

**SECOND CEPPOL SEMINAR ON MONITORING AND
CONTROL OF SANITARY QUALITY OF BATHING AND
SHELLFISH-GROWING MARINE WATERS IN THE
WIDER CARIBBEAN**

KINGSTON, JAMAICA, 9-13 AUGUST 1993





Caribbean Environment Programme

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REPORT OF THE MEETING

Introduction

1. Among the components of the CEPPOL Programme, Activity 4.4.3 "Monitoring and Control of the Sanitary Quality of Bathing and Shellfish Growing Waters", represents an important effort for the development of a monitoring and research programme that will ensure the sanitary quality of these waters and the development and enforcement of adequate quality criteria and control measures. To this end, and as a preliminary activity within the programme, this seminar was convened in Kingston, Jamaica, 9-13 August 1993, at the Regional Co-ordinating Unit of the Caribbean Environment Programme.
2. The main objectives of the Seminar were:
 - (i) To discuss the progress made in the implementation of the pilot studies sponsored by this activity leading to the development of suitable environmental quality criteria for the region;
 - (ii) To examine and provide comments regarding the monitoring programmes on the sanitary quality of bathing and shellfish growing waters actually being conducted in the Wider Caribbean region;
 - (iii) To formulate detailed recommendations for the prioritization of future studies geared to complete the objectives of the activity; and,
 - (iv) To discuss measures required to improve the control of microbial contamination by micro-organisms of waters and to diminish the public health risk from such contamination.

3. Seminar participants were selected by the CEPPOL Coordinator and were invited to attend as experts in their personal capacity. In selecting the participants, the RCU was guided by the experience of the participants to the subject matter of the Seminar. A list of the participants is provided in Annex I of this report.

Agenda Item 1: Opening of the meeting

4. The meeting was opened on Monday, August 9 1993 at 10:00 a.m., at the Conference Room of the United Nations Law of the Sea Office in Kingston by Mr. Richard Meganck, Coordinator of the Caribbean Environment Programme. Mr. Meganck welcomed the participants and highlighted the main aspects and importance of the CEPPOL Programme for the region. In particular, he emphasized the relevance of the subject matter for the Wider Caribbean as it affects the natural environment, the economic activities and the health and well-being of the population. He recognized that without the appropriate regional co-operation between countries and international organizations, it would be impossible to achieve the objectives of CEPPOL. He then encouraged the participants to consider this type of co-operation during the deliberations of the meeting. Moreover, Mr. Meganck emphasized the need to consider the socio-economic and policy implications resulting from the decisions adopted by the Seminar.

Agenda Item 2: Administrative Arrangements

Agenda Item 2.1: Adoption of Agenda

5. The meeting adopted the agenda (IOC/UNEP-WG. 12/1) with minor modifications, which is attached as Annex II to this report. The list of documents (IOC/UNEP-WG. 12/3) is appended as Annex III to this report.

Agenda Item 2.2: Election of Officers

6. The meeting unanimously elected the following officers:

Chairman: Mr. Enrique Mandelli
IOC Consultant

Rapporteur: Mr. Gary Toranzos
Associate Professor
University of Puerto Rico

Agenda Item 3: Present State of Development of Activity 4.4.3

7. The CEPPOL Coordinator presented the document "Summary of the Implementation of CEPPOL Activity 4.4.3 - Monitoring and Control of the Sanitary Quality of Bathing and Shellfish Growing Waters", for the period April 1991 through August 1993 (UNEP(OCA)/CAR WG.12/6).
8. Activity 4.4.3 was initiated with a Seminar on "Monitoring and Control of Sanitary Quality of Bathing and Shellfish-Growing Marine Waters in the Wider Caribbean", convened in Kingston, 8-12 April 1991. The participants in the Seminar, a total of fourteen (14), were selected by the CEPPOL Coordinator and invited to attend as experts in their personal capacity. Among the invited experts were representatives of CEPIS/PAHO and the MEDPOL Programme of UNEP'S Mediterranean Action Plan.
9. The report of the Seminar together with a Circular Letter (CAR 16/1991) inviting the submission of study proposals was sent to the CEPPOL focal points of all States and Territories participating in the CEP during August 1991. The Circular Letter indicated that the proposals should concentrate on the Seminar's recommendations as previously described.
10. As a result of this request, the RCU received the following proposals:
 - "Indicadores Sanitarios de la Calidad de las Aguas del Mar Caribe: Relaciones y Tasas de Mortalidad", Instituto Costarricense de Acueductos y Alcantarillados (A y A), Costa Rica;
 - "Coliphages and H₂S Strips as Alternate Indicators of the Microbiological Quality of Tropical Coastal Waters", Department of Biology, University of Puerto Rico;
 - "Pilot Prospective Epidemiological Study in Trinidad and Tobago", Institute of Marine Affairs (IMA), Trinidad and Tobago;
 - "A Comparative Assessment of Selected Bacterial Indicators of Faecal Contamination for Application in Sanitary Water Quality Monitoring Programmes in the Caribbean", (CEHI), St. Lucia and,
 - "Microbiological Indicators of Pollution in Bathing Waters in the U.S. Virgin Islands", Eastern Caribbean Centre, University of the Virgin Islands.

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11. Four (4) of the above-mentioned pilot studies were approved in 1992. It is important to indicate that the CEPPOL assistance to the IMA for the epidemiological study was an additional support to project TRI/87/003/A001, financed by UNDP and the Government of Trinidad and Tobago. The proposal presented by the Eastern Caribbean Centre was not approved due to limited funds available at the time the proposal was received.
12. The funds allocated in 1992 to the above-noted proposals totalled US\$41,000 - (US\$32,000 from Activity 4.4.3 and US\$9,000 from Activity 4.4.6). At the time of writing this document, the pilot studies proposed by A y A, Costa Rica and the Department of Biology of Puerto Rico were completed.
13. During 1993, three (3) additional proposals were received by the CEPPOL Coordinator, namely:
 - "Pilot Study to Assess the Status of Microbiological Pollution of Shellfish and Shellfish Growing Waters in Trinidad", Institute of Marine Affairs (IMA);
 - "Monitoring of the Sanitary Quality of Shellfish- Growing Waters in Jamaica", University of the West Indies (UWI), Jamaica and,
 - Vibrio cholerae Bacteriophages and their possible use to Monitor Waters for the presence of Enterotoxic Vibrios", University of Puerto Rico (UPR).
14. The above proposals were approved by the RCU Coordinator and funds made available for a total of US\$22,000.
15. In addition, the CEPPOL Programme prepared a questionnaire on the "Monitoring of the Microbiological Quality of Coastal Marine Waters in the Wider Caribbean Region (Annex I) that was distributed to the CEPPOL focal points. Approximately 50% of the states and territories of the Wider Caribbean completed the questionnaires. In this regard, some of the countries provided information collected by more than one agency. Most of the countries used faecal coliforms as indicator organisms. The frequency of sampling varied from occasional sampling to very strict schedules such as those observed by the Overseas Departments of France.
16. During 1993, the CEPPOL Programme received a document from CEHI/PAHO entitled "Assessment of Operational Status of Waste Water Treatment Plants in the Caribbean", covering mainly CARICOM Countries . The preparation of the above document was discussed during the First Seminar of Activity 4.4.3.
17. Concerning the future of Activity 4.4.3, the Sixth Intergovernmental Meeting of the Contracting Parties to the Convention for the Protection and Development of the Marine

Environment of the Wider Caribbean Region which was held in Kingston, 16-18 November 1992, approved a preliminary budget of US\$45,000 for the 1994-1995 biennium.

Agenda Item 4: PAHO/WHO Activities on Health-related Aspects of Marine Pollution by Micro-organisms in the Wider Caribbean

18. Mr. Henry Salas of PAHO/CEPIS initiated his presentation by identifying PAHO/CEPIS's role as a technical centre of the Panamerican Health Organization.
19. Microbiological criteria and/or standards for primary contact recreation and shellfish harvesting waters presently in use in Colombia, Cuba, Ecuador, Mexico, Puerto Rico and Venezuela were presented. The above criteria and/or standards for recreational use were primarily based on the US/EPA (pre 1986) and California, USA criterion to assess the merits of the present indicators, which are presently used. It was pointed out that modifications were made by various countries with respect to the percent of samples which should not contain more than a specified level of indicator organisms, for example, in Puerto Rico 20% of the samples should not exceed 400 faecal coliforms/100 ml, while in Venezuela 0% of the samples should have more than 400 faecal coliforms/100 ml. On the other hand, Brazil promulgated a water quality standard for primary contact recreation stating that 80% of samples should be less than 1000 faecal coliforms/100 ml., based on attainability and not epidemiological studies. Peru subsequently followed suit adopting the Brazilian standard.
20. A log-normal distribution comparison showed that the EEC mandatory guideline of 95% of the samples should be less than 2000 faecal coliforms/100 ml. is actually more restrictive than the EPA geometric mean guideline (pre-1986) of 200 faecal coliform/100 ml. in that the 95 percentile for this mean would be greater, (Kay et al, 1992). This fact, notwithstanding, it is recognized that the EPA exceedance criteria (10% of the samples not more than 400 faecal coliforms/100 ml.) would be the controlling factor. As such, care through rigorous statistical evaluations should be taken in selecting percent exceedance levels in that these are normally the controlling criteria and can significantly alter the engineering design and costs of adequate disposal systems necessary to comply with the standards. To support this point, cost curves for submarine outfalls were presented and typical values for outfall installation varied from US\$5,000 per metre (diameter = 2m) to US\$400 per metre (diameter = 0.25m) using high density polyethylene (HDPE) plastic; the latter case applicable to small communities. Submarine outfall length would be inversely proportional to the standard adopted.
21. Sewage disposal alternatives and present practices in Latin America and the Caribbean were briefly discussed. At present, there are around 80 outfalls with lengths of 500 meters or greater primarily distributed as follows: Venezuela (39), Puerto Rico (14), Brazil (13) and

- Mexico (9). Outside of Puerto Rico, only one other outfall of more than 500 meters exists in the Caribbean. The reader is referred to the CEP Technical Report No. 9 of 1991 of a previous CEPPOL Seminar on Monitoring and Control of Sanitary Quality of Bathing and Shellfish-Growing Waters in which this subject is discussed in more detail.
22. The results of an epidemiological "Cabelli Style" study conducted in the bathing waters of the state of Sao Paulo, Brazil 1991 were presented. The results were presented at the previous CEPPOL meeting in 1991 (Report No. 9) and unfortunately, the study has yet to be published. It was noted that this was the only epidemiological research on this subject matter conducted in Latin America and its comparison to the Trinidad & Tobago study would be interesting. Mr. Salas again stressed that further epidemiological research was needed in tropical waters.
 23. With regard to shellfish-harvesting water, the Latin American countries already mentioned have adopted the internationally accepted microbiological criterion of 70 total coliforms/100 ml., using a median MPN, with no more than 10% of the values exceeding 230 total coliforms/100 ml. or the faecal coliform equivalent of 14 and 43 faecal coliforms/100 ml. respectively. The only exception is Peru which has a standard of 80% less than 10 faecal coliforms/100 ml. and 100% less than 100 faecal coliforms/100 ml. for shellfish harvesting waters.
 24. The problems encountered (Ahmed, F. E. 1991) with current faecal coliform indicators in growing waters and the guideline for oyster meat (230 MPN faecal coliforms/100 g.) are the following:
 1. Non-Escherichia coli faecal coliforms and even non-sewage related bacteria may predominate in the faecal population analyzed by APHA (1985) methods. For example, a Louisiana oyster study showed that in warm months, Klebsiella pneumoniae isolates of environmental origin accounted for 86% of the non-E. coli faecal coliforms and often outnumbered E. coli 1000 to 1. This was confirmed in Gulf of Mexico studies.
 2. The faecal coliform indicator for water does not include the presence of non-sewage related naturally occurring aquatic bacterial pathogens, such as Vibrionaceae.
 3. The faecal coliform indicator does not correlate with the presence of human enteric viruses considered to be causative agents of illness.
 25. Processing and distribution aspects were discussed and the National Shellfish Sanitation Programme guidelines for monitoring interstate shellfish shipments presented. It was emphasized through examples that quality deterioration of shellfish could be handling abuse during transport of foodstuffs characteristic of developing countries.

26. The presentation ended with discussion regarding the cholera outbreak in Peru in 1991 and the measures taken. Basically, the community educational programmes assisted in controlling the outbreak. The basic sanitation situation has essentially remained unchanged in Peru. It was noted that there were minimal risks of cholera due to swimming in water contaminated with *Vibrio cholerae* because of the high infectious dose.
27. The Seminar was very interested in the epidemiological study conducted in Brazil following the Cabelli methodology. In the above-mentioned study, a correlation was observed between levels of enterococcus and health risks, but no correlation was observed with faecal coliform levels. The data obtained in this study seemed to indicate a higher incidence of overt disease than in the study conducted by Cabelli in the USA. The Seminar concluded that this maybe due to the percentage of pathogen excreters in the population. Mr. Dufour suggested a more detailed look at the data collected in the Brazil study (still unpublished), since this was not possible with the Cabelli USA study.
28. Mr. Dufour also discussed a French study on people exposed to unpolluted and polluted beach waters. These researchers compared the upper confidence limit of the mean illness rate of non-swimmers versus the lower confidence limit of the slope of the swimming-associated illness rate, water quality relationship. They suggested that the point at which the confidence limit lines intersected was the point above which statistically significant gastro-intestinal illness occurred. Mr. Dufour suggested that this statistical evaluation approach might be considered for analyzing data from the Trinidad recreational water quality studies.

Agenda Item 5: Review of Indicator Organisms for Bathing and Shellfish-Growing Waters and Health Studies from Non-point Sources

29. Mr. Alfred P. Dufour, Director of Microbiological Research Division of the USEPA/EMSL/CIN summarized the work being conducted by this organization for the development of microbiological water quality criteria for recreational and shellfish-growing waters.
30. In this regard, EPA is supporting a study in Hawaii which is examining the effectiveness of currently recommended indicator bacteria in tropical waters. Investigators in Puerto Rico, Hawaii and Hong Kong have suggested that these indicator bacteria may have extra-enteric sources that preclude their use as indicators of water quality. Alternate indicators will be examined to determine if they can be used in place of *E. coli* or enterococci.
31. Enterococci - A single step, 24 hour procedure has been developed to replace the 2-step procedure, 48 hour test for enterococci. Indoxyl-B-D-glucoside was incorporated into the mE medium. The hydrolysis of this chromogenic substrate results in a blue colour in

colonies of enterococci. This modified method will be approved by the EPA after completion of inter-laboratory evaluation studies.

32. *E. coli* - The two-step mTEC procedure has been modified by the addition of indoxyl-B-D-glucoside to the medium. Blue *E. coli* colonies result from the hydrolysis of this substrate. The substrate is highly specific for *E. coli*.
33. The derivation of the 35 enterococci per 100 ml guideline was reviewed. A simple ratio formula was presented to show how EPA arrived at the 35 number.
34. Data from multiple freshwater studies were presented to show that statistically significant correlations between two indicator bacteria could not be used to imply that each indicator would show a strong correlation with health effects in swimmers.
35. The current guidelines for water quality were reviewed and it was pointed out that because of the wording of the guideline, surface waters must be of a much higher quality than implied by the current upper limit of the indicator bacteria. For instance, if the geometric mean of 5 samples is not to exceed 35 enterococci per 100 ml, then the true geometric mean density of enterococci must be about 1 enterococcus per 100 ml.
36. Relative risks for gastro-intestinal and respiratory illness in swimmers were compared to enterococci densities in five (5) separate trials relating swimmers' illness to water quality. A significant correlation was found between indicator density and relative risk associated with gastro-enteritis. Relative risk associated with respiratory illness was not correlated with indicator densities, implying that gastro-enteritis is pollution related and respiratory disease is not. Skin, ear and eye infections, were similarly not correlated with water quality indicators.
37. A large scale study of the relationship between gastro-intestinal illness associated with the consumption of raw shellfish and the quality of the water from which the shellfish were harvested is currently being supported by funds from the U.S. National Marine Fisheries Service. This study, entitled "The National Indicator Study (NIS)", is being conducted in three (3) phases. The first phase covered the development of new methods for detecting and enumerating indicators and pathogens in water and shellfish meats, the preparation of a manual of available methods and the preparation of literature review covering all aspects of shellfish microbiology.
38. The second phase of the NIS will address the development of protocols for examining candidate sites from which to obtain shellfish for a feeding study, the development of an epidemiology study design and the identification of a contractor to carry out the feeding studies.

39. The third and last phase of the NIS will include feeding studies wherein participants will be exposed, i.e., fed, shellfish obtained from harvesting waters contaminated by point sources and non-point sources of pollution, and shellfish obtained from uncontaminated waters. Three (3) types of shellfish will be used: the eastern hardshell clam, the western hardshell clam and oysters.
40. Shellfish digestion - A procedure has been developed for producing filterable digests of oysters. The procedure involves homogenation, enzyme digestion and pre-filtration. The digested shellfish meat can be filtered through a 0.45 micron pore size membrane for subsequent placement on selective media for various indicators.
41. The study of non-point sources of pollution, with respect to infectious agents, is more meaningful if addressed in terms of the potential health risks associated with exposure to human faecal contamination versus exposure to animal faecal contamination. A study conducted by Yale University examined the relationship between the quality of water contaminated only by animals and gastro-intestinal illness in swimmers.
42. High densities of faecal indicator bacteria were not associated with illnesses in swimmers, indicating that the risk of illness from exposure to animal faecal contamination must be lower than that due to exposure to point source contamination (human). Illnesses in swimmers were shown to be associated with swimmer density and Staphylococcus aureus densities.
43. After Mr. Dufour's presentation, the discussion centered on how the indicator numbers were chosen to establish guidelines i.e. <200 FC/100 ml . In the case of selecting new emerging indicators, Mr. Dufour cautioned that the emerging indicators may not indicate all health risks. Concerning the selection of health risks to be included in studies related to primary contact with recreational waters, we must keep in mind that for enteric illnesses we can do something to prevent them by controlling the sources of contamination. However, other infections may be caused by resident microorganisms. Mr. Salas pointed out that taking into account the socio-economic conditions of developing countries, enteric illnesses risk associated with primary contact recreation are relatively insignificant when compared to infant mortality.
44. Considering coliphages as emerging indicators for the Caribbean, Mr. Dufour mentioned that they may be an alternative for the Caribbean as determined in a pilot study which is being supported by the EPA in Hawaii. However, a large study in the Caribbean should be able to determine if coliphages could in fact be used as indicators. Moreover, Mr. Dufour emphasized the importance of determining human versus animal faecal contamination. Several studies are being carried out using coproantibodies (sIgA) to differentiate between these two (2) sources. However, the need for simple and facile techniques should continue

to be research goals.

Agenda Item 6: Presentation of the Progress Reports of the Pilot Studies Under Implementation

Agenda Item 6.1: Relationships and Decay Rates of Indicators in Micro-organisms of Coastal Marine Waters of the Costa Rican Caribbean Sea

45. Mr. Darner Mora, Head of the Laboratory at the Institute of Aqueducts and Sewerage, Costa Rica, reported the final results of the above-mentioned pilot study. In order to fulfill the objectives of the study, eight (8) points of sampling were selected along the beach of "Ciudad Limón" - four (4) sampling points were located in the contaminated area and the rest in areas with reduced faecal contamination.
46. The collection of water and sediment samples was carried out in 1992 at monthly intervals. Levels of the following microbiological indicators were assessed: *Escherichia coli*, faecal coliforms: *Staphylococcus aureus*, *Streptococcus faecalis*, *enterococcus*, *Pseudomonas aeruginosa* and *Candida albicans*.
47. Additionally, the declination rates (**T90**) were determined in five (5) of the above indicators. It was observed that a high linear correlation ($r > 0.5$) existed between different pairs of indicators both in sediment and water. The relationship between faecal coliforms and faecal streptococcus demonstrated that the pollution observed in the waters of the beach comes from domestic discharges. For all the indicators, it was observed that the **T90** under light conditions was shorter than those obtained under dark conditions. The longest **T90** of all the organisms tested under light conditions was observed with *Candida albicans*. *E. coli* exhibited the longest under dark conditions.
48. According to the previous results, Mr. Mora concluded that faecal coliforms and *E. coli* appear to be the most adequate indicators for the evaluation of contamination of faecal origin in tropical waters.
49. The discussion generated by Mr. Mora's paper referred to the need to consider the examination, if possible, of opportunistic pathogens (e.g. *Candida albicans*). The importance of strict control of the time elapsed between the collection of samples and the time of analysis to a maximum of 6 hours at the appropriate temperature. Concerning the observed differences between **T90** of indicator microorganisms obtained under light and dark conditions, he stressed the need for more **T90** studies; however, it was the opinion of the Seminar that **T90** values obtained during the light period may provide information about these microbes as indicators of faecal pollution for possible health risk.

Agenda Item 6.2 : A Pilot Prospective Epidemiological Study to Assess the Health

Risks Associated with Bathing in Recreational Waters of Trinidad

50. Mrs. Christine Bullock-Ramsumair, Research Officer of the Microbiology Section of the Institute of Marine Affairs (IMA), provided information on the progress of the above-mentioned study.
51. In 1992, during the month of April in the dry season and July and August in the wet season, an epidemiological study was conducted in Trinidad to assess the health risks associated with sea bathing and to provide data to CEPPOL to assist in the development of environmental quality criteria for the Caribbean region.
52. There were six (6) survey areas in the study and these were located at four (4) beaches selected from a pre-pilot study. On each Sunday during the study period, water samples were collected, water parameters such as temperature, salinity, dissolved oxygen, pH and turbidity were measured and interviews were conducted at each survey area. Three (3) days after the initial beach interviews, follow-up interviews were conducted by telephone. Water samples were analyzed to assess the levels of water quality indicators: total coliforms, faecal coliforms, *Escherichia coli* and enterococci in the bathing water.
53. To date, the statistical analysis of the data obtained during this study has not been completed. Some information, however, has been obtained on the water parameters measured and on the bathing water quality at the survey areas during the study period. Crude symptom rates per 1000 persons have also been calculated from the questionnaire data.
54. Water temperature, pH and dissolved oxygen content did not vary considerably over the study period. However, a higher degree of variation was noted among salinity and turbidity values obtained during this period. With respect to the four (4) microbiological water quality indicators, levels obtained during the wet season were usually higher than in the dry season. Bacterial water quality indicator levels were notably higher on survey days in the wet season when there was heavy rainfall and increased river discharge rates.
55. A total of 10,204 beach interviews were conducted and 8,542 telephone follow-up interviews were completed. Only 8,369 of the completed telephone follow-up interviews, however, yielded usable data. The response rate was, therefore, 82 percent. Approximately 88 percent of the study participants were swimmers and 12 percent were non-swimmers. During the dry season, the wet season and for the overall study period, symptom rates were significantly higher for swimmers than for non-swimmers. Indeed in this study, 9 percent of the swimmers and only 4 percent of the non-swimmers became ill.

56. Generally, symptom rates for respiratory illnesses, gastrointestinal illnesses, highly credible gastrointestinal illnesses, eye infections, ear infections, skin infections and allergies were higher among swimmers than non-swimmers over the study period. Among swimmers, symptom rates were highest for respiratory illnesses followed by gastrointestinal illnesses. Respiratory illness represented about 47 percent of the overall illness incidence among swimmers. Over the study period, for all age groups, morbidity rates were higher among swimmers than non-swimmers. Among swimmers, morbidity rates were highest for study participants in the 16 to 20 age group. In addition, symptom rates for swimmers were higher among participants under 20 years than for those over 20 years.
57. Concerning the epidemiological study conducted the IMA, the data obtained in this study showed that there was a definite difference in health risks between the swimmer versus the non-swimmer groups.
58. The discussion was centered on the need to increase the size of non-swimmer control groups in future epidemiological studies. In addition, it was proposed that there should be an effort to correlate levels of indicators to health risks. Mrs. Bullock-Ramsumair replied that this is being looked at. The feeling of the seminar was that future studies should include emerging indicators as well as traditional ones.

Agenda Item 7: Special Presentations on Subjects Related to the Objectives of CEPPOL Activity 4.4.3

Agenda Item 7.1: Sanitary Quality of Water Sediments and Oysters from Coastal Lagoons in Tabasco and Campeche, Mexico

59. Mr. Jorge Romero from the (ICM Y L) of the University of Mexico described the work he conducted in the above-mentioned area. The work involved the determination of total and faecal coliforms in water, sediment and oysters. The results of the study indicated a heavy microbiological contamination beyond the accepted standards based on USEPA 1986 criteria. Consequently, the presence of pathogenic bacteria (*Salmonella* spp and *Vibrio* spp), and levels of heterotrophic bacteria and oil degrading bacteria were also determined.
60. The bacteria were isolated, purified and identified through biochemical tests. The isolated strains were exposed to antibiotics (Ampicillin, Kanamycin and Nalidixic Acid) and to HgCl₂ solutions in media containing NaCl at different concentrations. During the bioassay samples were allocated and DNA extracted to determine the presence of plasmids.
61. A correlation between the resistance to the above-mentioned agents and the presence of plasmids was determined.

Agenda Item 7.2: Report on the Consultation on Microbiological Quality of Coastal Recreational Waters (WHO/EURO Joint Project, MEDPOL Phase II) June 9-12 1993 Athens, Greece

62. Mrs. Christine Bullock-Ramsumair also prepared a brief on the MEDPOL Meeting convened in Athens, Greece. Under the framework of the Long-term Programme of Pollution Monitoring and Research in the Mediterranean Sea (MEDPOL Phase II), this consultation was convened to undertake recommendations proposed at a previous consultation in 1991 on "Health risks from bathing in marine waters".
63. The main objectives of the consultation were as follows:
1. To expand the WHO guidelines for prospective microbiological/epidemiological studies on the correlation between natural recreational water quality and effects on health to include complimentary designs such as the Randomized Controlled Clinical study design.
 2. To expand the scope of the above-mentioned guidelines by the inclusion of protocols for studies concerning specific recreational water activities other than bathing and,
 3. To review the results of recent microbiological/ epidemiological studies and to re-evaluate the scientific evidence for the recommendation of recreational water and beach quality criteria and standards.
64. The activities during the consultation included presentations by several participants who conducted epidemiological studies using two types of study designs.
65. The Cabelli type protocol was adopted during the previous consultation in 1991. Presentations were made by participants who had conducted studies using this protocol in Mediterranean countries and in temperate and tropical conditions climates.
66. A draft protocol for Randomized Controlled Clinical trials was reviewed for adoption as an alternative or complementary design for the Cabelli type protocol. In addition, there was a presentation on a study which was conducted in the United Kingdom using this type of study design.
67. In relation to a review of draft protocols for studies covering specific recreational water

activities other than bathing, it was indicated that recreational activities such as surfing and diving (including wind surfing and scuba diving) incur greater relative risks of reporting all classes of symptoms than swimming. There was also a presentation on research conducted in the United Kingdom on the health effects associated with of white-water canoeing.

68. From discussions during the consultation, it was recommended that the following factors be considered when conducting future epidemiological studies of this type:
- 1) It is generally considered that gastrointestinal symptomatic illness in bathers, related to water quality, are of viral origin and it would not be inconsistent to find that relative attack rates for such symptoms are related to counts of viral indicator, for example, enterovirus or possibly male-specific bacteriophage.
 - 2) The microbiological quality of wetted beach sand and sediments are important parameters in determining the environmental quality of bathing areas and should be an integral part of epidemiological-microbiological studies correlating recreational water quality with health effects.
69. The Seminar questioned the applicability of the Randomized Control Clinical Study. Mrs. Bullock-Ramsumair considered two (2) factors: it may be too expensive and labour intensive, and in addition, there may be too many pathogens not considered as risk factors, which may influence the results from the clinical analysis. Mrs. Bullock-Ramsumair also mentioned that because of ethical considerations only subjects 18 years old and above were surveyed, and therefore, a possible bias could be introduced.

Agenda Item 7.3: Coliphages as Alternate Indicators of the Microbiological Quality of Tropical Coastal Water

70. Mr. Gary Toranzos, Associate Professor of the Department of Biology of the University of Puerto Rico, provided information on the results of the above-mentioned pilot study. Data from a project financed by UNEP and currently being carried out was also presented.
71. A one year study was conducted in Puerto Rico at different beaches impacted and non-impacted by sewage. Coliphages were detected only from sewage-contaminated waters, (fresh as well as marine). In all cases, high concentrations of faecal coliforms and enterococci were detected but there seemed to be no correlation regarding the presence of point source pollution. The coliphage host strain used, permitted the detection of somatic and male specific coliphages. It was concluded that coliphages seem to be a good indicator of faecal contamination, therefore, any monitoring of waters should include coliphage detection. However, if results from one study are to be compared with another, they should use the same bacterial host and use standardized techniques. In conclusion, coliphages seem to be an excellent alternate indicator of faecal

contamination.

72. Regarding the second project, it was determined that the techniques used to detect *Vibrio spp* in the environment are susceptible to interference by background microbiota. The culture medium (TCBS) is easily overgrown and the detection of the target organisms is extremely difficult.
73. We have modified the TCBS by adding a buffer and different concentrations of salt. These modifications have reduced the interference caused by the background biota and allows for an easier detection of the target *Vibrio spp*.
74. The seminar generally agreed with the conclusions drawn from the project, however, it was felt that other countries in the region have expressed their interest in evaluating coliphages as possible indicators of faecal pollution. Discussion was centered on the modification of the TCBS and the possibility of using this medium to detect *Vibrio cholerae* 01 as well as *Vibrio parahaemolyticus* in the environment. Some use of molecular techniques conjointly with the modified media was discussed.

Agenda Item 7.4: Sanitary Quality of Water in Bays and Coastal Zones in Cuba

75. Mrs. Hortensia Mancebo, Research Officer of CIMAB in Cuba, provided information on the abovementioned subject.
76. During the past 15 years the results obtained from different bays and coastal zones in Cuba indicate that there is faecal pollution in certain areas. The indicators most commonly used in these studies were: total coliforms, faecal coliforms, faecal streptococci and *Clostridium perfringens*.
77. Coastal pollution is the result of poor domestic wastewater treatment systems and inadequate disposal practices. The environmental impact of these wastewaters discharged into coastal waters is minimal because of dispersion and dilution.
78. Generally, there is no evidence of pollution problems at Cuban recreational bathing beaches because they are not situated near sites of sewerage effluent discharges.

Agenda Item 8: Recommendations

79. The Seminar proceeded with the recommendations according to the following:

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- a) Types of indicators recommended for the region and justification (bacteria, fungi, virus)
 - b) Types of methodology according to the indicator
 - c) Guidelines, standards or criteria
 - d) Treatment systems
 - e) Monitoring programmes which need to be implemented in order to achieve or maintain the Water Quality of Bathing and Shellfish-Growing Activities
80. With reference to the type of indicators to be recommended, the Seminar felt that there is insufficient evidence to change the current standards in use. However, it is recommended that the enterococci group should be included in routine sampling because there is evidence indicating that is a good indicator of recreational water quality.
81. The Seminar also recommended the continuation of studies on male specific and somatic coliphages and Clostridium spp as indicators of faecal contamination in bathing and shellfish-growing areas within the region, taking into account the following:
- i) Their persistence in the tropical environment;
 - ii) identification of their sources (faecal material, sewage effluents, run-off, etc.); and
 - iii) their occurrence (their presence in polluted recreational and shellfish harvesting areas).
82. The Seminar encouraged the continuation of microbiological/epidemiological studies, taking into consideration traditional as well as newly emerging indicators of faecal contamination (more specifically coliphages and Clostridium spp).
83. These studies should use as a base the newly completed study in Trinidad and Tobago. The Seminar suggested that Cuba, Costa Rica, Jamaica, and Puerto Rico have suitable facilities and locations for conducting these studies.
84. In reference to the microbiological analyses of shellfish tissue, the Seminar recommended that new techniques for the preparation of the samples (such as enzyme digestion methods) as well as the above-mentioned emerging indicators should be included in the two pilot studies which are being implemented in Jamaica, Trinidad and Tobago and Puerto Rico.

Agenda Item 8.1: Types of Methodologies

85. Taking into account that the methods used for the detection of emerging indicators (coliphages and enterococci) were successfully evaluated in at least one country it is recommended that this emerging methodology be evaluated by laboratories in other countries of the region.

Agenda Item 8.2: Guidelines/Standards Criteria

86. The Seminar felt that there is not enough information at present to recommend any new guidelines.

Agenda Item 8.3: Treatment and Disposal Systems

87. Although this topic was thoroughly discussed, it is the consensus of the Seminar that the topic of sewage disposal and treatment is beyond the scope of this Seminar. Consequently, is recommended that a workshop be convened to discuss treatment and disposal systems during the 1994-1995 biennium.

Agenda Item 8.4: Monitoring Programmes

88. The Seminar recommends strongly that monitoring programmes be started or continued by the countries of the region. Monitoring programmes will benefit the economy of the region based on the following rationale:
- a) The economy of a great number of countries in the region is based on tourism as a source of income. Consequently, an unacceptable microbiological quality of recreational water may adversely affect the tourism industry;
 - b) many of the Wider Caribbean countries may have increased access to the international market in the future for the commercialization of shellfish; and,
 - c) based on previous experiences within the region (e.g. Costa Rica) long-term microbiological monitoring of coastal waters has provided the baseline data necessary for the negotiation of loans (e.g. IDB, World Bank, etc.) to finance appropriate sewage disposal systems.
89. The Seminar recommended that a protocol be prepared as a guide for monitoring practices in 3 phases:
- a) Preparation of a preliminary questionnaire which will be used to identify appropriate authorities to whom communications would be addressed;
 - b) preparation of a questionnaire to elicit information about the needs and problems relative to monitoring practices;
 - c) use the responses from the above survey to prepare a guidance document that addresses water quality monitoring issues in the region.

Agenda Item 8.5: Additional Recommendations in Priority Order for Actions to be Implemented during the 1994-1995 Biennium

90. To determine if traditional indicators of faecal pollution multiply in the tropical terrestrial or aquatic ecosystems
- 1) To determine if traditional indicators of faecal contamination have sources other than the enteric tract of warm blooded animals.
 - 2) To show if *E. coli* is capable of multiplying under environmental conditions using traditional cultural and molecular techniques.
91. To sponsor a cooperative programme among the states and territories of the Wider Caribbean to evaluate traditional and emerging methods for measuring faecal contamination in coastal waters
- . The objective of this effort is to conduct inter-laboratory studies to define the accuracy, specificity and reliability of microbiological methods used to measure the quality of tropical coastal waters
92. To conduct a follow-up microbiological/epidemiological study in Trinidad and Tobago using indicators appropriate for tropical climates
- 1) To develop indicators that can be used to reliably assess the risk to human population, and
 - 2) to develop a relationship between the quality of recreational water and health effects in swimmer population.
93. To demonstrate the effectiveness of alternative technologies for on-site treatment of domestic sewage in coastal areas of the Wider Caribbean
- 1) To identify the most effective tropical plant species for constructing artificial wetlands for on-site sewage treatment and,
 - 2) to demonstrate the use of artificial wetlands to improve the quality of wastewater normally discharged into shellfish-harvesting waters.

94. To conduct a workshop/seminar to define treatment and disposal practices applicable to coastal areas in the Wider Caribbean
- 1) The objectives are to discuss the benefits and problems of various options for disposal of sewage in tropical environments and to define research needs for the Wider Caribbean and,
 - 2) prepare a report summarizing the findings of the workshop/seminar.
95. To determine the occurrence and abundance of *Vibrio spp* in coastal waters of the Wider Caribbean
- 1) To determine seasonal variations and,
 - 2) to determine the importance of eutrophication on *Vibrio spp.* in the tropical marine/estuarine environment.
96. To educate citizens in coastal areas of the Wider Caribbean on the potential health risks and economic impacts of improper disposal of domestic sewage to recreational and shellfish growing waters
- . The objective is to encourage citizens to voice their concern regarding the treatment of domestic wastes before disposal in coastal areas.

Agenda Item 8.6: Suggested Activities

96. Prepare leaflets in the languages of the Wider Caribbean region for distribution during community gathering, festivals, etc.
97. For children preparation of colouring/workbooks for distribution in elementary schools, guides for high school teachers, and
98. Prepare slide presentation/script or video for use at community gathering, service club meetings, etc.

Agenda Item 9: Other Matters

99. No other matters were brought to the floor for discussion.

Agenda Item 10: Adoption of the Report

100. The Chairman presented the report of the Seminar to the meeting including its recommendations and requested that the participants adopt the report with its amendments, if required. The report was adopted with amendments.

Agenda Item 11: Closure of the Meeting

101. The meeting was closed on Friday, 13 August 1993, at 7:00 p.m. by Mr. Orjan Mohlund, Officer-in-Charge, in the absence of Mr. Richard Meganck, Coordinator, UNEP-CAR/RCU, who was on mission overseas. Mr. Mohlund presented the closing remarks and thanked all the participants for their valuable contributions, co-operation and interest in the Seminar. The Chairman thanked UNEP and the CEPPOL Secretariat on behalf of the participants, for a well organized and interesting meeting and the RCU staff for their support and kindness during their stay in Jamaica.

ANNEXES

ANNEX I

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ANNEX II

AGENDA

1. Opening of the meeting
2. Administrative arrangement
 - 2.1 Adoption of agenda
 - 2.2 Election of officers
3. Present state of development of CEPPOL Activity 4.4.3 CEPPOL
4. PAHO/WHO activities on health related aspects of marine pollution by micro-organisms in the Wider Caribbean
5. Presentation by the representative of USEPA Office of Research and Development
6. Presentation of the progress reports of the pilot studies under implementation
 - 6.1 Relationships and Decay Rates of Indicators in Micro-organisms of Coastal Marine Waters of the Costa Rican Caribbean Sea
 - 6.2 A Pilot Perspective Epidemiological Study to Assess the Health Risks Associated with Bathing in Recreational Waters of Trinidad
7. Special presentations on subjects related to the objectives of Activity 4.4.3
 - 7.1 Sanitary Quality of Water Sediments and Oysters from Coastal Lagoons in Tabasco and Campeche, Mexico
 - 7.2 Report on the consultation on Microbiological Quality of Coastal Recreational Waters (WHO/EURO Joint Project, MEDPOL Phase II), June 9-12 1993, Athens, Greece
 - 7.3 Coliphages as Alternate Indicators of the Microbiological Quality of Tropical Coastal Water

- 7.4 Sanitary Quality of Water in Bays and Coastal Zones in Cuba

- 8. Recommendations
 - 8.1 Types of Methodologies
 - 8.2 Guidelines/Standards Criteria
 - 8.3 Treatment and Disposal Systems
 - 8.4 Monitoring Programmes
 - 8.5 Additional Recommendations in Priority Order for Actions to be Implemented during the 1994-1995 Biennium
 - 8.6 Suggested Activities

- 9. Other Matters

- 10. Adoption of the Report

- 11. Closure of the Meeting

ANNEX III

LIST OF DOCUMENTS

Working Documents

UNEP(OCA)/CAR WG.12/1	Agenda and Timetable
UNEP(OCA)/CAR WG.12/2	Annotated Agenda
UNEP(OCA)/CAR WG.12/3	List of Documents
UNEP(OCA)/CAR WG.12/4	List of Participants
UNEP(OCA)/CAR WG.12/5	Report of the Meeting
UNEP(OCA)/CAR WG.12/6	Present state of development of Activity 4.4.3
NOAA, 1993	The National Indicator Study, prepared by The Interstate Shellfish Sanitation Conference for the National Oceanic and Atmospheric Administration, 1 July 1993

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Council Directive of 8 December 1975 concerning the quality of bathing water (76/160 EEC). Official Journal of the European Communities No. L31/1 - 4
2. WHO, 1977
Health criteria and epidemiological studies related to coastal water pollution, Athens, 1 - 4 March 1977 (English only)

3. UNEP/WHO/IAEA, 1985
The determination of total coliforms in sea water by the multiple test tube (MPN) method. Reference Methods for Marine Pollution Studies No. 21 (draft), UNEP 1985
4. UNEP/WHO/IAEA, 1985
The determination of faecal coliforms in sea water by the multiple test tube (MPN) method. Reference Methods for Marine Pollution Studies No. 22 (draft), UNEP 1985
5. UNEP/WHO/IAEA, 1985
The determination of faecal streptococci in sea water by the multiple test tube (MPN) method. Reference Methods for Marine Pollution Studies No. 23 (draft), UNEP 1985
6. WHO, 1990
Health risks associated with pollution of coastal bathing waters, WHO Geneva, Vol 43, No. 3, 1990
7. UNEP/WHO/IAEA, 1986
The determination of Staphylococcus aureus in sea water and sewage by the membrane filtration culture method. Reference Methods for Marine Pollution Studies No. 28 (draft), UNEP 1986
8. UNEP/WHO/IAEA, 1986
The determination of Pseudomonas auruginosa in sea water and sewage by the membrane filtration culture method. Reference Methods for Marine Pollution Studies No. 29 (draft), UNEP 1986
9. UNEP/WHO/IAEA, 1986
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10. UNEP/WHO/IAEA, 1988
Guidelines for monitoring the quality of coastal recreational and shellfish growing

- areas. Reference Methods for Marine Pollution Studies No. 1, Rev. 1, UNEP 1988
11. UNEP/WHO/IAEA, 1989
Statistical methods for the evaluation of results from monitoring the quality of coastal recreational and shellfish growing waters. Reference Methods for Marine Pollution Studies No. 55 (draft), UNEP 1989
 12. IOC/UNEP, 1990
Environmental Quality Criteria of Coastal Areas in the Wider Caribbean Region - A Compilation. IOC/UNEP-WQC-I/4, 1990
 13. IOC/UNEP, 1991
CEPPOL Regional Workshop on Coastal Water Quality Criteria and Effluent Guidelines for the Wider Caribbean region. San Juan, Puerto Rico, 5 - 15 November 1990
 14. IOC/UNEP, 1991
Report on the CEPPOL Seminar on Monitoring and Control of Sanitary Quality of Bathing and Shellfish-Growing Marine Waters in the Wider Caribbean, Kingston, Jamaica 8-12 April 1991. CEP Technical Report No. 9, UNEP Caribbean Environment Programme, Kingston 1991

**SECOND CEPPOL SEMINAR ON
MONITORING AND CONTROL OF
SANITARY QUALITY OF BATHING
AND SHELLFISH-GROWING MARINE WATERS
IN THE WIDER CARIBBEAN**

Kingston, Jamaica, 9-13 August 1993

REPORT OF THE MEETING