REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME OCTOBER 1995

Guidelines for monitoring the quality of coastal recreational and shellfish areas

Reference Methods For Marine Pollution Studies No. 1 (Rev.2)

Prepared in co-operation with

WHO

UNEP 1995

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PREFACE

The Regional Seas Programme was initiated by UNEP in 1974. Since then the Governing Council of UNEP has repeatedly endorsed a regional approach to the control of marine pollution and the management of marine and coastal resources, and has requested the development of regional action plans. The Regional Seas Programme at present includes 12 regions and has over 140 coastal states participating in it (1), (2).

One of the basic components of the action plans sponsored by UNEP in the framework of the Regional Seas Programme is the assessment of the state of the marine environment and of its resources, and of the sources and trends of the pollution, and the impact of pollution on human health, marine ecosystems and amenities. In order to assist those participating in this activity, and to ensure that the data obtained through this assessment can be compared on a world-wide basis and thus contribute to the Global Environment Monitoring System (GEMS) of UNEP, a set of Reference Methods and Guidelines for marine pollution studies is being developed as part of a programme of comprehensive technical support which includes the provision of expert advice, reference methods and materials, training and data quality assurance (3). The methods are recommended to be adopted by Governments participating in the Regional Seas Programme.

The methods and guidelines are prepared by, or in cooperation with, the relevant specialized bodies of the United Nations system as well as other organizations, and are tested by a number of experts competent in the field relevant to the methods described.

In the description of the methods and guidelines the style used by the International Organization for Standardization (ISO) is followed as closely as possible.

The methods and guidelines, as published in UNEP's series of Reference Methods for Marine Pollution Studies, are not considered as final. They are planned to be periodically revised taking into account the development of our understanding of the problems, of analytical instrumentation and the actual need of the users. In order to facilitate these revisions, the users are invited to convey their comments and suggestions to:

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which is responsible for the development and preparation of microbiological and other health-related Reference Methods.

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(1) UNEP: Achievements and planned development of the UNEP's Regional Seas Programme and comparable programmes sponsored by other bodies. UNEP Regional Seas Reports and Studies No. 1, UNEP, 1982.

(2) P. HULM: A strategy for the Seas. The Regional Seas Programme: Past and Future, UNEP 1983.

(3) UNEP/IAEA/IOC: Reference Methods and Materials: A Programme for comprehensive support for regional and global marine pollution assessments. UNEP, 1990.

This revised issue of Reference Methods for Marine Pollution Studies No. 1 was prepared by the World Health Organization (WHO) within the framework of the Long-term Programme for Pollution Monitoring and Research in the Mediterranean Sea (MEDPOL Phase II). These Guidelines are designed as an introduction to those UNEP/WHO Reference Methods dealing with microbiological and related aspects of coastal water quality. Although originally produced with the Mediterranean environment in view, in which region they have been extensively applied by a large number of scientists, their application is not limited to this particular region. Their global application is in fact contemplated throughout the Regional Seas Programme. The assistance of all those who contributed to the preparation of the guidelines is gratefully acknowledged.

CONTENTS

1. INTRODUCTION

The overall objectives of the Long-term Programme of Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase II), which constitutes the environmental assessment component of the Mediterranean Action Plan, adopted by the governments of the region in 1975, include the assessment, on a continuing basis, of the state of pollution of the Mediterranean Sea, the identification of the sources, pathways and effects of pollutants entering into it, and the establishment of temporal trends of pollution levels.

Apart from this general objective of assessing the state of pollution at overall regional level, another, perhaps more important, objective of the MED POL Phase II Programme is to enhance the capabilities of national institutions in the region, particularly those in developing countries, to participate to the fullest extent possible in marine pollution monitoring and research within the framework of national monitoring programmes adapted to specific national and local situations and requirements. Activities necessary for the attainment of this objective include, apart from the basic necessities of laboratory upgrading and manpower development, the formulation of common methodology and the implementation of quality assurance and control programmes to ensure both reliability and comparability of data obtained.

In order to achieve the aim of data comparability, a set of reference methods and guidelines has been developed through the combined efforts of UNEP's Regional Seas Programme and the major UN Specialized Agencies (mainly FAO, WHO, IOC, WMO and IAEA) as well as other organizations wherever appropriate. In these methods and guidelines, the style used by the International Organization for Standardization (ISO) is followed as closely as possible. Responsibility for preparation and updating of microbiological and related methods and guidelines has been entrusted to the World Health Organization (WHO) by virtue of its mandate in the field of international health.

The original version of this part of the present guidelines and its first revision were prepared by WHO within this general framework in 1983 and 1988 respectively. Both were issued by UNEP as No.1 and No.1/Rev.1 in its series of Reference Methods for Marine Pollution Studies. The present version, which will also be issued separately as No.1/Rev.2 within the same series, has been substantially modified to comply with recommendations made during consultation meetings organized by WHO within the framework of the MED POL programme between 1989 and 1993.

2. SCOPE AND FIELD OF APPLICATION

These general guidelines constituting Part I of this publication are designed for use in the planning, design and implementation of programmes for monitoring the quality of coastal marine recreational and shellfish areas in connection with any actual or potential health hazard arising from microbiological or related pollution of such areas. They can be used either with the aim of conducting baseline studies on the levels of pollution, if any, prevailing in any particular coastal area likely to be affected by sewage or other discharges as a tool for the development and implementation of remedial action at source, or for the design and implementation of routine programmes to ensure whether or not pollution levels conform with established criteria and standards.

Although primarily designed for application to temperate and subtropical seas and, more specifically, to conditions prevailing in the Mediterranean region, these guidelines may be broadly applied to seas with other environmental conditions.

The scope of the monitoring programmes detailed in these guidelines is mainly concerned with microbiological, as distinct from chemical, pollution of coastal recreational and shellfish areas.

3. DEFINITIONS

3.1 **Coastal Recreational Areas** can be defined as areas of the coastline used for bathing or other recreational activities bringing the users into direct or indirect contact with the water and/or the sand on the beaches. They can also include areas where the water is used for therapeutic purposes.

3.2 **Shellfish Areas** can be defined as coastal waters used for culturing shellfish (aquaculture areas) or for harvesting natural shellfish populations.

3.3 **A Health Effect Water Quality Indicator** can be defined as a microbiological, chemical or physical agent, substance or quality which indexes the potential risk of infectious disease coincident with man's use of the aquatic environment for recreational purposes or food production. Theoretically, the best indicators would be those showing the highest degree of correlation with associated health effects. Such a correlation should be based on properly-designed and conducted epidemiological studies. In the design of such studies, selection of potential indicators should be based on the following requirements:

3.3.1 Indicators should be consistently and exclusively associated with the source of pathogens and, occasionally, noxious substances;

3.3.2 Indicators should be present in sufficient numbers or quantities without proliferation or somatic/genetic changes, so as to provide a reasonable estimation of the presence of pathogens and the actual or potential existence of health risks;

3.3.3 Indicators should approach the degree of resistance to disinfectants and environmental stress (including that resulting from toxic materials deposited into the marine environment) shown by the most resistant pathogen potentially present at significant levels in the pollution source;

3.3.4 Indicators should be quantifiable in environmental samples by reasonably easy and relatively inexpensive methods, and with considerable accuracy, precision and specificity.

3.4 **A Water Quality Criterion** can be defined as a quantifiable exposure-effect relationship between the density of the indicator in the water (or other matrix) concerned and the potential risks to human health involved in the use of such

water. Above all, it is a set of facts or a relationship on which a judgement can be based.

3.5 **A Water Quality Standard** is derived from the criterion, and is an accepted maximum level for the density of the indicator in the water associated with acceptable health risks (i.e. levels above such maximum would be considered as presenting unacceptable health risks). The concept of acceptability implies that in addition to medical factors, which remain the principal ones, social, cultural, economic and political factors are also taken into account, and that all of these may vary both spatially and temporally.

3.6 **A Pollution Source** can be defined as a river or effluent discharging in the coastal zone, or any other anthropogenic activity (including dumping of wastes or other material) capable of affecting the quality of any specific coastal area.

4. PROGRAMME RATIONALE

4.1 Health Risks from Polluted Recreational and Shellfish Waters

The main channels of human exposure to pollutants discharged into coastal waters are:

4.1.1 Through contact with, or ingestion of, microbiologically contaminated water while swimming, bathing, or indulging in any other aquatic recreational activity, including scuba diving, windsurfing, water-skiing, etc;

4.1.2 Through contact with microbiologically contaminated sand on beaches;

4.1.3 Through consumption of chemically or microbiologically contaminated seafood;

The monitoring of chemically-contaminated seafood is beyond the scope of this document.

Potentially, all the diseases which are spread by the faecal-oral route and whose aetiological agents are shed in the faeces of all infected individuals or carriers could be contracted by bathing in sewage-polluted waters or by the consumption of shellfish grown in, or harvested from such waters. These diseases can be bacterial, viral, or caused by a variety of protozoan or metazoan parasites. Insofar as the actual ingestion of water during swimming or bathing is necessarily limited, with the exception of pathogens with a relatively low infective dose, most diseases can be contracted more easily through the consumption of raw or partially-cooked shellfish. There are other pathogens whose native habitat is the sea which are also capable of causing human infections through this route.

Apart from diseases affecting the gastro-intestinal tract, a number of diseases and disorders affecting the eye, ear, skin and upper respiratory tract have also been

associated with bathing. These may cause infection as a result of being forced into breaks or tears in the skin, or into ruptures in delicate membranes in the ear or nose resulting from the trauma associated with diving into water. Contraction of such diseases or disorders may not necessarily, however, depend on the quality of bathing waters themselves, as either the bather may have been carrying the microorganism(s) in question beforehand, or infection can spread from one individual to another in high bather density beaches.

Microorganisms which are actual or potential pathogens of man and can be transmitted through bathing in polluted (and sometimes non-polluted) waters, or through consumption of contaminated seafood include bacteria, viruses, fungi and a variety of protozoan and metazoan parasites.

4.2 Pathogenic Bacteria

The main bacterial pathogens present in the Mediterranean include *Salmonella*, *Shigella*, *Vibrio*, *Staphylococcus*, *Pseudomonas*, *Clostridium, Campylobacter*, *Yersinia*, *Aeromonas*, enteropathogenic *Escherichia coli*, and a number of streptococci.

Salmonella species, which are the agents of typhoid and paratyphoid fevers, food poisoning and gastroenteritis, have a worldwide distribution and are abundantly represented in the Mediterranean. While major attention has been paid to *S. typhi*, *S. paratyphi* A and *S. paratyphi* B, a large number of other serotypes have been isolated in the region. Salmonellas do not survive long in seawater, and infection directly as a result of bathing or other recreational activities is not very likely due to the relatively high infective dose required in the case of most serotypes. On the other hand, the infective dose for *S. typhi* and *S. paratyphi* A and B is considerable lower. Consumption of raw or partially-cooked seafood is a different problem, as the bacteria are concentrated either by filter-feeding shellfish or on fish gills. In shellfish, the concentration may be 50 times that in the surrounding water. With consumption of raw seafood, such as oysters, this route obviously assumes importance, and attention should therefore be directed at *Salmonella* in the monitoring of shellfish waters.

Shigella species, which are the agents of bacillary dysentery, are also, like *Salmonella*, widely distributed throughout the world. Cases reported from the Mediterranean region call for a more comprehensive assessment of the situation than has hitherto been undertaken, and statistics on the occurrence of *Shigella* species in sewage should be the first step taken, followed by correlation of this with cases of dysentery. A number of reports attribute a relatively short survival time in the marine environment to *Shigella*. Other reports state that no major differences in survival time appear to exist between *Shigella flexneri* on the one hand, and *Salmonella typhi*, *S. wien* and *Escherichia coli* on the other.

Cholera is one of the major diseases associated with the consumption of sewage-contaminated shellfish, and the causative agent, *Vibrio cholerae*, is widespread in the Mediterranean, where a number of cholera outbreaks have been recorded. NAG (non-agglutinable) vibrios, which cause gastroenteritis, are also frequently found in shellfish in the region. A large number of *Vibrio* serotypes have been identified in effluent, seawater and mussel samples, the main ones of sanitary interest being *V. fluvialis*, *V. cholerae* (non-01) and *V. metschnikovii*.

Two other *Vibrio* species widespread in the Mediterranean are natural to the marine environment, and no correlation exists between their presence and pollution of the sea by sewage. In the case of *V. parahaemolyticus*, the main cause of infection is again through shellfish, although wound infection by contact with seawater is another route of transmission. *V. alginolyticus* causes otitis, sore throat and wound infections. It occurs in coastal marine areas and its main route of transmission to man is through contact with seawater and sediments.

Staphylococcus aureus and related species (particularly *S. epidermidis*) are potential pathogens associated with skin, skin glands and mucous membranes of warm-blooded animals, including man. They are found in swimming pools and natural bathing waters, and coagulase-positive strains cause a wide range of infections and intoxications, including boils, abscesses, furunculosis, pyaemia, osteomyelitis, otitis, suppuration of wounds and food poisoning. *S. aureus* is salt-tolerant, and can survive in the marine environment. Ear infections due to *S. aureus*, as well as others affecting the skin and naso- pharyngeal tract, are suspected of being transmitted by bathing water after being shed by bathers. Although *S. aureus* is linked with food-poisoning in general, mention of its transmission through shellfish is relatively sparse.

Pseudomonas aeruginosa can be recovered from about 10% of normal human stools, and is consequently frequently found in sewage, where concentrations may reach 10^6 per 100 ml. It causes ear and eye infections, and wound, burn and urinary tract infections, as well as enteritis, Like *Staphylococcus aureus*, it is prevalent in the Mediterranean. The route of transmission was originally considered to be infected swimming pools, but the organism is now becoming increasingly implicated in ear, throat and skin infection through bathing in contaminated seawater. Numerous cases of folliculitis, dermatitis, ear and urinary tract infections due to *P. aeruginosa* acquired through bathing in contaminated water have also been reported.

Clostridium perfringens is discharged in significant amounts in sewage, where it is mainly of human origin. It is more resistant than other indicators, but its detection in seawater is difficult. It does not multiply in sediments, but survives longer in the marine environment than *E. coli*, and the two organisms can therefore be considered as complementary. As there are significant differences in *C. perfringens* counts near to and far from sewage discharge points, interpretation of the counts appears to be feasible, The use of this species as a monitoring parameter, however, is still a matter for discussion.

Campylobacter has recently been recognized as an important bacterial pathogen of man, *C. jejuni* and *C. coli* causing diarrhoea and fever. They can both be transmitted through seafood consumption or ingestion of water. *C. jejuni* and, to a lesser extent, *C. faecalis*, have been isolated from sewage outfalls and polluted seawater during the summer months in the Mediterranean. Considerable study on these organisms is still required.

Yersinia has been recognized as an important type of bacterium in terms of epidemiology and zoonoses. It has been demonstrated as present in seawater, as well as in other media, and pigs and rodents are considered to be the main natural reservoirs. Its status can be considered to be similar to that of *Shigella* in that more information is required, the examination of sewage being the necessary initial step.

Aeromonas hydrophila causes septicaemia in immunosuppressed hosts, diarrhoea, pneumonia, abscesses and wound infections. It can be transmitted through contact with, or ingestion of, seawater, or through consumption of contaminated seafood.

Other bacterial pathogens or potential pathogens recorded in the Mediterranean capable of causing varying degrees of gastroenteritis through consumption of contaminated shellfish or, to a lesser extent, ingestion of polluted seawater while bathing include enteropathogenic *Escherichia coli*. Members of Lancefield's Group D of streptococci (*Streptococcus faecalis*, *S. faecium*, *S. bovis* and *S. equinum*) have also been considered responsible for outbreaks of food-borne diseases associated with mainly non-marine sources.

4.3 Viruses

More than 120 different virus types are known to be excreted in human faeces by infected persons, whether or not they manifest illness. These viruses belong to various groups, including enteroviruses (polioviruses, coxsackieviruses, echoviruses and hepatitis A virus), reoviruses, adenoviruses, and parvoviruses (adeno- associated viruses). The frequency of isolation and quantity of virus recovered from sewage depends not only on the infections caused by normally- occurring viruses and those induced by oral poliovirus vaccine, but also on the efficiency of the recovery procedures. The serotypes that can be detected at any specific time in sewage except for polioviruses, reflect to a greater or lesser extent those viruses circulating in the community with the highest frequency. However, in countries using the Sabin vaccine to immunize against poliomyelitis, it is expected that all three strains of polioviruses will be present in urban sewage, and it has been suggested that they may constitute an adequate indicator of the virological quality of water. Enteroviruses have been recorded in sewage effluents and/or in seawater in many parts of the Mediterranean. In more than one Mediterranean country, polioviruses are detected in every sewage sample tested.

As even a single plaque-forming unit (PFU) or (as alternatively termed) cytopathogenic unit (CPU) of virus may lead to infection when swallowed, the presence of human viruses in seawater has to be taken seriously, and the danger of infection as a result of bathing in polluted waters is therefore not imaginary. Although epidemiological studies have not, so far, shown any clear correlation between swimming in polluted waters and viral epidemics, sporadic cases of infection cannot be ruled out. Most research work performed in the Mediterranean on viruses in sewage effluents or seawater has been qualitative as distinct from quantitative.

The role of shellfish as vectors in human enteric virus disease is well documented. Viruses which have been shown epidemiologically to be transmitted by shellfish are Hepatitis A, non-A, non-B hepatitis, Norwalk, Snow Mount agent, astroviruses, coxsackie virus and small round viruses. Of these, Hepatitis A and Norwalk viruses appear to be of chief concern to public health officials. There are a number of reports worldwide of gastrointestinal disease due to eating shellfish for which no causative agent has been identified, and many of these cases were believed to involve and unidentifiable viral agent, rather than a bacterial pathogen.

It should be noted that clinical diagnosis of virus diseases depends on isolation of the virus and a positive seroreaction. The relative lack of availability of the necessary specialized diagnostic facilities on a routine basis in many Mediterranean countries indicates that the extent of virus disease, particularly on an individual case basis, is still largely unknown.

4.4 Pathogenic Fungi

A number of fungal species are pathogenic to man, causing superficial, sub-cutaneous or deep mycoses according to the eventual location of the pathogen within the host after infection. The most common one associated with infection through contact with beach sand and, to a lesser extent, seawater is *Candida albicans*, a yeast considered responsible for a number of superficial and deep mycoses. A number of other genera are also considered important, again mainly from the point of view of infection via beach sand.

Candida albicans, together with other *Candida* species, has been isolated from a number of sandy beaches in the Mediterranean. Its presence in seawater is currently under investigation in a number of areas. Work has also been performed in the region on the identification of other fungi, and genera isolated so far include *Penicillium*, *Aspergillus* and *Cladosporium*, the latter two of which contain pathogenic species, as well as *Mucor*, *Fusarium* and *Rhizopus* species in seawater. These are opportunistic pathogens, but attention is drawn to *Fusarium*, which is toxinogenic and one of the major causes of eye infections.

4.5 Other Parasites

Relatively little information is available on risks to human health arising from the presence of animal parasites in the marine environment. The eggs of the nematode worms *Ascaris*, *Toxoplasma*, *Oxyuris* and *Trichurus* (all of which are prevalent in the Mediterranean region) are able to survive for months in seawater, and ingestion of a single egg is enough to cause infection. The eggs are discharged in the faeces of infected individuals, and transmission by swimming in polluted water is considered a possibility.

Protozoan parasites of either worldwide distribution or present in the Mediterranean region include *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli* and *Naegleria* species among those present in sewage and constituting a potential health hazard. It is recommended that particular attention be devoted to these, as well to nematode eggs, when monitoring shellfish harvested in the vicinity of sewage outfalls.

4.6 Algal Biotoxins

Blooms of toxic algal species are common occurrences in shellfish areas worldwide, the algal species involved, which produce potent toxins, mainly belonging to the dinoflagellate group. The shellfish accumulate the toxic cells during filter feeding, becoming vectors in various forms of shellfish poisoning. Of all shellfish consumed, mussels probably pose the greatest threat with regard to shellfish poisoning. Diseases include paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP) and diarrhoeic shellfish poisoning (DSP). PSP toxins constitute a well-characterized group of tetrahydropurines, saxitoxin being the first component identified, and are produced

by a well-defined dinoflagellate group, mainly *Gonyaulax* and *Gymnodinium* species, occurring in both tropical and temperate seas, NSP is caused by *Gymnodinium breve*, with symptoms similar to, but milder than, PSP. DSP is caused by a number of toxic components isolated from shellfish associated with human symptoms characterized by diarrhoea, nausea, vomiting and abdominal pain. The algae responsible are considered to be *Dinophysis* and related species. A further form - Amnesic Shellfish Poisoning (ASP), produced by toxins causing abdominal cramps and neurologic responses involving memory loss and disorientation has also been described.

Algal biotoxins appear to be a relatively recent problem in the Mediterranean area. DSP caused by *Dinophysis sacculus* in shellfish was a problem in the northwestern region between 1987 and 1989. DSP has also been reported as widespread in the Adriatic. PSP toxins caused by *Gonyaulax tamarensis*, *Gymnodinium catenatum* and *Alexandrium minutum* have also been recorded in mussels and other shellfish from various parts of the region during the past five years. Blooms of *Gymnodinium breve* (responsible for NSP) have also been recorded in the Northwestern and Eastern Mediterranean.

4.7 Quality Criteria and Standards

Standards are considered to be one of the most effective regulatory instruments to control the quality of recreational and shellfish waters within acceptable limits. From the purely technical aspect, the development of water quality standards represents a relatively complicated issue. As the main objective is to ensure adequate health protection which, in turn, has to be translated into the definition, through the application of selected criteria, of an upper tolerance limit to the degree of pollution by sewage, a satisfactory quality-effect relationship has to be established. In the case of bathing and other recreational waters, apart from selection of the appropriate criteria on which standards would eventually be based, there is also the problem of deciding what would constitute an acceptable (or unacceptable) health risk in terms of large populations with a wide range of exposure patterns and durations, not to mention age and health status variations. The effects of local environmental conditions on the frequency and duration of exposure would also appear to call for different standards to be applied to various geographic regions, although there would certainly be problems in deciding on appropriate lines of demarcation. In the case of shellfish waters, the situation is slightly less complicated, but the health risk arising from pollution is decidedly greater, and even though the main objective is area, rather than produce acceptability (the latter is normally performed as an independent public health exercise after harvest), the fact that concentrations of microorganisms in shellfish are generally many times higher than those in the ambient water body due to continuous accumulation renders it necessary to use the shellfish themselves as the main monitoring matrix for assessing the quality of the area, apart from the water itself.

4.7.1 Bathing water quality criteria and standards

Criteria and standards for bathing water quality in the Mediterranean region differ from country to country. From this point of view, countries can be broadly divided into two types: Member States of the European Community (France, Greece, Italy and Spain) which are bound by, and have their relevant national legislation based upon, the 1976 EEC Directive on the Quality of Bathing Waters, form the first group, along with a number of other countries which, although not EEC Member States, have based their

legislation on the provisions of the EEC Directive. The second group is composed of those other countries whose legislation is either *ad hoc* (i.e. based on the evaluation of local requirements) or, in some cases based either on the recommendations of the 1974 WHO Bilthoven Working Group on guides and criteria for recreational quality of beaches and coastal waters, or on the interim criteria for bathing waters adopted by Mediterranean States in their capacity of contracting parties to the 1976 Barcelona Convention for the protection of the Mediterranean Sea against pollution and its related protocols, adopted at their fourth Ordinary Meeting in Genoa in 1985.

In 1983, and again in 1985, within the framework of the Long-term Programme of Pollution Monitoring and Research in the Mediterranean (MED POL Phase II), WHO and UNEP jointly proposed interim microbiological quality criteria and standards for bathing waters in the region. These proposals are given in Table 1. In October 1985 in Genoa, Mediterranean States accepted only part of these proposals as an interim measure. The criteria adopted are shown in Table 2.

The microbiological standards within the 1976 EEC Bathing Water Directive are reproduced in Table 3, and the physico-chemical ones in Table 4. Conformity with the provisions of the Directive in no ways any Member State from adopting stricter measures, which each country has in fact done, especially with regard to the mandatory or imperative values. In some countries, recreational water is classified into a number of quality categories, ranging from high to just acceptable.

Table 1. Interim quality criteria for Mediterranean bathing waters proposed by WHO and UNEP in 1983.

Parameter	Concentration per 100 ml not to be exceeded 50% of the time	90% of the time	Minimum number of samples	Analytical method	Interpretation method
Faecal coliforms	100	1000	10	Membrane filtration, m-FC broth or agar incubated at 44.5±0.2° C for 24h	Graphical or analytical adjustment to a lognormal probability distribution
Faecal streptococci	100	1000	10	Membrane filtration, KF-streptococcus agar incubated at 36±0.5° C for 48h	

Table 2. Interim quality criteria for bathing waters adopted by Mediterranean states in 1985.

Parameter	Concentration per 100 ml not to be exceeded 50% of the samples	90% of the samples	Minimum number of samples	Analytical method	Interpretation method
Faecal coliforms	100	1000	10	WHO/UNEP Reference Method No. 3, "Determination of Faecal Coliforms in seawater by the Membrane Filtration Culture Method", or WHO/UNEP Reference Method No. 22, "Determination of Faecal Coliforms in seawater by the Multiple Test Tube Method".	Graphical or analytical adjustment to a lognormal probability distribution

Table 3. Microbiological criteria and standards contained in the 1975 EEC Directive on the quality of bathing waters.

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

G = Guide
I = Mandatory
(0) Provision exists for exceeding the limits in the event of exceptional geographical or meteorological conditions.
(1) When a sampling taken in previous years produced results which are appreciably better than those in this Table and when no new factor likely to lower the quality of the water has appeared, the competent authorities may reduce the sampling frequency by a factor of 2.
(2) Concentrations to be checked by the competent authorities when an inspection in the bathing area shows that the substance may be present or that the quality of the water has deteriorated.

Table 4. Physico-chemical criteria and standards contained in the 1975 EEC Directive on the quality of bathing waters.

	Parameters	G	I	Minimum sampling frequency	Method of analysis and inspection
1	pH	-	6 to 9 (0)	(2)	Electrometry with calibration at pH 7 and 9
2	Colour	- -	No abnormal change in colour (0) -	Fortnightly (1) (2)	Visual inspection or photometry with standards on the Pt.Co scale.
3	Mineral oils mg/litre	- ≤0.3	No film visible on the surface of the water and no odour -	Fortnightly (1) (2)	Visual and olfactory inspection or extraction using an adequate volume and weighing the dry residue.
4	Surface-active substances (lauryl-sulfate) reacting with methylene, blue mg/litre	- ≤0.3	No lasting foam -	Fortnightly (1) (2)	Visual inspection or absorption spectro-photometry with methylene blue.
5	Phenols (phenol indices) C_4H_5OH mg/litre	- ≤0.005	No specific odour ≤0.05	Fortnightly (1) (2)	Verification of the absence of specific odour due to phenol or absorption spectrophotometry 4-aminoantipyrine (4 AAP) method.
6	Transparency m	2	1(0)	Fortnightly (1)	Secchi's disc.
7	Dissolved oxygen % saturation O_2	80 to 120	-	(2)	Winkler's method or electrometric method (oxygen meter).
8	Tarry residues and floating materials such as wood, plastic articles, bottles, containers of glass, plastic, rubber or any other substance. Waste or splinters	Absence		Fortnightly (1)	Visual inspection.
9	Ammonia mg/litre NH_4			(3)	Absorption spectrophotometry, Nessler's method, or indophenol blue method.
10	Nitrogen Kjeldahl mg/litre N			(3)	Kjeldahl method.

G = Guide

I = Mandatory

(0) Provision exists for exceeding the limits in the event of exceptional geographical or meteorological conditions.

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

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

(3) These parameters must be checked by the competent authorities when there is a tendency towards the eutrophication of the water.

4.7.2 Shellfish water quality criteria and standards

As is the case with recreational waters, quality criteria and standards for shellfish waters vary from country to country, both globally and within the Mediterranean region. Mediterranean countries which are member states of the European Community are bound by the terms of EEC 1979 Directive on the quality of shellfish waters, which is reproduced in Table 5. However, each state has its own legislation which, in every case, is stricter than the terms of the Directive.

Table 5. Microbiological criteria and standards contained in the 1979 EEC Directive on the quality of shellfish waters.

	Parameter	G	I	Reference methods of analysis	Minimum sampling and measuring frequency
1	Faecal coliforms/100 ml	≤300 in the shellfish flesh and intervalvular liquid(1)		Method of dilution with fermentation in liquid substrates in at least three tubes in three dilutions. Subculturing of the positive tubes on a confirmation medium. Count according to MPN (most probable number). Incubation temperature 44° C ± 0.5° C	Quarterly

G = guide
I = mandatory
(1) However, pending the adoption of a Directive on the protection of consumers of shellfish products, it is essential that this value be observed in waters in which live shellfish directly edible by man.

In 1983 and 1986, WHO and UNEP proposed interim microbiological quality criteria and standards for shellfish waters and shellfish in the Mediterranean. The former are reproduced in Table 6. These were not accepted by Mediterranean states, and an expert consultation jointly convened by WHO and UNEP in 1987 made alternative recommendations which were identical to the 1979 EEC Directive. These were adopted by Mediterranean states on a common basis later the same year.

It should be stressed that although the terms of both the EEC 1979 Directive and (as a result) the joint measures adopted by all Mediterranean states in 1987 utilize examination of shellfish alone (examination of the ambient water is recommended, but is not mandatory), the criteria and standards are limited to the acceptability or otherwise of the area for shellfish growing and/or harvesting. In no way do they determine the acceptability of shellfish for human consumption, this being determined through appropriate sanitary measures in each country.

Table 6. Microbiological quality criteria and standards for Mediterranean shellfish waters and shellfish proposed by WHO and UNEP in 1983.

Faecal coliforms in SHELLFISH-GROWING WATERS			
Concentration of faecal coliforms per 100 ml not to be exceeded 80% 100% of the samples	Minimum sampling frequency	Analytical method	Interpretation method
10 100	In winter monthly; in summer fortnightly	Membrane filtration m-FC broth or agar incubated at 44.5±0.2° C for 24h	Graphical or analytical adjustment to a lognormal probability distribution
Faecal coliforms in SHELLFISH FLESH			
Concentration of faecal coliforms per gram of flesh	Minimum sampling frequency	Analytical method	Interpretation method
2: sale permitted between 3 and 10: temporary prohibition of sale 10 and above: sale prohibited	In winter monthly; In summer fortnightly	Multiple tube fermentation and counting according to MPN. MacConkey broth incubated at 36±0.5°C for 24h and then at 44.5±0.2° C for 24h	By individual results histograms or graphical adjustment of a lognormal probability distribution

5. PROGRAMME DESIGN

5.1 Monitoring Objectives

5.1.1 The objectives of a coastal recreational and shellfish water quality monitoring programme would normally be:

(a) Initially, if no studies have been carried out in the area in question, to carry out a spatial and temporal survey of existing pollution levels, and to try and link such levels with an identifiable pollution source. The results of such a baseline study, which should preferably include an attempt at correlation of the presence and (if possible) density of selected epidemiologically-significant pathogens with indicator organism density, should be utilized to determine any remedial action necessary with regard to the source, as well as to decide on water

quality criteria and standards applicable to the specific area in question.

(b) To control water (and, where applicable, sand or seafood) quality in accordance with established criteria and standards.

(c) To assess the effectiveness of pollution control measures in course of implementation.

(d) To regularly assess the presence and (if possible) density of epidemiologically-significant pathogens in relation to levels of indicator organisms monitored, in order to acquire the data required for any amendments to existing criteria and/or standards to conform to changes in morbidity patterns among local populations.

5.1.2 Water use

A coastal water quality monitoring programme should be initiated only after careful consideration of the general and specific objectives pertinent to the region or area involved. In this regard, three different types of waters may be distinguished on the basis of use:

(a) **Bathing waters.** In such cases, the area to be monitored would have to include beach sand, which in many instances is utilized to the same, or even greater, extent than the water itself. In view of the primary aim of minimizing risks to human health, water and sand monitoring should normally form part of an overall control programme covering the hygienic conditions prevailing in the general beach area in question, including any catering establishments or other relevant amenities present within the beach complex itself.

(b) **Waters used for other recreational purposes.** The recreational use of water has recently extended to activities other than bathing, such as windsurfing, scuba diving, etc. In indicated areas, monitoring programmes should also cover water quality in the locations where such activities are pursued, and sampling points will have to be adjusted accordingly.

(c) **Shellfish waters.** A distinction will have to be made between commercial culture areas (which are generally under varying degrees of control, and from which the product has to pass through statutory food-safety procedures prior to sale and consumption) and areas in which naturally-growing shellfish populations are harvested. In this latter case, the products of commercial harvesting should also be normally subject to food-safety regulations, but control over the aquatic environment in which the shellfish grow is obviously less than that holding good for *ad hoc* culture areas, so that more care and attention is required. In addition, a number of areas exist where shellfish populations are not large enough to attract the attention of commercial harvesters, and where varying numbers of individual shellfish are collected by amateur fishermen and/or other members

of the public for their own consumption. In this last case, it is normally not feasible to monitor all the areas in which such activity could possibly occur, and public health measures normally take the form of official warnings to the public not to eat raw shellfish collected in this manner.

(d) **Reference waters** are areas relatively unaffected by the pollution source, and are used for comparison purposes.

5.1.3 Monitoring areas

The monitoring areas selected should primarily be those which are most frequented by bathers and/or most used for other recreational water activities, and those which are intensively used for harvesting or collection of shellfish and, in each of the above cases, exposed to discharges of wastewaters or other pollution loads.

5.2 Area and Problem Identification

The carrying out of a problem assessment is an essential prerequisite for any monitoring programme, and for the preparations thereof. Prior to establishing the programme, the impact of pollution (both actual and potential) on the various uses of the coastal waters in question should be determined through the acquisition of relevant data (area assessment). The area assessment should include both landward and seaward descriptions of the area, and the data obtained should be noted either on a fact sheet, or on a descriptive map, or on both, depending on circumstances.

5.2.1 Area assessment

From the landward side, the following should be noted, wherever appropriate with regard to the aims and objectives of the programme:

(a) **Land use:** categories of land use within the general area, including use of immediate coastal areas, e.g. industrial, residential, forestry, agricultural, recreational or mixed;

(b) **Run-off:** identification of rivers and streams, including location, flow and individual monthly discharge into the sea, as well as areas where erosion is known to occur;

(c) **Wastewater discharges and outfalls:** outfall sites, beach and offshore, including type, e.g. industrial, domestic or mixed, and total daily flow. Industrial discharges should be specified;

(d) **Waste treatment:** location of treatment plants, capacity in m^3 per day, and degree of treatment;

(e) **Dumping sites:** identification of dumping sites in the vicinity of the beach, indicating whether for solid waste, sewage disposal or both, and giving volume of deposit per year;

(f) **Coastline:** sand, rock, gravel, cliffs. Also, whether shallow or deep water.

From the seaward side, the following should be noted, again wherever appropriate with regard to the aims and objectives of the programme:

(g) **Shellfish areas:** indicate site and type of shellfish. In the case of commercial areas, also indicate tonnage per year;

(h) **Fishing grounds:** indicate type of fish and (if possible) catch;

(i) **Dumping sites:** location, materials, quantity per year;

The following meteorological and oceanographic observations will also have to be made, wherever relevant:

(j) **Winds:** drawing up of seasonal wind roses;

(k) **Precipitation and air temperatures:** annual precipitation in tabular form. the same table to include average monthly air temperatures;

(l) **Currents and tides:** description and seasonal fluctuation of currents, tidal cycles where applicable;

(m) **Salinity and temperature:** from existing studies (the data should be sufficient to provide information on water column stratification and its seasonal variations;

(n) **Depth contours:** from nautical charts;

(o) **Buoys and other navigational aids:** these, as well as any important obstacles such as wrecks or rocks, should be indicated.

Of the above, (b), (c), (d), (e), (f), (i), (l), (m) and (n) should be considered as important items for the interpretation of results obtained from monitoring of coastal recreational waters. (g) and (h) should be added in the case of shellfish waters.

In the case of coastal recreational areas, detailed observations should be carried out on the whole of each beach complex (land and sea), including beach amenities.

5.2.2 Maps

The use of adequate maps and nautical charts is an essential prerequisite for a monitoring programme. The first step to be taken is the drawing up of a detailed map of the areas selected for monitoring. Such maps should incorporate as much as possible of the information collected during the area assessment, in particular:

(a) Sewage outlets and any waste or other discharge points;

(b) inshore and offshore solid waste dumping sites;

(c) local; currents in the coastal waters relative to point sources and beach locations;

(d) meteorological and oceanographic conditions.

If insufficient quantitative information exists, discharge measurements (flow gauging) or current measurements will have to be made prior to, or in conjunction with, the sampling and analytical programme in the area. the importance of coastal currents, their speed, direction and rebound on the coast have to be particularly noted because of the important role they play in the transport of pollutants, especially those found on floating matter which is submissive to the movement of the currents.

The most recent geodetic and nautical maps of the coastal area to be studied should be obtained. The nautical charts will normally be of prime interest. The situation and use of each map wi;; normally define the appropriate scale. A map of practical size could be the European A3 format (approximately 42 x 60 centimetres). Many copying machines allow for direct reduction from A3 to A4, resulting in economic reproduction and presentation of results.

Each map should be clearly identified by location, coordinates, scale and orientation. This must be assured before any copying or reproduction is made. Such identification should include:

(a) **Location:** use the name of a typical town or conspicuous landmark. Always indicate the country;

(b) **Coordinates:** give the approximate latitude and longitude of the location;

(c) **Scale:** this should be graphed, e.g. in divisions of 100 metres or in kilometres, not numerical, as the latter may change with enlargement or reduction;

(d) **Orientation:** indicate N for north, or give lines for latitude and longitude of the main location;

(e) **Date:** give date of preparation of map, if available.

5.2.3 Initial reports

An initial report on area and problem assessment should be made. This should be as concise and factual as possible, and should include the local assessment of problems and goals, maps, fact sheet indications, wind data, graphs and tables, and summaries of previous studies. The kind of map useful in the initial reporting phase is shown in Figure 1.

5.3 General Design

Prior to the actual implementation of the monitoring programme, it is essential to decide on:

(a) the matrices to be monitored;

(b) the parameters to be monitored in each matrix;

(c) the number and location of sampling points;

(d) the frequency of sampling.

The extent of the monitoring programme will depend entirely on already-existing resources and on extra resources which can be made available to meet the required demand. These resources will consist in:

(a) trained manpower for sampling and analysis;

(b) laboratory facilities (apparatus, equipment and materials);

(c) transport facilities.

It should be borne in mind that in practically all cases, the essential minimum is dictated by the provisions of international conventions or other similar legal instruments (such as EEC Directives). In most countries, national legislation provides for coverage over and above this minimum, to conform with local requirements.

5.4 Monitoring Matrices and Parameters

For recreational areas, matrices monitored should be the seawater itself and, wherever appropriate, beach sand. For a complete picture, sediments immediately beneath the water sampling points should also be monitored, though this is not normally a statutory requirement. Within organized beach complexes, the whole area, including its facilities, should be monitored. However, aspects concerning food sold within the area and potable water are normally dealt with by the appropriate authorities as part of Public Health programmes, and are not covered by this document.

For shellfish areas, both the ambient seawater and the shellfish themselves should be monitored. It should be emphasized that such examination of shellfish within the framework of a shellfish area monitoring programme does not normally constitute an evaluation of the shellfish themselves for human consumption (which is an aspect covered by Public Health regulations), but simply an evaluation of the acceptability or otherwise of the area concerned for shellfish culture or harvesting. Monitoring of sediments, though again not normally covered by regulations, is more important here than in the case of recreational areas.

For both recreational and shellfish areas, the basic microbiological parameters in seawater are bacterial indicator organisms and pathogens. In the former, fungal examination of beach sand may also be necessary. In the latter case, monitoring for algal biotoxins would be required whenever circumstances (such as eutrophication phenomena) so dictate.

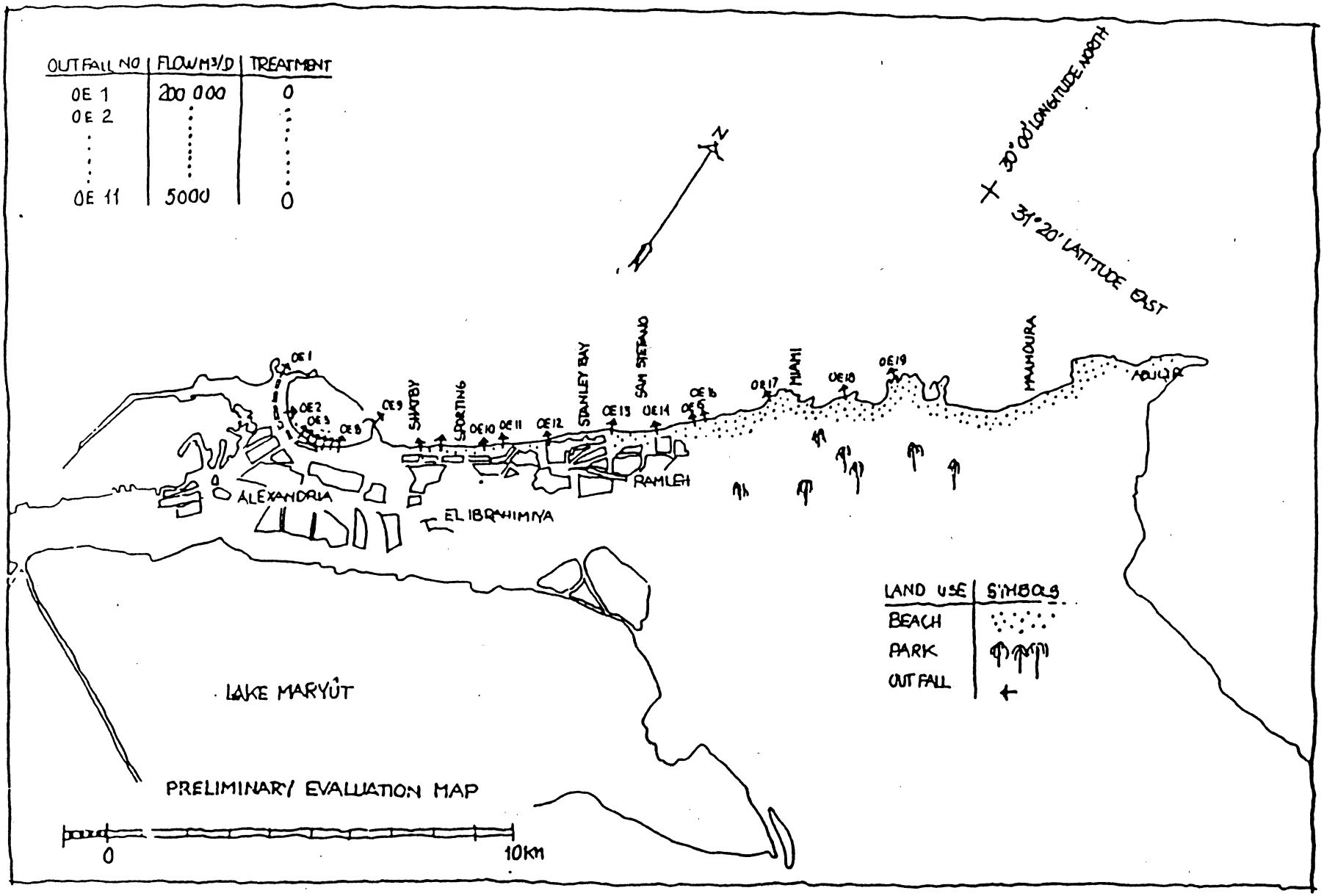

Figure 1. Preliminary evaluation map of monitoring area.

5.4.1 Bacterial indicator organisms

The main parameters used for monitoring the microbiological quality of coastal recreational and shellfish areas are bacterial indicator organisms. The main ones recommended are the following:

(a) **Faecal coliforms:** these, wherever found, will generally indicate recent faecal pollution, as they do not normally propagate in the marine environment. Though good indicators in many respects, their major problem is their relatively short survival time in seawater, which may call for the use of supplementary indicators. *Escherichia coli* is also in common use whenever a more specific indicator within this group is required;

(b) **Faecal streptococci:** species of the Lancefields group D streptococci occurring in human and animal faeces are likely to be found in polluted waters. They include *Streptococcus faecalis*, *S. faecium*, *S. bovis*, and *S. equinum*. The ratio of faecal coliforms to faecal streptococci may vary for different types of polluted waters. There is normally no need for species identification of faecal streptococci in water pollution monitoring. Density ratios in relation to faecal coliforms are generally adequate to assign the probable source of wastewater discharge, whether domestic, farm animal or wildlife.

The value of **total coliforms** as a parameter indicating the sanitary quality of coastal recreational and shellfish waters is doubtful. Methods of determination which are currently available also detect coliforms of non-faecal origin, and consequently do not constitute a specific test indicating pollution by faecal material. Therefore, although the use of total coliforms as indicators of coastal pollution is widespread and traditional, it is hardly justified with regard to modern indicator criteria, and in view of more specific alternatives now available.

5.4.2 Pathogens and algal biotoxins

Apart from bacterial indicator organisms, it would be necessary to monitor the presence and (if possible) density of one or more bacterial pathogens causing diseases of the gastrointestinal tract, e.g. *Salmonella*, if there is reason to believe that any of these are being discharged into the local receiving waters in significant amounts. Similarly, it could be advisable to monitor for at least one enterovirus, though this may be rendered difficult both because of the relatively sophisticated techniques required, and the comparatively high cost. Most microbiological laboratories performing quality analysis of seawater on a routine basis are not equipped for virus determinations.

Several studies have been performed on the correlation between the density of bacterial indicators and the presence (and density) of pathogens in seawater. In every study, bacterial pathogens have been found to occur when concentrations of indicator organisms reach specific levels. Results have been generally constant for each individual area studied. It is not, however, possible to utilize correlations obtained from different areas, as the density of indicator organisms is an index of the degree of sewage pollution, while the presence and density of each specific pathogen in seawater

depends on the proportion of infected individuals within the local population. In addition, the distance between the sewage outlet and the recreational area, and the prevailing hydrodynamic conditions, will influence the pathogen/indicator relationship.

In view of the fact that contact infection may be even more important than gastrointestinal infection through ingestion, at least one appropriate pathogen causing infection through contact should be monitored. The selection of any particular pathogen or pathogens should be made in the light of prevailing local circumstances, preferably after an initial screening exercise.

In areas prone to periodic eutrophication, it would be advisable to obtain identification of toxic algal species, and to monitor shellfish for biotoxins if there is any reason to believe these may be present.

5.5 Sampling

5.5.1 Sampling point locations

In large beaches, as well as in relatively extensive shellfish areas, multiple sampling (i.e. the taking of a number of samples from within the same overall location) will have to be performed. For recreational waters, normal locations of sampling points for multiple sampling are generally considered to be best spaced at intervals of 250 metres along the coastline, 10 metres seaward from the low tide mark. However, the exact location of sampling points in any monitoring programme, including the distance between them,would be expected to vary with each individual beach, the particular requirements of which would have to be determined by the local responsible authorities. In addition, the criterion for determination of the actual location relative to the shoreline should be water depth, rather than distance from shore. As the most critical part is that immediately near the shoreline, those areas where the water is 20-30 cm deep should invariably be monitored. One other good indication as to where to monitor is to look for those parts where bathers tend to congregate most. In sandy beaches, the two most important points to monitor from the point of view of distance from shore would be where the water is at approximately waist-high level (where most adults will be found) and in the shallows near the shoreline (where small children would normally be).

Rocky beaches present more variations, as there may be no regular bottom gradient, and the bottom could be rocky, sandy or mixed. Large shallow pools where children are found should be monitored. Where the water depth exceeds 5 metres, water samples should preferably be taken at surface, bottom and intermediate points. Where the bottom is composed of sand, silt or mud, a sediment sample should also be taken. Multiple sampling points should also include points remote from direct (outfalls) and indirect (rivers, creeks, etc.) wastewater discharges. Results from such points would serve as references for other observations in the area, and would also serve for comparison between areas. A seaward sampling point, lying approximately 1 kilometre offshore, should also be established for every kilometre of beach being monitored (remote background multiple sampling point). An example of the possible location of multiple sampling points is shown in Figure 2 and Figure 3.

The location of sampling points in areas used for recreational activities other than bathing, i.e. windsurfing, canoeing, scuba diving, etc., would have to be

determined on an *ad hoc* basis, depending on the circumstances prevailing in each particular locality.

For shellfish areas, sampling point locations should be situated:

(a) in the shellfish culture or harvesting area;

(b) in the culture or harvesting area surroundings;

(c) close to sources of pollution affecting the culture or harvesting area.

Selection of the sampling locations will depend on the exact aims of the monitoring programme, on the resources made available for monitoring, and on the specific characteristics of the area itself. Where adequate resources for comprehensive monitoring are not available, or where the area itself does not permit the coverage described above, modifications should be made, keeping in mind that the primary aim of the whole programme is to determine the suitability of the area for recreational purposes or for the harvesting of shellfish for human consumption from the public health viewpoint, i.e. to ensure that no significant health risks are attached to bathing in, or consuming shellfish from, the area in question.

Adjustments to the monitoring programme, such as alterations in the number or location of sampling points, may be realistically considered only after the completion of at least one season's monitoring.

5.5.2 Sampling frequency

For coastal recreational waters, observations and measurements at the sampling points should be made at two-week intervals (as a minimum) during the bathing season, as well as after every considerable rainfall in beaches located in the vicinity of an outfall. Under special circumstances, a higher frequency may have to be applied during specific periods. For example, measurements would have to be taken immediately after any accident or other circumstance (such as leaks in sewerage systems) likely to affect water quality and continued at a frequency dictated by the situation itself until it reverts back to normal. Heavily-populated beaches should be monitored at least once a week **as a routine measure** during the bathing season. There is no definition as to what constitutes a crowded or heavily-populated beach in quantitative terms, and this would have to be determined by local authorities in each individual case. The bathing season proper will vary from country to country, and its official duration for monitoring purposes will have to be determined locally. During the rest of the year, i.e. outside the bathing season, observations and measurements should be carried out at the same points at three equally-spaced intervals, which would be approximately every three months under Mediterranean conditions.

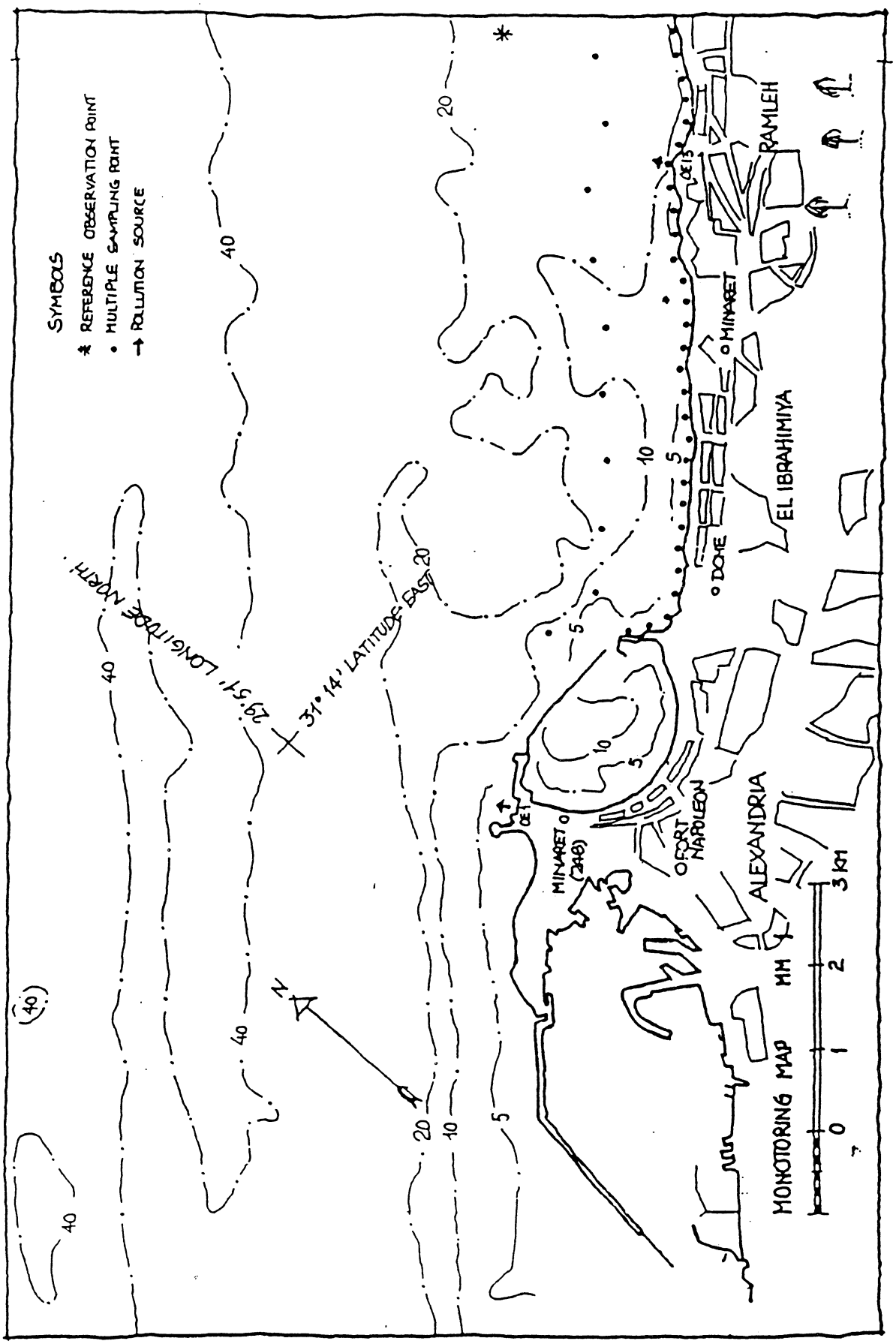

Figure 2. Location of sampling points in a typical monitoring programme.

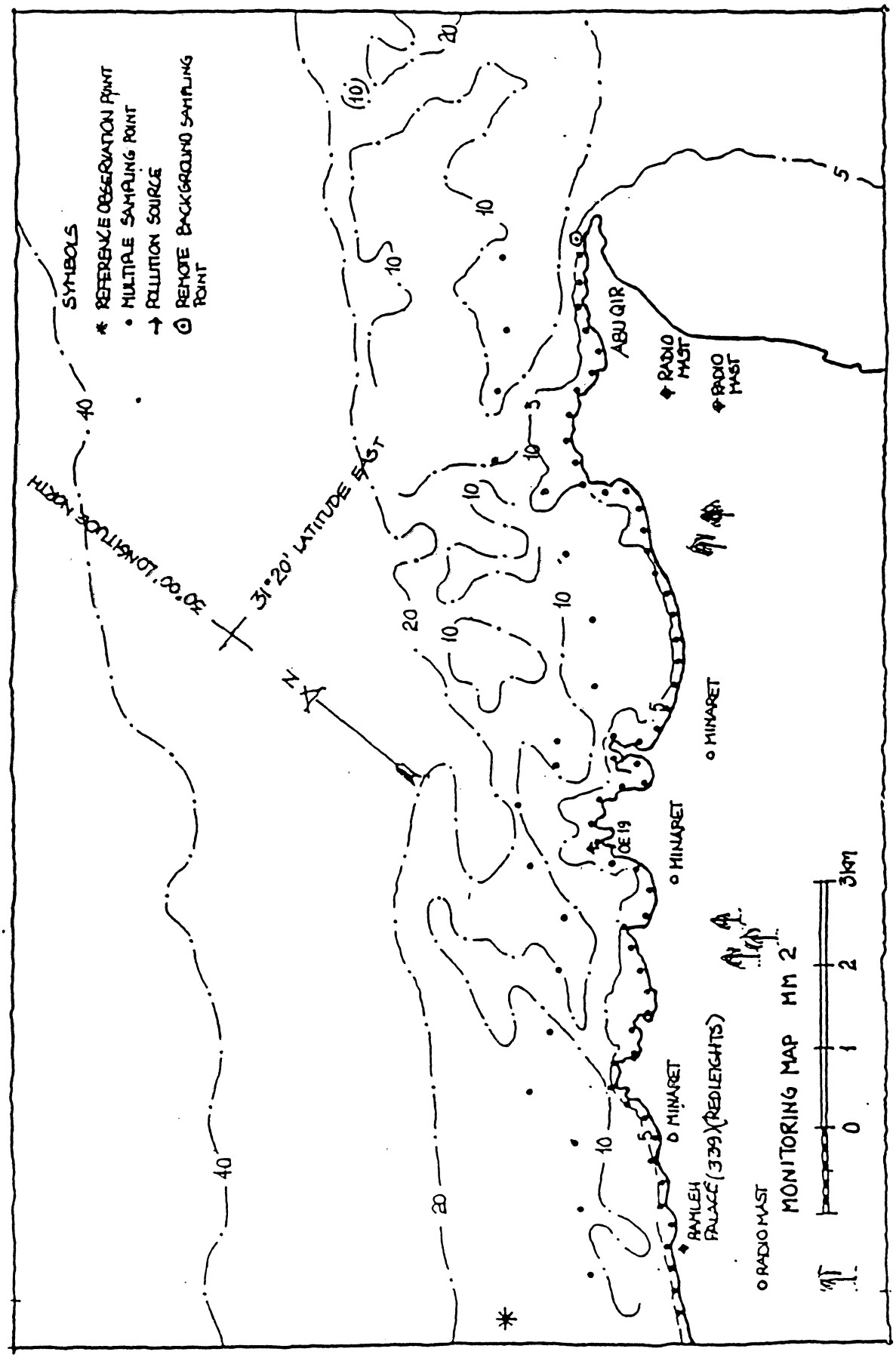

Figure 3. Location of sampling points in a typical monitoring programme.

For commercial shellfish areas, observations/measurements should be carried out once a week during the peak consumption period when shellfish are harvested. When no harvesting occurs, monitoring may be carried out at 2 to 3-month intervals. For large-scale (over 500 tonnes per year) shellfish areas, monthly monitoring during the non-harvesting period is recommended, in addition to the weekly monitoring during the harvesting period.

For natural shellfish areas, observations/measurements should be carried out once a week during the season in which the shellfish are collected, and three times at equally-spaced intervals for the rest of the year.

5.5.3 Sampling equipment

Details of the relatively simple types of sampling equipment is contained in each recommended method for determination of specific bacterial indicators or pathogens in Parts II and III of these guidelines. For surface and subsurface sampling, the containers utilized should be bottles of dark-coloured borosilicate glass of 200 to 300 ml capacity, wide-mouthed and with ground glass stoppers. A common method of sampling directly beneath the surface is shown in Figure 4. The same type of bottle may be used for subsurface sampling with the addition of an extension arm and clamp, as in Figure 5. One type of equipment for sterile subsurface sampling is shown in Figure 6.

For sediments, two types of bottom samplers are shown in Figure 7 and Figure 8 respectively. Other, simpler types of equipment which can be used for collection of samples of sediments for microbiological analysis are also available on the market.

5.6 Analysis and Quality Control

Recommended methods of analysis for determination of concentrations of (a) bacterial indicator organisms and (b) selected pathogenic bacteria are provided in Parts II and III of these guidelines respectively. Each method contains a list of necessary equipment and expendable materials, and the approximate cost of these would have to be taken into consideration, especially in the case of new laboratories, when planning the monitoring programme, to ensure that resources are sufficient to meet analytical requirements.

Quality assurance and control is an essential part of ant monitoring programme, as important decisions may have to be taken on the basis of results obtained. This aspect is comprehensively described in Part V of these guidelines.

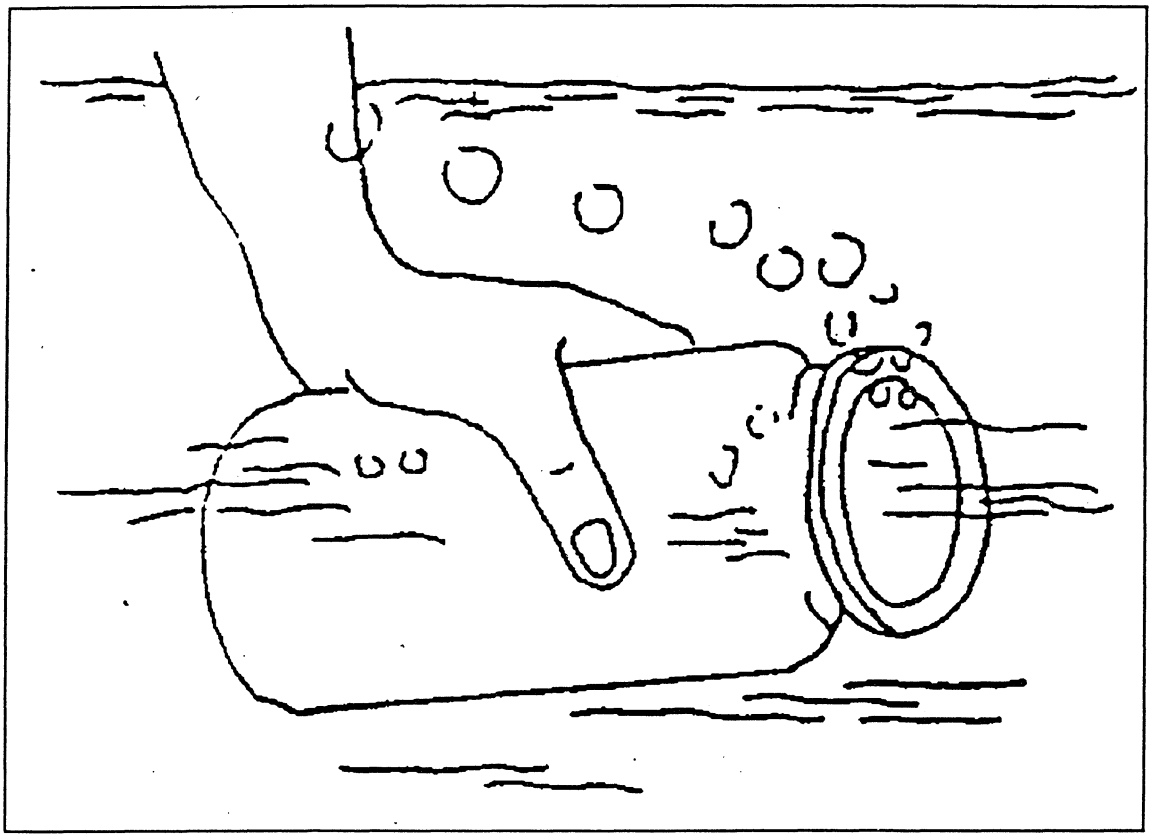

Figure 4. Subsurface sampling by hand.

5.7 Interpretation of Results

Details for interpretation of results obtained from specific analyses are contained in the relevant sections of Parts II and III of these guidelines. Interpretation of overall results will normally be performed by the competent authorities, particularly in the case of compliance monitoring. Results obtained will indicate (a) whether the quality of the recreational or shellfish area complies with stipulated standards, and (b) what remedial action is necessary in appropriate cases. Remedial action can be long-term, particularly when it involves the establishment of sewage treatment plants and submarine outfall structures.

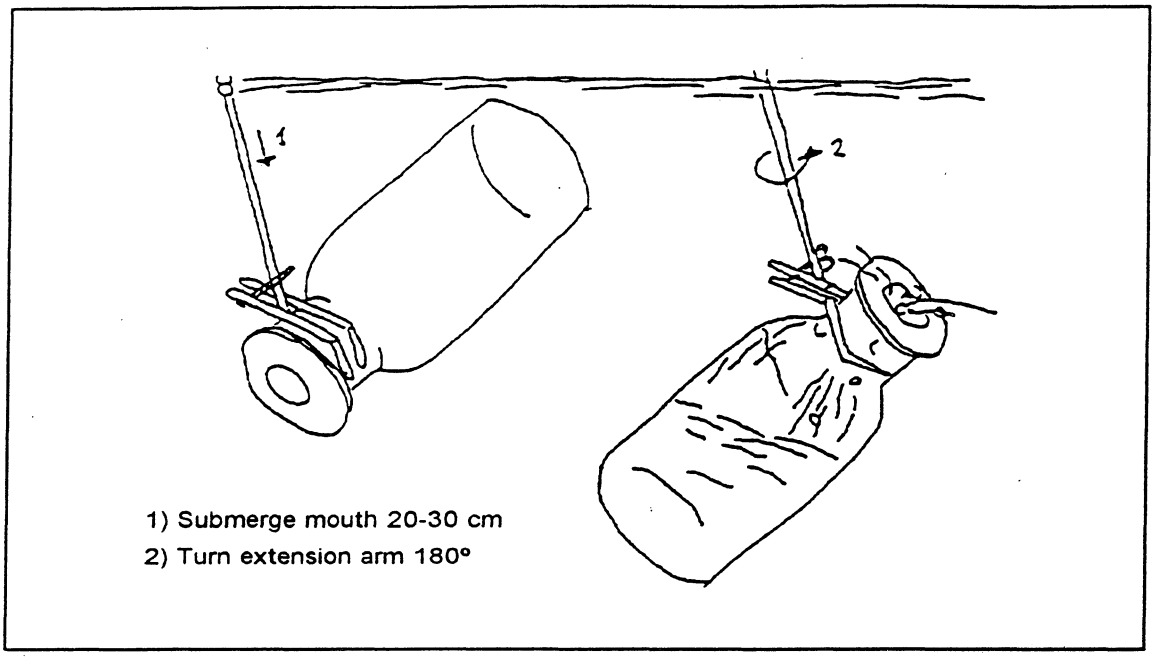

Figure 5. Subsurface sampling with extension arm.

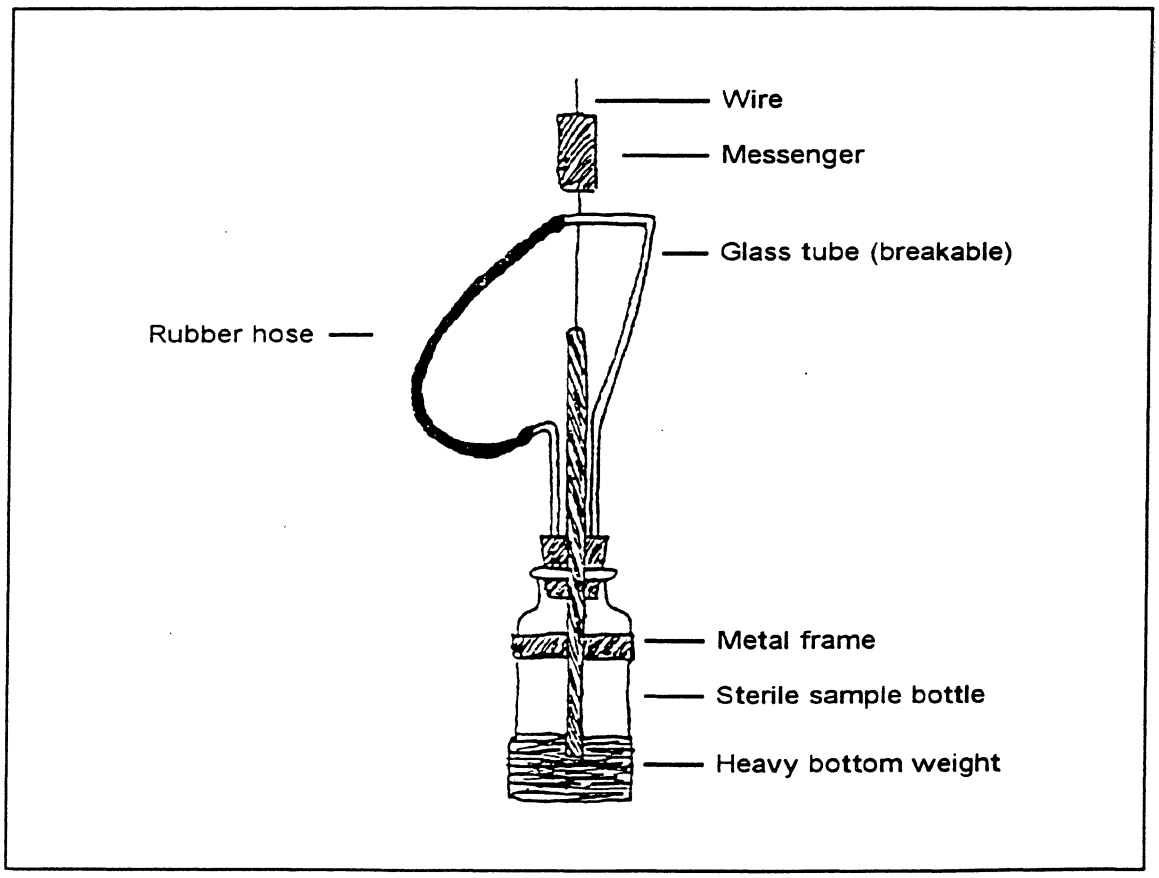

Figure 6. Sampler for sterile subsurface sampling.

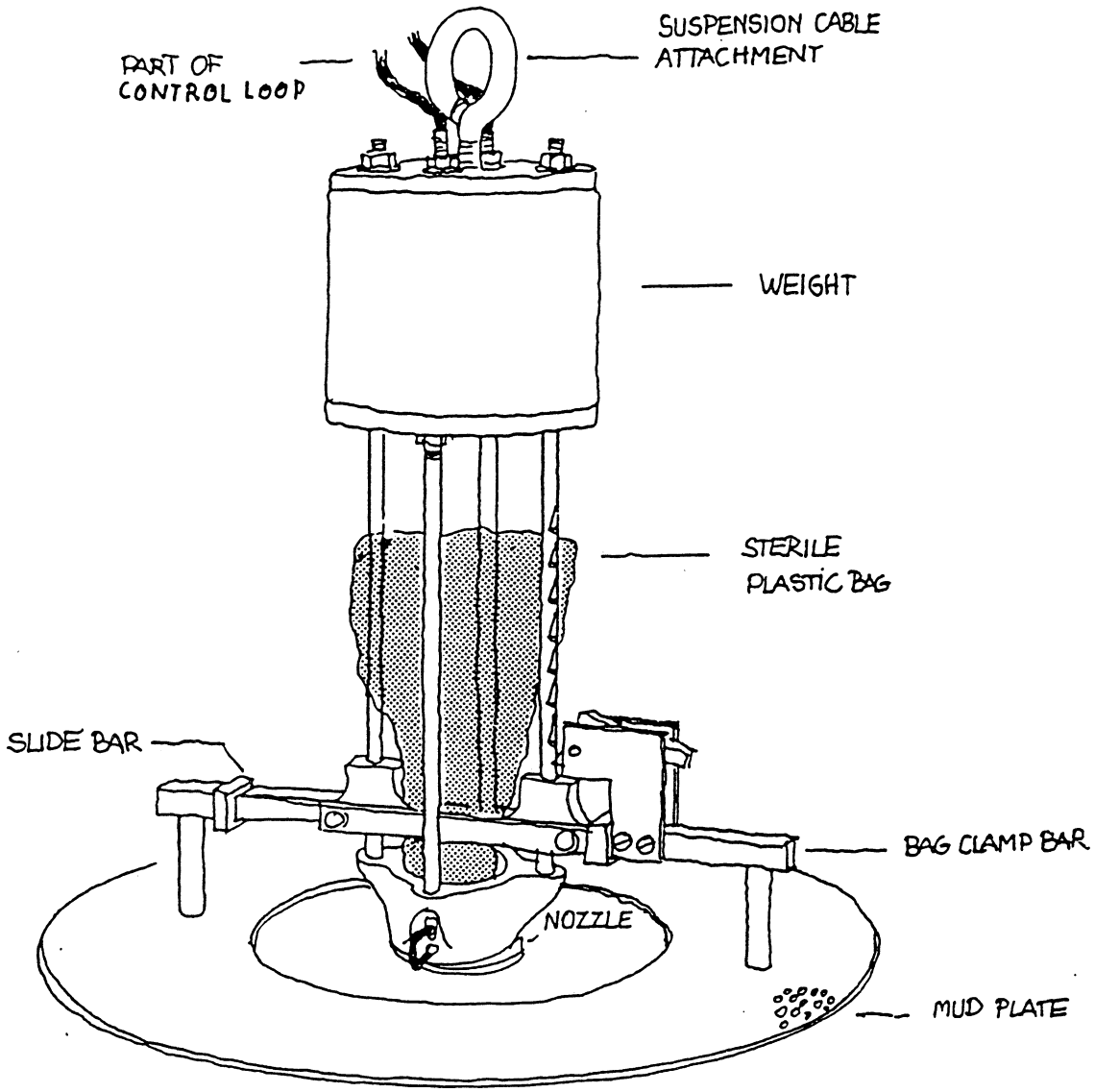

Figure 7. The Van Donsel-Geldreich bottom sampler.

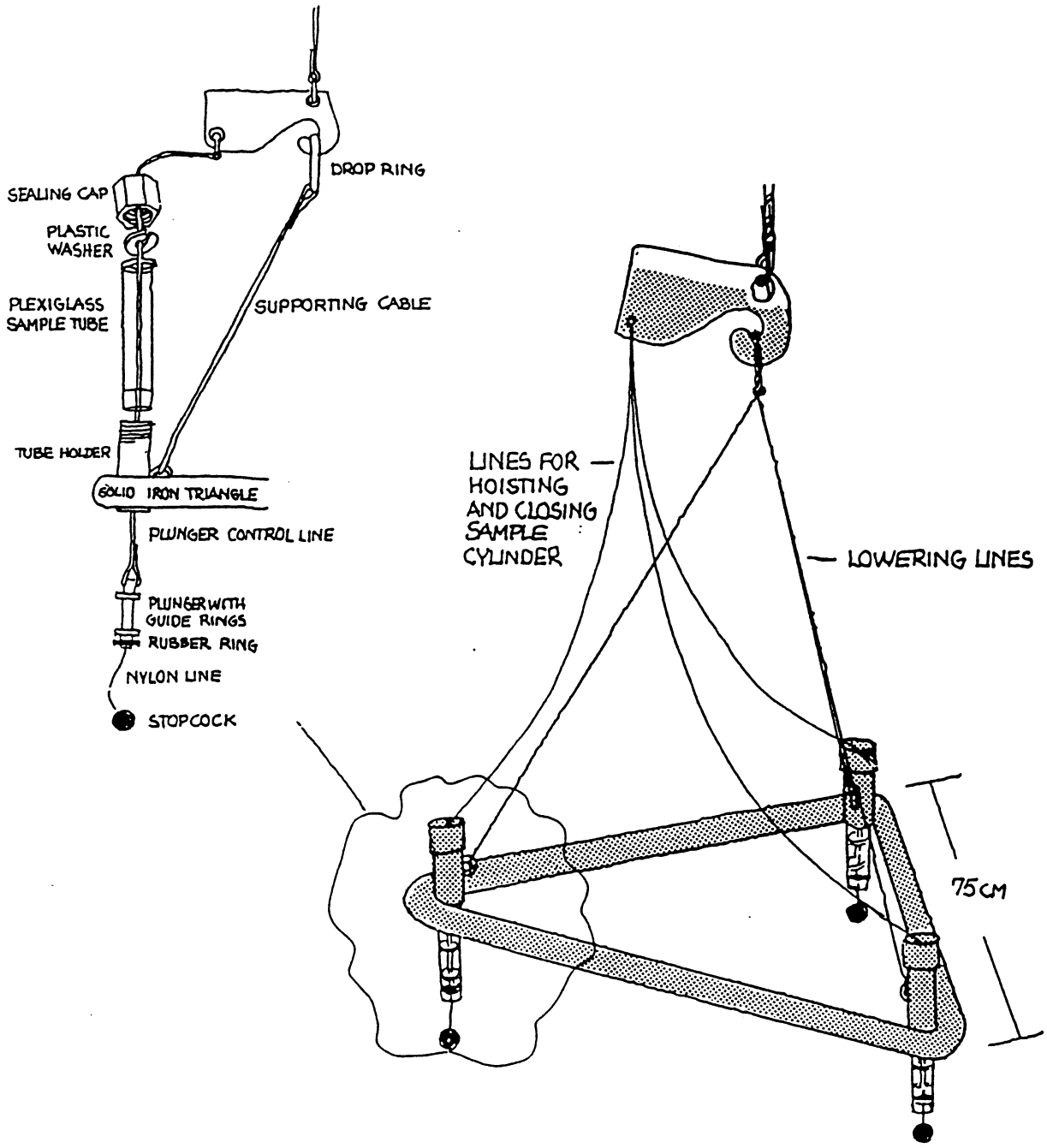

Figure 8. Three-core Albrechtsen bottom sampler.

6. PROGRAMME IMPLEMENTATION

6.1 Sampling

Sampling should be performed in a systematic manner to reduce variation between individual results. For this reason, it is necessary to maintain as many factors as possible constant. These include both the actual period of sampling (i.e. time of day) and the actual sampling method, as well as the location and depth of individual sampling points.

The actual sampling period (i.e. time of day) in the same station should not vary by more than 3 (preferably 2) hours. The actual optimum sampling time would depend on a number of factors. Two approaches are possible in determination of the optimum sampling time: (a) coincidence with peak use, and (b) correlation with the effects of intermittent-flow sources, such as sewage outlets. Eventual selection of the optimum sampling time will depend on the particular approach adopted in the light of the overall objectives of the monitoring programme, i.e. to ensure adequate health protection for bathers.

Examination of a large number of samples together with their analysis for a relatively small number of important microbiological parameters would be preferable to the reverse, i.e. a small number of samples analyzed for many parameters.

Every effort should be made to achieve the fullest possible degree of standardization between all sampling stations.

Specific sampling procedures are contained in the recommended methods for determination of specific indicator organisms and pathogenic bacteria described in Parts II and III of these guidelines. The points below are intended as a general outline for overall guidance.

(a) Microbiological sampling (as well as handling and examination) requires great care, because a large number of steps are necessary before results (e.g. concentration of organisms in the sample) become available, and at each step there is a risk of spoiling the whole procedure, for example through contamination or by non-controlled growth of microorganisms. Sterility and correct temperature (including cooling) are of prime importance in the performance of microbiological investigations.

(b) Sampling proper can be considered as completed once the sample is transferred to the sterile container, whether on the beach or aboard a sampling vessel. Dark-coloured bottles should be used to protect the sample from light.

(c) Sterile sample containers are primarily the responsibility of the microbiological examination laboratory, where all necessary preparations should take place. These preparations include the provision of:

- sterile equipment
- sample labels
- enumerated sample containers

- insulated cooling boxes
- cooling elements necessary to maintain a constant temperature of 4°C throughout sampling and transportation.

(d) The sampling team or crew should be well-trained in micro-biological laboratory practice in order to appreciate the importance of sterile handling of sampling equipment and sample containers. All storage should be in the shade. Sampling vessels should have sufficient working space and a safely-fixed position for any equipment to cater for possible rough seas.

(e) Cooling of samples during storage (in the vehicle or vessel) and subsequent transportation to the laboratory is largely ensured by correct handling of the equipment and cooling containers prepared in the laboratory. The sampling team or crew should ensure that everything possible is done to maintain low temperatures inside the cooling boxes by securing shade, keeping lids tightly closed, etc. It is important to protect samples against ultra-violet radiation: they should always be kept inside a closed container. In addition, temperatures should be controlled at 3 to 4-hour intervals and any irregularity recorded. Samples to be analyzed for vibrios should be kept at ambient temperatures.

(f) Transportation to the laboratory should take place as early as possible. Normally, the time between actual sampling and receipt of the sample in the laboratory should not exceed 24 hours. After 30 hours, a sample is normally considered useless for microbiological examination.

(g) In general, water sampling should be commenced at the farthest point of the sampling area with respect to a pollution source, at which point the pollution should be lowest. This strategy is particularly important when operating from a vessel, as it involves the shortest possible storage time for the more polluted samples before analysis. Surface sampling will be the commonest procedure for most microbiological water monitoring, although subsurface sampling would be necessary in a number of cases.

(h) In commercial shellfish areas, sampling could most economically be carried out by joining local shellfish producers on their boat in their regular routine. This might not be possible in the case of areas where shellfish grow naturally.

(i) Full details regarding sampling should always be properly recorded. Such details should always include the station number, location, depth, distance from shore, date and time of day.

6.2 Recreational and Shellfish Water Quality Monitoring

Monitoring programmes limited to bare compliance with existing national regulations would involve sampling and analysis of only a few indicator and pathogenic organisms. Resources required would be relatively slight, but only a basic public health assessment and control would be achieved with such an approach. In a programme

of this basic nature, three types of parameters or observations should be respectively determined or recorded;

(a) General observation of selected local meteorological and hydrographic conditions relevant at the actual time of observation;

(b) Determination of selected local hydrographic parameters at reference points

(c) Determination of bacterial counts for a particular location, medium and depth, selected as multiple sampling points.

6.2.1 General observations

The following parameters of a general nature should be observed on a continuous basis, preferably at an existing weather station, adequately and regularly sampled:

(a) **Wind:** continuous records of direction and velocity;

(b) **Tide:** tide- tables for monitoring periods (if applicable);

(c) **Currents:** current patterns from local records;

(d) **Water level:** variations to be registered continuously or at least observed every two hours, if appropriate.

Records of these parameters should be retained, wherever possible, as copies of original recorder strips or similar material. In particular, wind and current directions are very important, and should always be recorded.

6.2.2 Reference point measurements

Reference point measurements provide background data for interpretation of multiple point sampling. They should commence well before, and be carried out continuously through, each individual point sampling period, and should be made at the beginning and end of each multiple point sampling period. The location of the reference point should be as representative as possible of the entire sampling area. Adjustments may be necessary after experience has been gained on the characteristics of the area.

Reference point observations and measurements should include:

(a) **Surface water drift:** observations of surface water speed and direction relative to a fixed point;

(b) **Waves:** periodical estimation of wave height, and estimation of sea condition as calm, rough or very rough;

(c) **Salinity and temperature:** measurements at the sampling points;

(d) **Turbidity:** observations of disappearance and reappearance depths of Secchi disc in metres;

(e) **Oxygen:** observations at the surface, bottom and intermediate depth;

(f) **Wind:** observations of direction and velocity, taking into account the considerable shifts that may be registered by local winds;

(g) **Air temperature:** measurements.

Secchi disc readings ((d) above) are not of practical value in very shallow waters. In these circumstances, the use of Secchi discs can be substituted **either** by measurement of suspended solids, which would be useful for characterization of the nature of the particulates, **or** by the use of turbidity meters.

6.2.3 Matrices and Microbiological parameters

In basic monitoring programmes for both recreational and shellfish areas, the minimum microbiological parameters which should be determined are faecal coliforms, faecal streptococci, **at least** one bacterial pathogen causing diseases of the gastrointestinal tract, and, if necessary, one bacterial pathogen causing infection through contact. This does not preclude the necessity for measuring other parameters if local circumstances so dictate. For recreational areas, the water itself should be monitored, and for shellfish areas, **at least** the shellfish themselves. In the latter (i.e. shellfish areas, there may be a case for determining viruses and/or algal biotoxins in specific localities.

More extensive monitoring programmes involve sampling and analysis of several indicators as well as a number of pathogens, and automatically include the minimum parameters. Such programmes are more resource-demanding, and often involve an element of research. A series of extended monitoring programmes often lead to new developments in pollution control, which may cause modification and redefinition of the scope of activities, and of the programme itself.

Extensive coastal water quality control programmes of this nature should only be designed after careful consideration of the objectives and available resources. The degree of refinement of such programmes will depend on specific problems within the area itself. In general, they require shorter intervals between observations and measurements envisaged in relation to the minimum programme and the monitoring of additional parameters. The choice of parameters monitored would be expected to vary in each individual case, and it would be difficult to generalize on (a) the precise organisms to be included, (b) the media to be sampled. (c) the locations used and (d) the frequency of sampling in each individual case.

In addition to pathogens, indicator organisms and other parameters mentioned in preceding paragraphs, the monitoring of a certain number of extra parameters is recommended in extensive monitoring programmes, although these are only indirectly related to human health aspects of coastal water quality. These supporting parameters may be of importance in that they may change the survival conditions of microorganisms in coastal waters, and may significantly influence the microbiological counts and assessment of human health risks. In a more direct way, a number of parameters, such as odour and turbidity also influence the recreational value of coastal waters from the aesthetic viewpoint.

The determination of biochemical oxygen demand (BOD), chemical oxygen demand (COD) and settleable matter would be mandatory in monitoring of effluents themselves, but would not be necessary for recreational waters or "clean" reference areas.

Parameters specifically related to eutrophication include:

(a) **Biomass and density of phytoplankton:** chlorophylls;

(b) **Nutrients:** total phosphorus, phosphate, ammonia, nitrite, nitrate, organic nitrogen;

(c) **Indications of anoxic conditions:** relationship of $H_2S/O_2/E_h$.

Although the monitoring of these parameters would certainly be useful in a comprehensive monitoring programme of an extended nature, and would equally certainly be essential in the monitoring of effluents and other pollution sources, the complexity of the nutritional balance and the primary production in a coastal area is such that their inclusion in a sampling strategy for the monitoring of coastal recreational and shellfish waters can be justified only in exceptional heal-oriented monitoring programmes.

6.3 Sediment Analysis

In extensive monitoring programmes for coastal recreational waters, sampling and analysis should not be confined to seawater alone, but should include bacteriological and, where appropriate, fungal determinations of (a) sand on beaches and (b) sediments. The latter can be considered as the sea bottom material immediately beneath the water sampling area. In the sampling of sand and sediments in recreational beaches, double sampling should be practiced, with selection of two particular depths, one frequented mostly by adults and the other by young children and elderly people (the latter two constituting the groups most susceptible to infection). Samples of sand should be taken of five centimetres thickness, under the best possible aseptic conditions.

6.4 Beach Quality Monitoring

Sanitary surveillance of beaches frequented by the public can be considered as an integral part of a coastal recreational water monitoring programme, as a significant proportion of the total time is spent on the beach itself. Experience in systematic beach surveillance as a type of pollution control is relatively limited. The nature of the beach is important. In general, a classification system for examining the visual appearance of beaches can be adopted. The McKay procedure, a modified version of the Garber classification, has been successfully employed for several years by different workers, with identical results that have proves useful for improving beach appearance. The classification is given in Table 7.

6.4.1 Basic monitoring

The full range of observations listed in Table 7 would be beyond the capabilities of most institutions carrying out only basic monitoring programmes, and these have therefore been divided into priority and non-priority parameters. Priority parameters should be recorded, and such non-priority ones as may be possible with available resources.

Observations should be conducted once a month during the bathing season, and at three equally-spaced intervals throughout the rest of the year. They should be made on days and at times when the visitor-density is low. During the bathing season, observations should preferably be made both before and after beach activity.

Microbiological examination of beach sand is recommended, since it may reveal the presence of coliform organisms and fungi in varying densities. As a general rule, monitoring should be carried out for the same micro-organisms as for seawater, with the addition of fungi.

6.4.2 Extended monitoring

In relatively extensive monitoring programmes, thorough sanitary surveillance of beaches assumes considerable importance and, apart from all the priority parameters listed in Table 7, as many as possible of the other parameters should be recorded, as well as faecal coliforms and fungi.

To obtain simple and accurate reporting of observation results, copies of maps should be obtained during surveillance, and notes should be made directly on to them. Figures 9, 10 and 11 are provided as models. In each case, these have been reproduced from a normal chart, enlarged to a suitable scale, and orientated in such a way as to have the coastline placed at the centre, so as to simplify observation and to allow notes to be made equally easily both to landward and to seaward of the central axis. On each, a number of observation positions have been marked for demonstration purposes.

Observation points should be established where a classified contaminant (i.e. one listed in Table 7) is observed in significant quantities. Contaminants not classified in the Table 7 may also be registered by separate notes on the beach surveillance map, but every effort should be made to use existing classes of contaminants, rather than establishing new classes.

The map allows observations to be conducted offshore as well as on the beach. Both types of observations should be recorded on the map. The frequency of observations should be the same as for basic monitoring.

The uses that a beach is put to are important for evaluating beach surveillance results. If possible, therefore, peak visitor densities for the beaches surveyed should also be reported, possibly on the maps, where dates and times would indicate peak visitor densities relative to regular beach surveillance operations.

Table 7. Modified McKay procedure for beach classification.

Code	Identification	Application, examples
Priority contaminants		
HS	Human faecal matter	Intact faeces must be differentiated from animal waste
HL	Human faecal matter	L = Landborne S = Seaborne
R	Refuse, including garbage from beach and land use	Domestic trash such as cartons, cans, boxes, bottles, and garbage from use of beach recreation areas
TR	Floating trash and garbage from boats and ships	Similar to R above, but judged to originate from boats or ships
NS	Noxious odours, fumes or gases-sewage	Sewage or treated sewage odours present in water or along beach
M	Murky-dirty	Water dirtied by causes other than plankton blooms. M_1 approx. 2 m.Secchi, M_2 approx. 1.5m. Secchi. M_3 approx. 1 m. Secchi
Other contaminants		
C	Clear water	Clear, no off-colour water or particulate matter
D	Ocean debris	Driftwood
K	Sewage debris	Match-sticks, hair, sludge floc, some garbage
ST	Sanitary towels	
CC	Rubber goods	Condoms and rings
S	Seaweed	Any kind of seaweed
B	Dead bird	Any dead marine bird
ML	Dead marine life	Fish or other marine animal
P	Plankton blooms or rafts	Plankton bloom discoloration of water
SP	Spores	Usually kelp spores that appear as a surface scum or film
O	Oil	Mineral oil from ships or other sources. Ship bilge pumping, fuel spills, etc.
OS	Mineral oil scum	Mineral oil slicks associated with natural oil seeps.
G	Particulate grease, sewage origin	Grease particles or balls near waste outlet
GS	Grease sum, sewage origin	Slick appearing to originate at a sewage discharge point
T	Tar	Mineral oil tar
N	Noxious odours, fumes or non-sewage gases	Mercaptans, sulfides, smog odours from industrial activities
F	Outflow of water to ocean from land	Usually storm-drain outflow which can affect ocean water condition
Quantity		
1	Small amount	Traces of the coded materials
2	Moderate amount	Some of the coded materials at intervals. Usually not objectionable
3	Large amount	Enough of coded materials to be objectionable
Example:	H2, P2, O1, GS1, T1	This result should be entered directly on the copy of the map (Figs. 9 to 11)

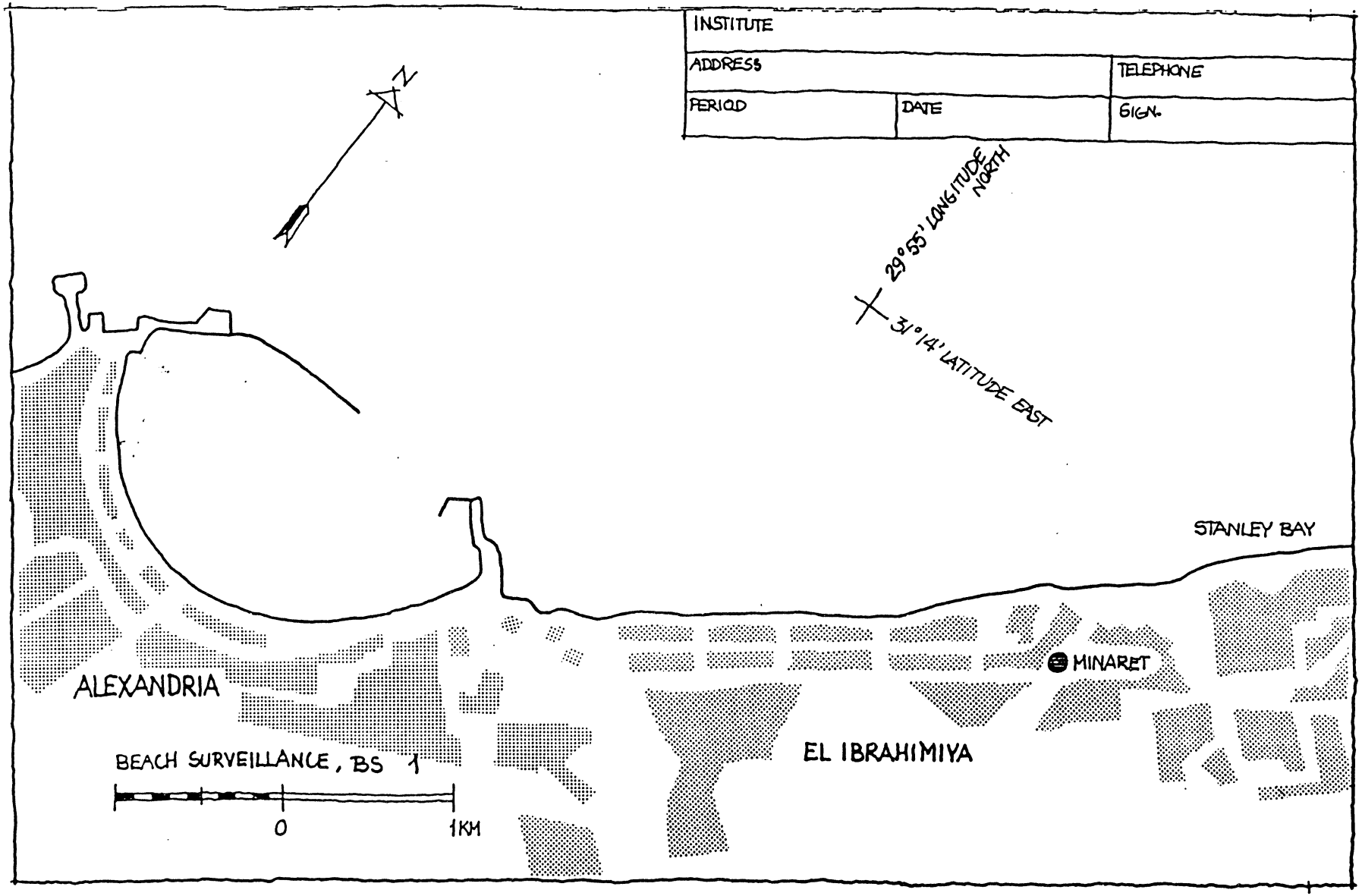

Figure 9. Beach surveillance observation sheet 1.

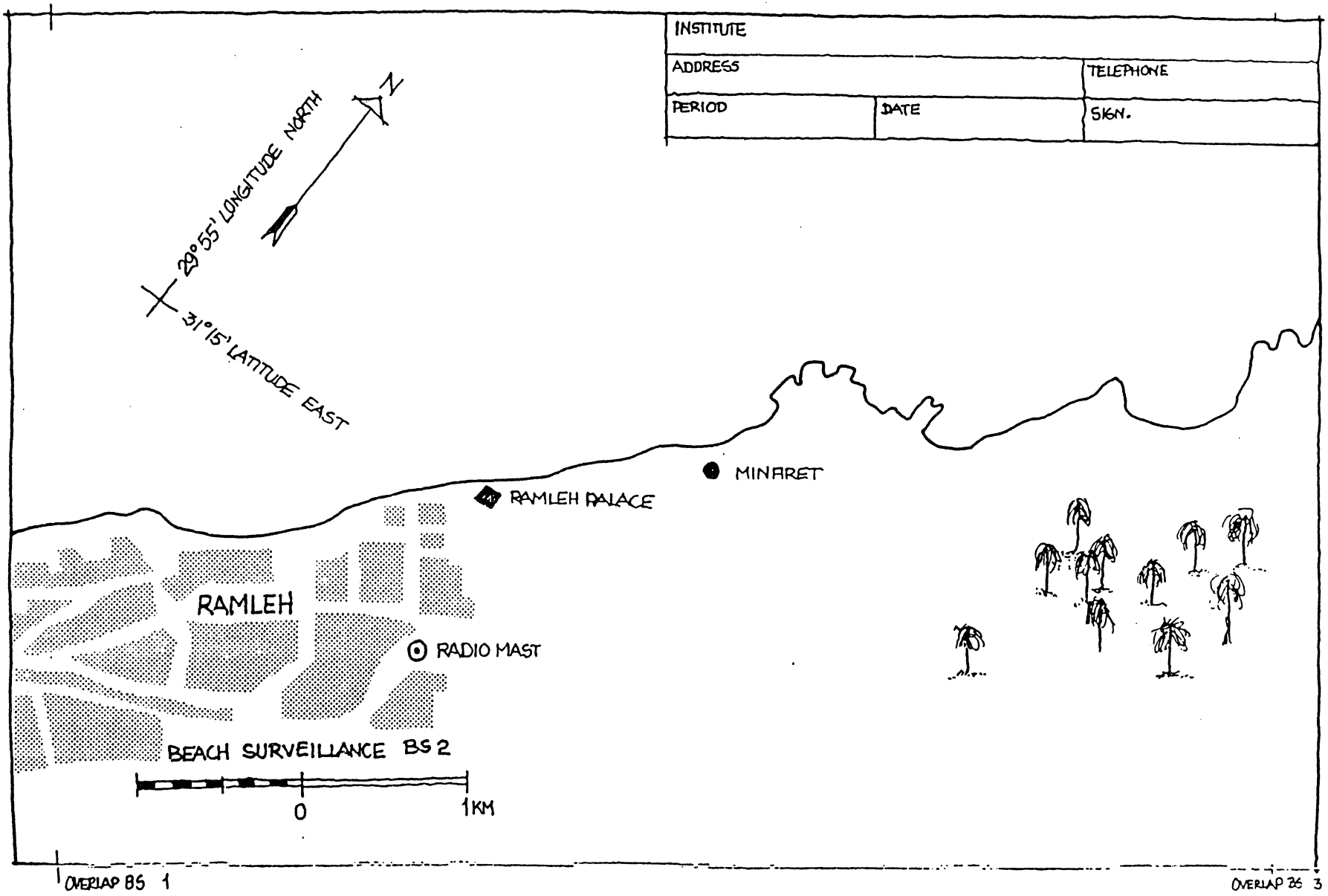

Figure 10. Beach surveillance observation sheet 2.

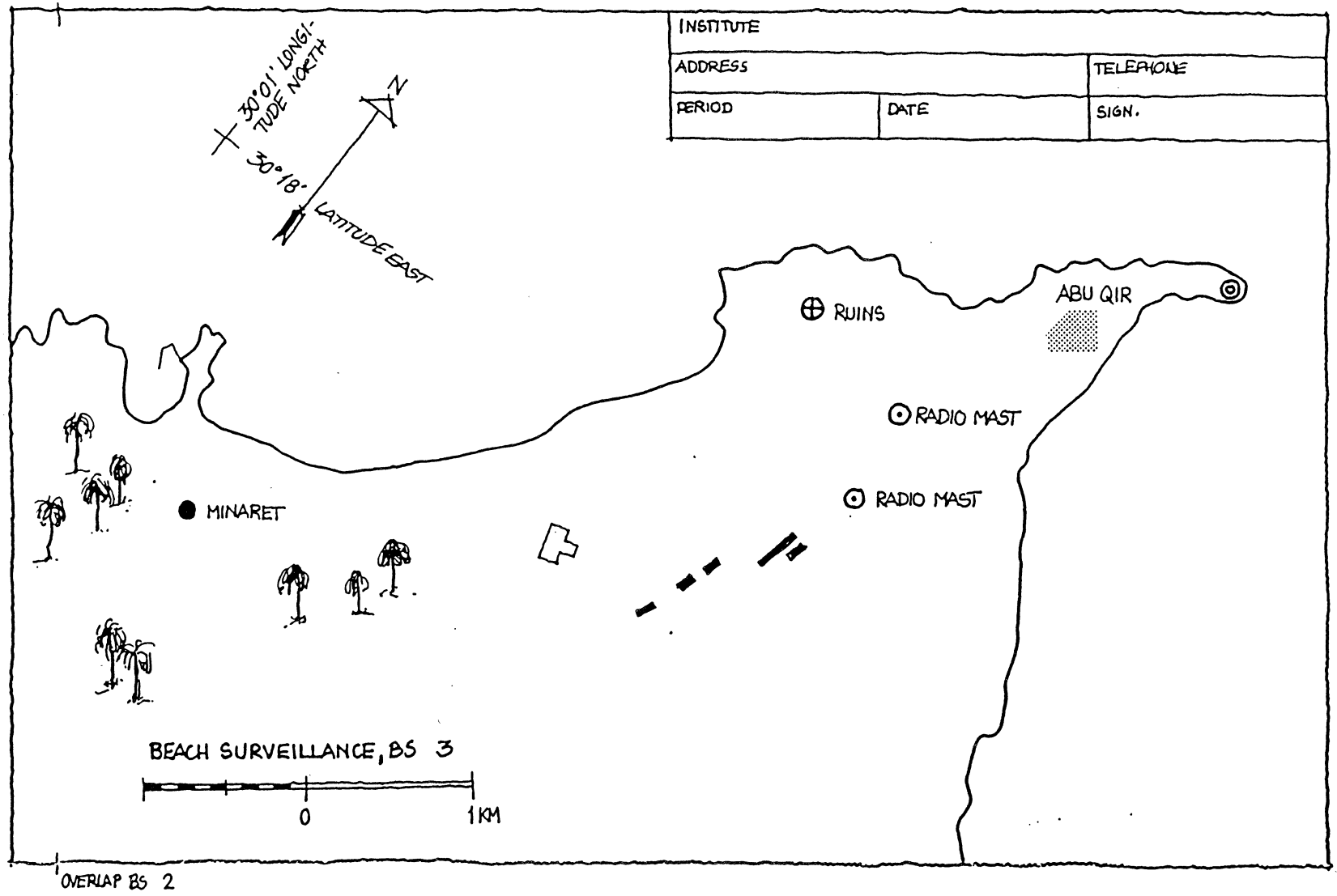

Figure 11. Beach surveillance observation sheet 3.

6.5 Recording and Reporting of Data

All data relevant to the observations and measurements forming part of a monitoring programme should be carefully recorded on standardized forms. These forms should include:

(a) Identification labels for sample containers;

(b) Records on currents;

(c) Records on basic hydrographic and meteorological conditions;

(d) Records on multiple sampling points;

(e) Test reports on microbiological analysis of environmental samples.

In any monitoring programme, apart from continuous reports on analytical results and other relevant matters to enable national or local authorities to take any appropriate action dictated by circumstances (such as closing a beach temporarily, or withholding shellfish from sale), periodical reports must be prepared, in order to evaluate and assess results obtained during the period with respect to the goals defined before commencement of field and laboratory work. Such periodical reports should consist of:

(a) Maps indicating the location of sampling stations;

(b) Exact dates and times of sampling for each station;

(c) Data on microbiological and complementary parameters, and on basic oceanographic and meteorological observations, throughout the sampling period;

(d) An overall evaluation of the results obtained, and an assessment of the sanitary quality of the area monitored in relation to stipulated criteria and standards.

As a model, the log form for reporting data on microbial pollution of seawater, which is currently in use un the Long-term Programme of Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase II), is reproduced in Annex 1 to this part of the guidelines.

6.6 Evaluation and Processing of Data

Monitoring usually creates considerable amounts of data that must be handled in orderly fashion, and often by using advanced methods of condensing data and expression of results. Considering the comprehensiveness of any monitoring programme for coastal recreational and shellfish-growing waters, a certain degree of statistical evaluation will be essential keeping in mind that:

(a) the microbiological quality of coastal waters can be adequately interpreted by a lognormal probability distribution model;

(b) correct compliance with any statistically-expressed water quality standard requires comparison of two probability distributions and not only two pairs of frequencies;

(c) the standard deviation of the concentrations of microbial indicators approaches quite closely that implied by some proposed interim coastal water quality criteria such as those developed during the pilot phase of MED POL;

(d) the standard deviation of a microbial indicators concentration, at a sampling station, is a useful and sensitive parameter for detecting discontinuous sources of pollution;

(e) a standard deviation estimate derived from sets of 12-14 weekly concentration values can be interpreted as follows:

$s \leq 1$: sampling station with very stable microbiological quality, either satisfactory or unsatisfactory,

$1 < s \leq 3$: : sampling station with a temporal variation of its microbiological quality within the range most commonly observed,

$s > 3$: sampling station with quite variable microbiological quality, due to a discontinuous source of microbial pollution or to variable coastal circulation pattern.

A relatively simple system of elaboration and interpretation of data obtained from microbiological analysis is based on a statistical method the aim of which is to obtain the characteristic parameters of the microbiological quality of the water, starting with a chronological series of results obtained in a systematic manner from one particular sampling station.

The characteristic parameters so obtained allow:

(a) determination of the classification of the sampling station in accordance with the water quality standards applied.

(b) detection of peculiar aspects of the water mass in which the sampling station is situated, such as the presence of differential current regimes, the existence of intensity flows and characteristics varying considerably with time.

The operative process consists of the following steps:

(a) selection of a chronological of results corresponding to one sampling station. The more systematic the manner in which the processes of collection, conservation and analysis of samples have been performed, the more precise will be the interpretation of the results obtained. Experience has demonstrated the importance of having, as a minimum, a series of 8 to 10 analytical results from each sampling station.

(b) the analytical results should be ranked in ascending order (i.e. from lowest to highest), and each assigned a number in consecutive order starting from unity.

(c) each analytical result should be assigned a value of the accumulated frequency, F, obtained from the formula:

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

where: F is the accumulated frequency, expressed as a percentage
i is the successive number of the analytical result
n is the total number of results of which the sample is composed

(d) using a normal log/probability paper, plotting graphically the pairs of values obtained for the microbial concentrations in the ordinates, and the accumulated frequency in the abscissae.

(e) in the great majority of cases, the points obtained follow a linear pattern. Should there be a notable discrepancy with this pattern, the sample requires a more specific interpretation, the explanation of which is beyond the scope of this brief method-description.

(f) a straight line should be drawn by interpolation, adjusting it to the centre of the pattern of points on the graph. Generally, one or more of the initial and/or final points deviates from this linear behaviour. The process of interpolation can be considered adequate when more than 80% of the points (approximately) adjust themselves to the straight line drawn.

(g) and adequate criterion for interpolating a straight line through the pattern of points on the graph consists in the visual siting of such a straight line in a manner by which the areas limited, to right and left, by an imaginary polygonal line connecting successive experimental points, are equal. Another possible criterion, practically equivalent, consists in leaving approximately the same number of points on either side of the straight line drawn.

(h) the line obtained represents an estimation of the statistical distribution of the quality of the sampling station. From the graph, it is possible to deduce by direct reading those concentrations which do not exceed a determined percentage of cases. For example, the mean is defined as the concentration which does not exceed 50% of the cases. In the same manner, the concentration associated with F = 90% is proportional to the value which is not exceeded in 90% of the cases.

(i) apart from the parameters associated with a determined frequency, the standard deviation, S, constitutes a characteristic parameter of the variability of the quality during the period of observation. The estimated value of the standard deviation is obtained by means of the following formula:

S = 1n XX84 - 1n XX50
or, equally
S = 1n XX50 - 1n XX16

where S is the standard deviation, and 1n XX84, 1n XX50 and 1n XX16 are respectively the Napierian (or natural) logarithms of the microbial concentrations (XX) which are not exceeded in 84%, 50% or 16% of the cases.

(j) determination of the interval in which a certain percentage of concentrations observed in any sampling station are situated can be obtained directly from the distribution graph of accumulated frequencies. Thus, for example, 90% of the microbial concentrations observed will be comprised within the values

(XX05; XX95)

where:

X represents the abbreviation of the microorganism under consideration
XX05 and XX95 are the microbial concentrations, expressed in number of colonies per 100 ml which do not exceed, respectively, 5% and 95% of the cases.

In a similar manner, the confidence interval of the mean of the microbial concentrations which can be obtained from each of the possible series of 'n' microbiological results from a sampling station is defined as follows:

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

where:

'XX50' is the mean of the microbial concentrations in a sampling station, in XX/100 ml.

"S" is the standard deviation of these concentrations, obtained from the corresponding normal logarithmic distribution: S=1n XX84-1n XX50.

'n' is the number of results obtained (microbial concentrations or densities).

'$t_{(1-a/2,\ n-1)}$ is the value of student's 't' with n-1 degrees of freedom (Table 8).

'a' is the significance level.

'(1-a) X100' is the confidence interval, expressed as a percentage.

Example

Figure 12 illustrates the application of the method in a chronological sample of 12 concentrations of faecal streptococci (FS), obtained from a station on the coast of Malaga in the summer of 1979. The chronological data, expressed as FS/100 ml are the following:

16, 170, 3390, 450, 450, 590, 740, 190, 1180, 6700, 2800, 600

The estimated values of the graph for those parameters characteristic to this station are the following:

FS 50 = 680 FS/100 ml

FS 90 = 4700 FS/100 ml

S = 1.50

The confidence interval of 95% (a - 0.05) of the concentrations of faecal streptococci is defined by the corresponding concentrations in the accumulated frequencies of 2.5% and 97.5% respectively, and are seen in the graph

50; 6000 (approx.)

The confidence interval of 95% of the mean of this series of concentrations can be obtained from the following values:

FS 50 = 680 FS/100 ml

1n FS 50 = 6.52

S = 1.50

n = 12

$t_{(0.975,11)}$ = 2.20

The limits will therefore be:

$$\exp\left[\left(6.52 - \frac{1.50}{\sqrt{12}}\,2.20\right)\right] ; \left[\exp\left(6.52 + \frac{1.50}{\sqrt{12}}\,2.20\right)\right]$$

that is to say

(260; 1760)

After obtaining the characteristic parameters of the microbiological quality of each station, a comparison to be made with established limits and water quality criteria, thereby obtaining the final category of the microbiological quality of the waters in a specified beach.

7. PROGRAMME EVALUATION

Implementation of monitoring programmes should be evaluated every year. There may be a number of reasons for periodic adjustments to any programme of monitoring coastal water quality. These reasons may include the attainment of initial objectives and the consequent reorientation of the programme from a baseline study to routine, as well as the establishment of the relation between specific discharges and the state of contaminated beaches, etc. New objectives may also be established, requiring either supplementary monitoring parameters or substitution of existing activities, or combinations thereof. Results obtained from early phases may also require alteration in the number of sampling points or in sampling frequency.

Relevant adjustments to the monitoring programme should be based on a critical assessment of needs, reflected in periodic reports. The rationale for making adjustments to monitoring programmes should be clearly stated.

Table 8. Values of Student's "T".

Degrees of freedom	Prob (t_n $t_{1-a;n}$) = (1-a)											
	.005	.01	.025	.05	.1	.15	.2	.25	.3	.35	.4	.45
1	63.657	31.821	12.706	6.314	3.078	1.963	1.376	1.000	.727	.510	.325	.158
2	9.925	6.965	4.303	2.920	1.886	1.386	1.061	.816	.617	.445	.289	.142
3	5.841	4.541	3.182	2.353	1.639	1.250	.978	.765	.584	.424	.277	.137
4	4.604	3.747	2.778	2.132	1.533	1.190	.941	.741	.569	.414	.271	.134
5	4.032	3.365	2.571	2.015	1.476	1.156	.920	.727	.559	.408	.267	.132
6	3.707	3.143	2.447	1.943	1.440	1.134	.906	.718	.553	.404	.265	.131
7	3.499	2.998	2.365	1.895	1.415	1.119	.896	.711	.549	.402	.263	.130
8	3.355	2.896	2.306	1.860	1.397	1.108	.899	.706	.546	.399	.262	.130
9	3.250	2.821	2.262	1.833	1.383	1.100	.883	.703	.543	.398	.261	.129
10	3.169	2.764	2.228	1.812	1.372	1.093	.879	.700	.542	.397	.260	.129
11	3.106	2.718	2.201	1.796	1.363	1.088	.876	.697	.540	.396	.260	.129
12	3.055	2.681	2.179	1.782	1.356	1.083	.873	.695	.539	.395	.259	.128
13	3.010	2.650	2.160	2.771	1.350	1.079	.870	.694	.538	.394	.259	.128
14	2.977	2.624	2.145	1.761	1.345	1.076	.868	.692	.537	.393	.258	.128
15	2.947	2.602	2.131	1.753	1.341	1.074	.876	.691	.536	.393	.258	.128
16	2.921	2.583	2.120	1.746	1.337	1.071	.865	.690	.535	.392	.258	.128
17	2.898	2.567	2.110	1.740	1.333	1.069	.863	.689	.534	.392	.257	.128
18	2.878	2.552	2.101	1.734	1.330	1.067	.862	.688	.534	.392	.257	.127
19	2.861	2.539	2.093	1.729	1.328	1.066	.861	.688	.533	.391	.257	.127
20	2.845	2.528	2.086	1.725	1.325	1.064	.860	.687	.533	.391	.257	.127
21	2.831	2.518	2.080	1.721	1.323	1.063	.859	.688	.532	.391	.257	.127
22	2.819	2.508	2.074	1.717	1.321	1.061	.858	.686	.532	.390	.256	.127
23	2.807	2.500	2.069	1.714	1.319	1.060	.858	.685	.532	.390	.256	.127
24	2.797	2.492	2.064	1.711	1.318	1.059	.857	.685	.531	.390	.256	.127
25	2.787	2.485	2.060	1.708	1.316	1.058	.856	.684	.531	.390	.256	.127
26	2.779	2.479	2.056	1.706	1.315	1.058	.856	.684	.531	.390	.256	.127
27	2.771	2.473	2.052	1.703	1.314	1.057	.855	.684	.531	.389	.256	.127
28	2.763	2.467	2.048	1.701	1.313	1.056	.855	.683	.530	.389	.256	.127
29	2.756	2.462	2.045	1.699	1.311	1.055	.854	.683	.530	.389	.256	.127
30	2.750	2.457	2.042	1.697	1.310	1.055	.854	.683	.530	.389	.256	.127
	2.576	2.326	1.960	1.645	1.282	1.036	.842	.674	.524	.385	.253	.126

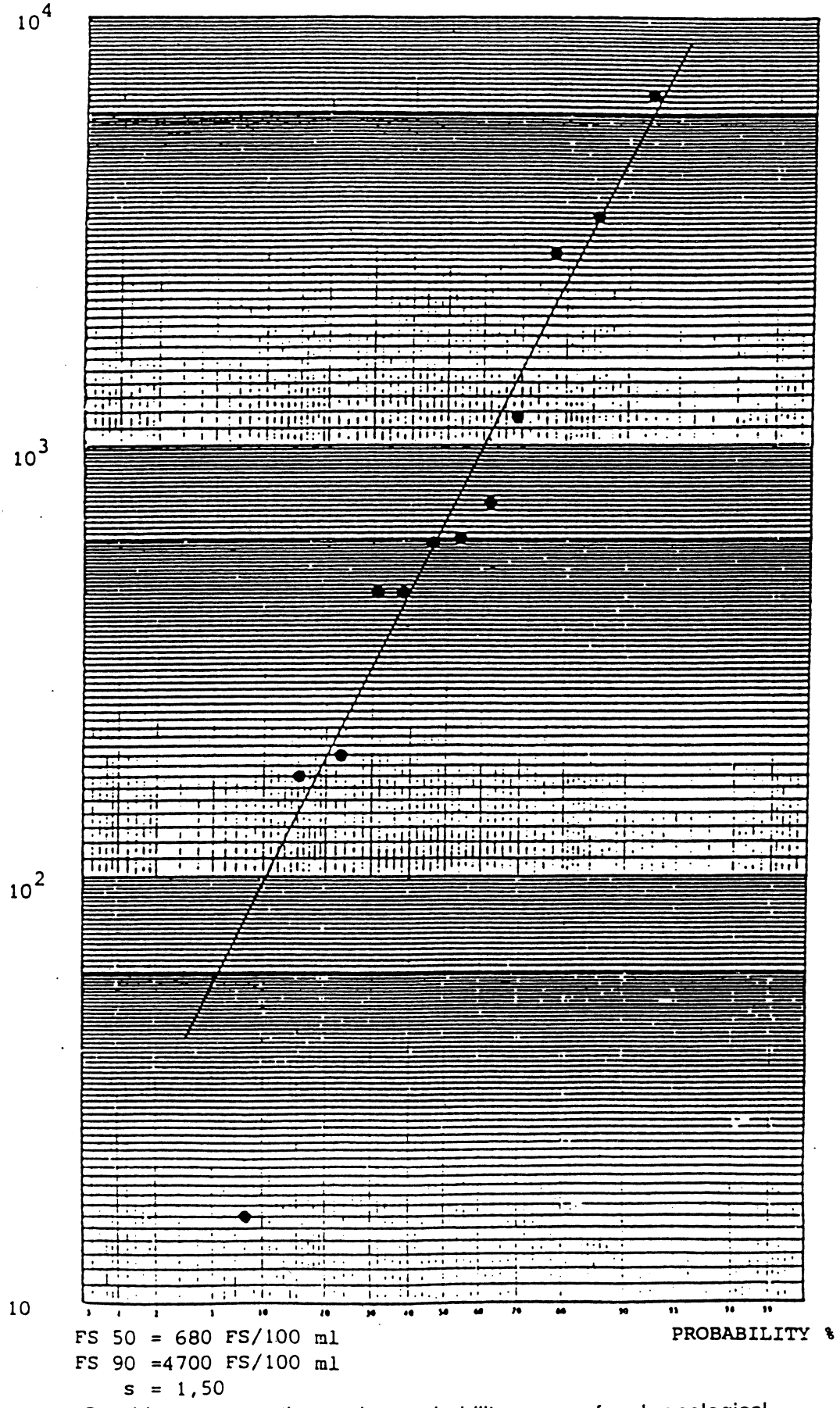

Figure 12. Graphic representation on log-probability paper of a chronological sample of twelve concentrations of faecal streptococci.

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ANNEX 1

MODEL FORMS FOR DATA REPORTING

The form reproduced in figure 13 is the one normally used by Mediterranean laboratories participating in the MED POL Phase II monitoring programme. It is designed to provide results of determinations of microbial pollutants in seawater sampled and analyzed according to the procedures detailed in Parts II and III of these guidelines. Reporting is as follows:

For the purposes of this form, a sampling point means a specific location, including a specific depth. Samples taken at the same point, but at different depths, are considered to represent different sampling points.

One line should be used to report the result of each analysis or the arithmetic mean and standard deviation of analyses of replicate samples from the same sampling point. Averaging of results from various sampling points should not be reported in this form.

If more than one line is used for a single sampling station, the **sampling information** and the **basic oceanographic and meteorological information** should be reported only in the first line for that station. If more than one bacterial indicator or pathogen is reported for the same sampling point, **sampling depth, temperature, salinity** and **oxygen** may be written down only in the first line for that sampling point. Where more than one bacterial indicator or pathogen is determined, the proper code for each should be specified in each lime.

The following specific instructions are also issued:

SAMPLING INFORMATION

Station number: Use the code number for the station as defined in your national monitoring programme.

Sampling date: Enter the date on which the sample was collected in numerical format, e.g. I July 1993 as 01.07.93.

Sampling time: Enter the time at which the sample was collected in 24-hour format, e.g. 2.30 pm as 1430.

Sampling location: Enter the geographic coordinates of the location where the sample was collected in degrees and minutes, and enter E or W in the E/W column, e.g. 40 15 30 E.

Distance from shore: Enter the distance from shore in metres.

Bottom depth: Enter depth of water in metres to the nearest 0.5 metre, e.g. 2.5 or 3.0.

Sampling Information													Bacteriological and Complementary Information						Basic Oceanographic and Meteorological Observations					Comments
Station Number	Date			Time		Location					Distance from Shore	Bottom Depth	Sampling Depth	Indicator Bacterial	Bacterial Count	Temperature	Salinity	Oxygen	Wave Height	Wind		Surface Current		
	Day	Month	Year	Hour	Minute	Lat		Long												Direction	Speed	Direction	Speed	
						Degree	Minute	Degree	Minute	E/W	(m)	(m)	(m)		(No/100ml)	(°C)	o/oo	ml O2/l	(m)	(d)	m/sec	(d)	cm/sec	

Figure 13. MED POL Log form for reporting data on microbiological pollution of seawater.

BACTERIOLOGICAL AND COMPLEMENTARY INFORMATION:

Sampling depth: Enter depth at which sample was taken to nearest 0.1 metre.

Bacterial indicator: Enter the indicator's or pathogen's code name as follows:

AH	*Aeromonas hydrophila*
CM	*Campylobacter*
FC	Faecal coliforms
FS	Faecal streptococci
PA	*Pseudomonas aeruginosa*
SA	Staphylococcus aureus
SM	*Salmonella*
TC	Total coliforms

Bacterial count: Enter the result as number of bacterial colonies per 100 ml.

Temperature: Enter in degrees centigrade.

Salinity: Enter in parts per thousand.

Oxygen: Enter in ml dissolved oxygen per litre.

BASIC OCEANOGRAPHIC AND METEOROLOGICAL OBSERVATIONS

Wave height: Enter height of waves in metres to the nearest 0.5 metre, i.e. 2.5 or 3.0.

Wind direction: Enter the direction from which the wind blows, in degrees.

Wind speed: Enter in metres per second

Current direction: Enter the direction towards which the surface current goes, in degrees.

Current speed: Enter surface current speed in metres per second.

COMMENTS

Use this section for any relevant information, such as analytical method from recommended one, etc. If comments take up more than one line, start any further data on other stations or parameters in the line following that in which comments end. Long comments may be written on the back of the form with proper reference to the appropriate line.

A new format has recently been developed in line with computerization of MED POL data. This is shown in table 9.

Table 9. MED POL Data transfer format for microorganisms in seawater.

Seq. No.	Column Name	Requisite?	Description	Column Type/Length
			Area & Station Information	
1	YEAR	YES	Monitoring year (Format is XX for 19XX)	NUM (2)
2	COUNTRY	YES	Monitoring country code (See country list, Appendix I)	CHAR (3)
3	AREA CODE	YES	Monitoring area code	CHAR (6)
4	AREA DESCRIPTION	-	Monitoring area description	CHAR (30)
5	STATION CODE	YES	Monitoring station code	CHAR (6)
6	STATION DESCRIPTION	-	Monitoring station description	CHAR (30)
7	STATION TYPE	YES	Station type code (See Volume 1, CODES)	CHAR (1)
8	BOTTOM DEPTH	-	Bottom depth in meters (Format is 99999.9)	NUM (7,1)
			Sampling Information	
9	SAMPLING DATE	YES	Date of sampling	DATE
10	HOUR	YES	Hour of sampling (00 to 23)	NUM (2)
11	MINUTE	YES	Minute of sampling	NUM (2)
12	LATITUDE DEGREE	YES	Latitude degree (sampling coordinate - applies to Seq. Nos. 12 - 19)	NUM (2)
13	LATITUDE MINUTE	YES	Latitude minute coordinate	NUM (2)
14	LATITUDE SECOND	YES	Latitude second coordinate	NUM (2)
15	LATITUDE HEMISPHERE	YES	Latitude hemisphere (Code N=North, S=South)	CHAR (1)
16	LONGITUDE DEGREE	YES	Longitude degree coordinate	NUM (3)
17	LONGITUDE MINUTE	YES	Longitude minute coordinate	NUM (2)
18	LONGITUDE SECOND	YES	Longitude second coordinate	NUM (2)
19	LONGITUDE HEMISPHERE	YES	Longitude hemisphere (Code W=West, E=East)	CHAR (1)
20	SAMPLING DEPTH	YES	Sampling depth in meters (Format is 99999.9)	NUM (7,1)

Table 9. (continued)

Seq. No.	Column Name	Requisite?	Description	Column Type/Length
21	SEA TEMPERATURE	-	Sea temperature in Celcius (Format is 99.99)	NUM (5,2)
22	AIR TEMPERATURE	-	Air temperature in Celcius (Format is 99.99)	NUM (5,2)
23	SALINITY	-	Salinity (Format is 99.99)	NUM (5,2)
24	OXYGEN	-	Dissolved oxygen in mL02/L (Format is 999.99)	NUM (6,2)
25	SEA STATE	-	Sea State (CODE, see below [1])	NUM (1)
26	WAVE HEIGHT	-	Wave height in meters (Format is 9.99)	NUM (4,2)
27	WIND DIRECTION	-	Wind direction in compass degrees	NUM (3)
28	WIND SPEED	-	Wind speed in m/sec (Format is 99.9)	NUM (4,1)
29	CURRENT DIRECTION	-	Surface current direction in compass degrees	NUM (3)
30	CURRENT SPEED	-	Surface current speed in cm/sec (Format is 99.9)	NUM (4,1)
31	SAMPLING INST CODE	-	Sampling institute code (See Supplementary Data chapter for clarification)	NUM (2)
			Parameter Analysis Information	
32	PARAMETER	YES	Micro-Organism code (See Volume 1, CODES)	CHAR (5)
33	CONCENTRATION	YES	Concentration in scientific format (9.99999E99, see Volume I, CODES for units)	CHAR (10)
34	INEXACT	-	If concentration is inexact (Code GT=Greater, LT=Less only)	CHAR (2)
35	ANALYSIS METHOD	-	Method used (See Volume I, CODES)	CHAR (5)
36	ANALYSIS DATE	-	Date of analysis	DATE
37	ANALYZING INST CODE	-	Analyzing institute code (See Supplementary Data chapter for clarification)	NUM (2)

[1]SEA STATE:

0 - Calm-glossy (0m)
1 - Calm-rippled (0 to 0.1m)
2 - Smooth-wavelet (0.1 to 0.5m)
3 - Slight (0.5 to 1.25m)
4 - Moderate (1.25 - 2.5m)
5 - Rough (2.5 to 4m)
6 - Very rough (4 to 6m)
7 - High (6 to 9m)
8 - Very high (9 to 14m)
9 - Phenomenal (>14m)

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