	Wilcoxon	0.68
	MPN and MF	Friedman	0.80
S. aureus	MPN and MF	Wilcoxon	0.31

Table 3. Value of T_1 test for dispersion between duplicate measurements per observer

Observer	Total coliforms	Faecal coliforms		*E. coli*		Enterococci	*P. aeruginosa*	*S. aureus*
		FC	LSA	FC	LSA			
1	0.00	0.06	0.05	N/A	N/A	0 23	0.00	0.00
2	0.15	0.33	5.26	0.33	5.26	1.21	7.36	3.20
3	0.36	0.31	1.00	0.31	1.00	0.89	0.53	3.00
4	2.61	2.91	0.00	2.91	0.00	0.00	1.64	0.15
5	2.61	0.44	4.00	0.44	4.00	3.61	37.35	0.00
6	8.80	2.46	0.36	2.46	0.36	0.20	0.24	0.25
7	0.53	0.39	1.81	0.39	0.39	0.00	1.17	0.33
8	0.57	1.32	1.38	1.32	1.38	0.53	1.25	2.86
9	2.46	0.04	0.05	N/A	N/A	1.80	1.28	2.00
10	0.53	0.39	1 96	0.39	1 96	0.11	0.03	0.44
Total	18.82	8.65	15.87	8.55	14.35	8.58	13.50	12.23
P	0.043	0.57	0.10	0.38	0.07	0.57	0.14	0.27

Notes: 1. Duplicate measurements not made by observer 11.
2. Total for *P aeruginosa* excludes observer 5.

Bonferroni tests for individual observers:

P. aeruginosa $X^2_1 = 37.35$, $P < 0.00001$

$X^2_1 = 7.36$, $P = 0.007 \times 9 = 0.063$

Total coliforms: $X^2_1 = 8.86$, $P = 0.003 \times 10 = 0.03$

Table 4. Comparison of within- and between-observer variation; ANOVA results and estimated repeatability

Microorganism	Filter	Dilution	No. of observers	P in ANOVA	Repeatability
Total coliforms		1:100	10	0.87	15.8
Faecal coliforms	FC	1:100	8	0.19	10.0
	LSA	1:100	9	0.92	13.6
E. coli	FC	1:100	6	0.51	10.7
	LSA	1:100	8	0.91	13.7
Enterococci		1:100	6	0.10	6.5
		1:10	5	0.17	26.3
P.aeruginosa		1:100	9	0.019	18.5
S. aureus		1:10	10	< 0.0001	10.8

Note: P = level of statistical significance for between- versus within-observer variability.