Statistical variation of Microbiological quality of coastal waters : Regulatory Implications*

by

R. MUJERIEGO, J. M. BRAVO, M. PINAS and B. SANCHEZ MURIAS

Environmental Health Service, Ministry of Public Health and Social Security, Madrid (Spain)

Abstract

A programme to evaluate the microbiological quality of the coastal waters has been carried out during 1979 at the MED-VII Pilot Zones of Malaga and Tarragona. The three indicator organisms considered were total coliforms, fecal coliforms and fecal streptococci. Microbial concentrations were measured by the membrane filtration technique. A total of 74 sampling stations were systematically monitored, 6-8 times during the February-May period, and 12-14 times during the June-September period.

From the results obtained the following conclusions can be drawn : **1.** The microbiological quality of coastal waters can be adequately interpreted by a lognormal probability distribution model, **2.** Correct compliance with any statistically expressed water quality standard requires comparison of the two probability distributions and not only two pairs of frequencies, **3.** Consistent application of presently accepted EEC standards classifies the vast majority of the sampling stations as unsatisfactory, **4.** The standard deviation of the concentrations of the three indicators approaches quite closely that implied by the WHO coastal water quality criteria, while notably disagrees with that implied by the EEC water quality standards, **5.** The standard deviation of a microbial indicator concentration, at a sampling station, is a useful and sensitive parameter for detecting discontinuous sources of pollution, **6.** A standard deviation estimate, derived from sets of 12-14 values, which lies outside the 1 to 3 interval can be likely associated to a singular water sampling station.

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Introduction

A programme to evaluate the microbiological quality of the coastal waters of Spain was established by the Environmental Health Service in 1977. The programme is operating within the framwork of the MED-VII Pilot Project, a part of the Mediterranean Action Plan, jointly coordinated by the United Nations Environment Programme (UNEP) and the World Health Organization (WHO).

Two MED-VII Pilot Zones were established in Malaga and Tarragona, where expensive studies have been carried out during 1979, thanks to the continuous support and encouragement of UNEP and WHO officials, as well as to a research grant from the United States-Spanish Joint Committee for Scientific and Technological Cooperation.

Objective

The main objective of the programme is to obtain statistically significant data, scientific information and technical principles to evaluate the degree of pollution at the beaches and coastal areas of Spain, particularly in those aspects related to public health.

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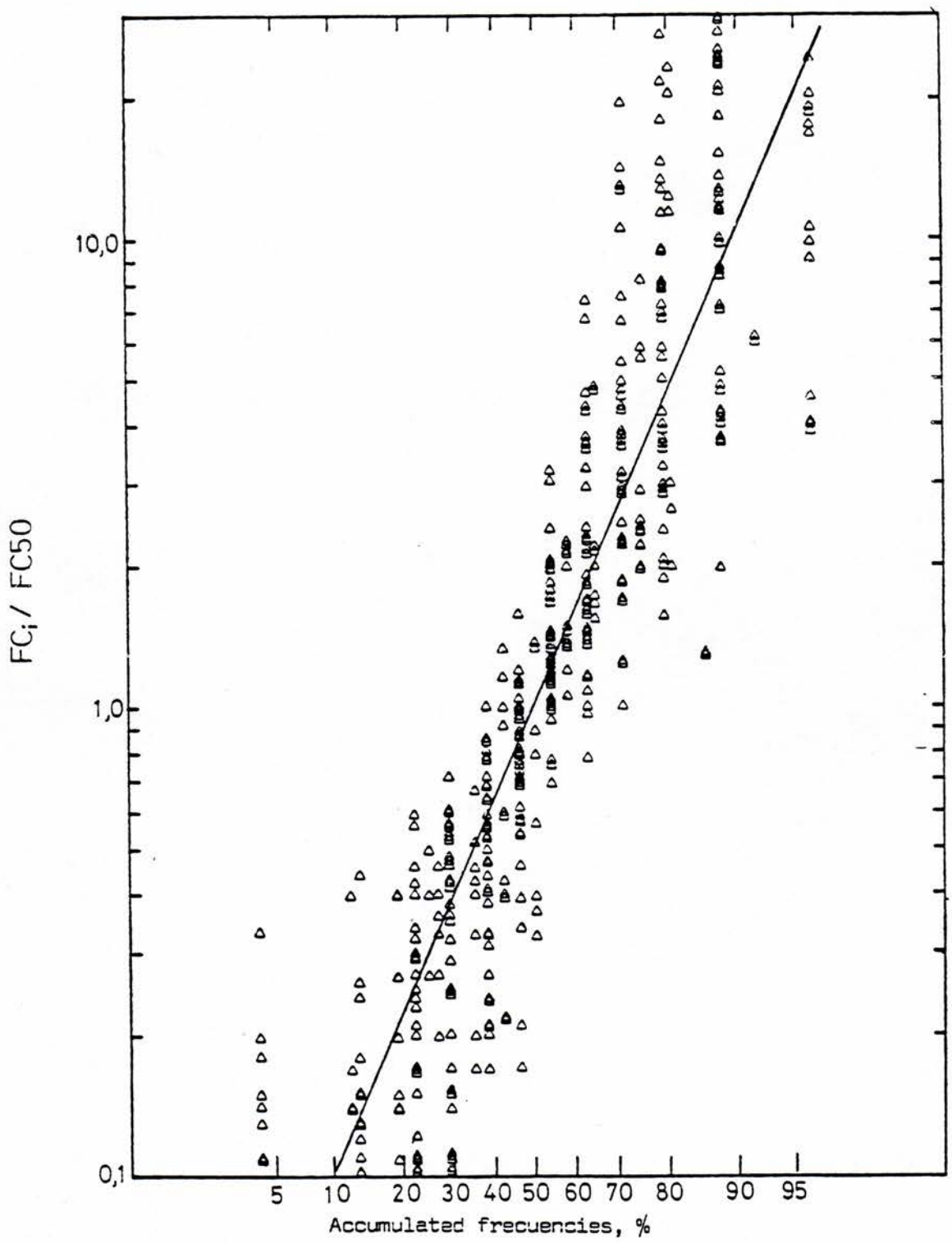

FIGURE 1 LOGNORMAL FREQUENCY DISTRIBUTION OF FECAL COLIFORMS CONCENTRATIONS, NORMALIZED BY SAMPLING STATION. COASTAL ZONE OF MALAGA, SUMMER 1979.

Some of the specific objectives of the programme are : **1.** to establish a statistical model of the microbial indicator concentrations, based on a systematic water quality monitoring programme, **2.** to appraise coastal water quality at the two Pilot Zones, based on existing microbiological criteria and standards, and **3.** to compare the experimentally observed variation of microbial indicators with that inherent to the criteria ans standards considered.

Experimental methods

Microbiological water quality was evaluated in terms of three microbial indicators : total coliforms (TC), fecal coliforms (FC), and fecal streptococci (FS). The method of analysis employed was the membrane filtration technique, according to the Guidelines for Health Related Monitoring of Coastal Water Quality (WHO, 1977 a), Biological Analysis of Water and Wastewater (1973) and Standard Methods (1975).

The filter membranes used for the three types of microorganisms were Millipore standard HAWG, with 47 mm diameter and 0.45 um pore size. The culture media and the incubation conditions were the following :

1. Total coliforms : M.-Endo MF broth, in absorbent pad placed in sealed 47 mm petri dish, and incubated in an oven at 35 C for 24 hours.

2. Fecal coliforms : M-FC broth, in absorbent pad placed in sealed 47 mm petri dish, and incubated in a thermostatic bath at 44.5 C for 24 hours.

3. Fecal streptococci : M-Enterococci Agar, in sealed 47 mm petri dish, and incubated in an oven at 35 C for 48 hours.

Statistical model

The statistical model considered for interpreting microbiological water quality has been a lognormal frequency distribution. Adjustment of the model to each sampling station data was graphically performed, using lognormal probability paper. The formula used to obtain the frequency associated to each microbial concentration was that of HAZEN (HAZEN, 1914) whose numerical expression is :

$$F(X_i) = \frac{i - 0.5}{n} \times 100$$

where :

$F(X_i)$: cummulative frequency associated to the i-th ordered observation.

i : order number of an observation.

n : total number of observations.

The straight line representing the cummulative frequency distribution of each set of observations was visually drawn, following the criterium of equalling the areas defined, at both sides of the line, by the imaginary polygonal connecting the central cloud of data points. Results gathered from more than 1.000 of these graphs show an excellent agreement between the model and the data, on top of the economy of time and effort of the visual method as compared to more elaborated techniques, such as the least squares method.

Water quality criteria and standards

The criteria and standards considered to assess the microbiological quality of coastal waters were the following :

1. The World Health Organization criteria (WHO, 1974, 1977 b). Established in terms of *E. Coli*, they can be expressed as EC50 = 100 EC/100 ml, and EC90 = 1 000 EC/100 ml.

2. The Spanish standards (MOPU, 1977). Established in terms of *E. Coli*, they can be expressed as EC50 = 200 EC/100 ml, and EC90 = 1 000 EC/100 ml.

3. The European Economic Community standards (EEC, 1975). Based on the general interpreta-

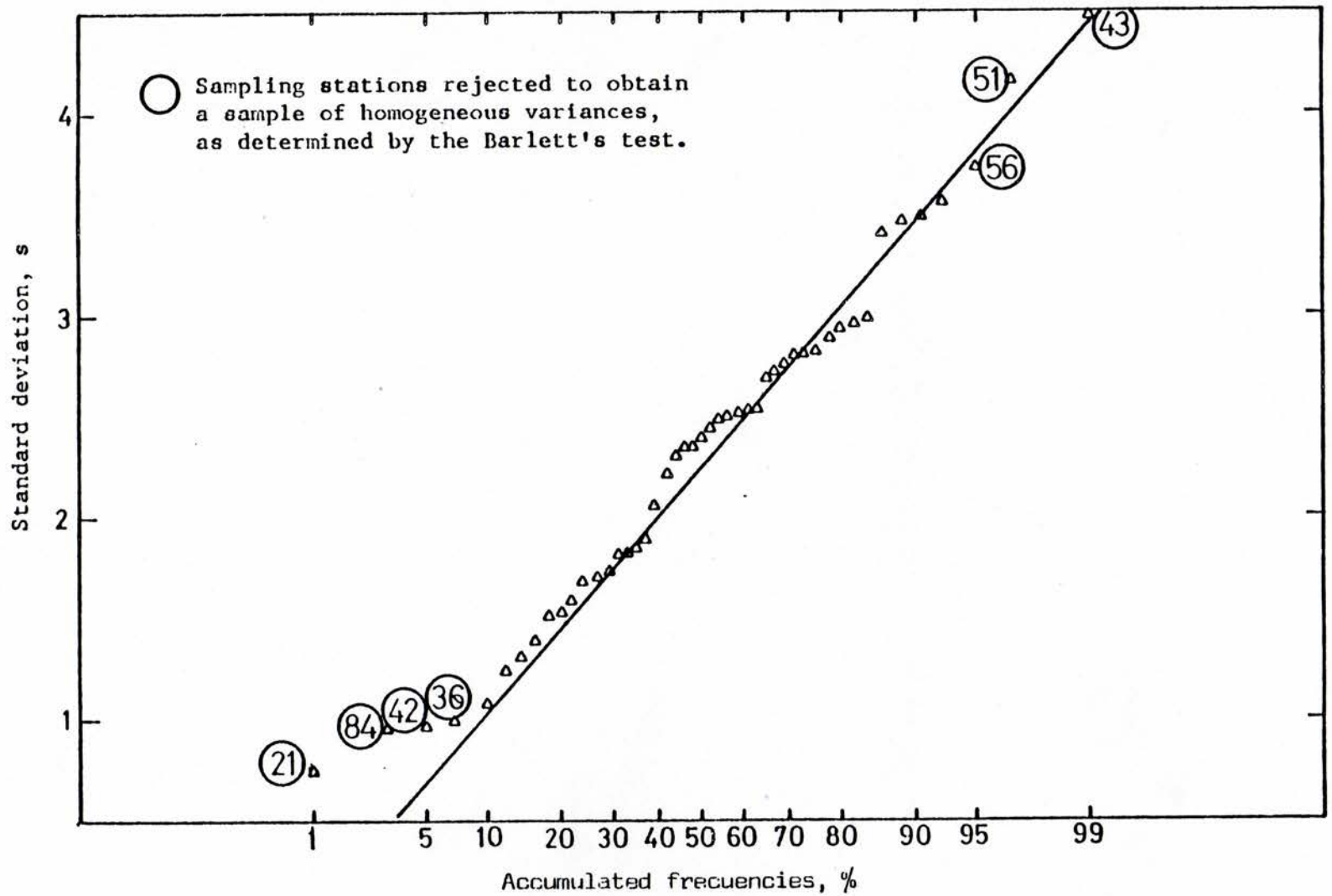

FIGURE 2 NORMAL FREQUENCY DISTRIBUTION OF THE STANDARD DEVIATIONS OF THE FECAL COLIFORMS CONCENTRATIONS. SAMPLING STATIONS AT THE COAST OF MALAGA, SUMMER 1979.

tion given by EEC members to the EEC Directive, they can be expressed as follows :

3 a. TC80 = 500 TC/100 ml, TC95 = 10 000 TC/100 ml.
3 b. FC80 = 100 FC/100 ml, FC95 = 2 000 FC/100 ml.
3 c. . FS80 = 100 FS/100 ml.

4. The California standard, established by the California State Department of Public Health (1943), and expressed as TC80 = 1 000 TC/100 ml.

Results and discussion

Statistical Model

The total number of sampling stations surveyed has been 74, from which 47 belong to the coast of Malaga and 27 to the coast of Tarragona. From the 47 sampling stations of Malaga, 3 are background water quality stations.

The experimental information gathered at each sampling station was classified according to two sampling seasons : **1.** the spring season, from February to May, and **2.** the summer season, from June to September. Sampling frequency during the spring season was twice a month, providing from 6 to 8 experimental results for each microbial indicator. Sampling frequency during the summer season was once a week, providing from 12 to 14 experimental results for each microbial indicator at each sampling station.

Water sampling was systematically carried out from 1 200 to 1 300 hours, from 15-20 cm below the water surface at points with water depth ranging from 1.0 to 1.2 m. These criteria represent a compromise between the accessibility to the sampling point and the time and place where most recreationists are.

Experimental data from each sampling station have been interpreted according to the proposed statistical model, providing a frequency distribution of each of the three microbial indicators considered. There has been an excellent agreement between the data and the proposed model. Some discrepancies arise at stations with microbial concentrations well below 100 microorganisms per 100 ml. The agreement between the data and the model improves then the number of experimental results increases from 6 to 14.

From previous results, it can be concluded that a lognormal frequency distribution, visually adjusted, is a practical method for accurately interpreting the microbiological quality of coastal waters.

Microbiological Quality of Coastal Waters

To establish a comparative criterium between water quality standards expressed in terms of *E. Coli* and in terms of fecal coliforms, 15 typical blue colonies obtained from fecal coliform analyses were biochemically identified (INVIC), resulting all of them to be *E. Coli*. Accordingly, and for the purpose of this study microbial concentrations in terms of *E. Coli* have been considered equivalent to microbial concentrations in terms of fecal coliforms (FC).

Tables I and II summarize the microbiological water quality at the sampling stations of Malaga and Tarragona during the 1979 summer season. Conformity with the criteria and standards has been obtained by two methods : 1) numerically, comparing the pair of frequencies of the standard with the two corresponding values of the sampling station, and 2) graphically, comparing the frequency distribution defined by the standard and that of the sampling station.

From the results summarized in Tables I and II the following conclusions can be drawn :

1. Consideration of the WHO criteria and the MOPU standards provides a classification of the sampling stations practically equivalent. This result was to be expected in view of the similarity of the WHO criteria and the MOPU standards.

2. Consideration of EEC standards provides a considerable different result depending on whether the numerical or the graphical method is used. This can be explained considering that, even if the two microbial concentrations at a sampling station are lower than those of the standard, the experimental frequency distribution may lie above that of the standard, at frequencies below those specified by the standard. As an illustration, the EEC fecal coliforms standard defines a frequency distribution whose FC50 value is close to 5 FC/100 ml, well below those of the WHO criteria.

Table I. — Microbiological quality of coastal waters. (Malaga 1979)

Walter Quality Criteria	Total	Number of beaches Satisfactory	
		Numerically	Graphically
Spring			
WHO, 1974	39	32 (82 %)	28 (72 %)
MOPU, 1977	39	32 (82 %)	31 (79 %)
EEC, 1975 (*)	39	25 (64 %)	15 (38 %)
California, 1943	39	—	32 (82 %)
Summer			
WHO, 1974	47	23 (49 %)	19 (40 %)
MOPU, 1977	47	23 (49 %)	21 (45 %)
EEC, 1975 (*)	47	8 (17 %)	1 (2 %)
California, 1943	47	—	18 (38 %)

(*) Without considering the fecal streptococci standard.

Table II. — Microbiological quality of coastal waters. (Tarragona, 1979)

Walter Quality Criteria	Total	Number of beaches Satisfactory	
		Numerically	Graphically
Spring			
WHO, 1974	27	24 (89 %)	24 (89 %)
MOPU, 1977	27	24 (89 %)	24 (89 %)
EEC, 1975 (*)	27	22 (81 %)	21 (78 %)
California, 1943	27	—	26 (96 %)
Summer			
WHO, 1974	27	26 (96 %)	26 (96 %)
MOPU, 1977	27	26 (96 %)	25 (93 %)
EEC, 1975 (*)	27	4 (15 %)	0 (0 %)
California, 1943	27	—	15 (56 %)

(*) Without considering the fecal streptococci standard.

3. While 95 % of the sampling stations at Tarragona can be considered satisfactory, according to the WHO criteria and the MOPU standards, only 50 % of the sampling stations of Malaga deserve the same classification.

4. Consideration of the EEC standards, in terms of total coliforms and fecal coliforms, gives very similar results for Malaga and Tarragona, with practically all the sampling stations being considered as unsatisfactory.

EEC Standards

A detailed analysis of the limiting character of each of the statistical parameters of the EEC standards, in terms of total coliforms and fecal coliforms, reveals the restrictions imposed by TC80 and FC80 are the most severe of the four considered, and particularly the TC80. Simultaneous consideration of the above two limits determines the overall classification of the sampling stations considered ; additional consideration of TC95 and FC95 does not modify the classification established by TC80 and FC80.

Temporal Variation of Microbiological Quality

The parameter used to evaluate the temporal variation of the microbiological quality of coastal waters has been the standard deviation of the corresponding lognormal frequency distribution.

The standard deviation « s » of the three microbial indicators considered has been estimated from the experimental data gathered at the Pilot Zones of Malaga and Tarragona, during the spring and the summer seasons of 1979. The standard deviations were obtained at two sampling levels : **1**. overall or coastal level, considering simultaneously all the data available at each Pilot Zone, and **2**. sampling station level, considering each sampling station independently.

1. Overall analysis

The joint analysis of data from sampling stations with quite different water quality has been carried out by previous normalization of the original data. The normalization process at each sampling station consists in dividing each microbial concentration by the median microbial concentration, obtained graphically from the corresponding lognormal frequency distribution.

The series of values thus derived were plotted in lognormal probability paper. The image emerging from this new cloud of points illustrates the « natural variation » investigated. Figure 1 illustrates the statistical behavior of the fecal coliforms concentration at the 47 sampling stations of the coast of Malaga, during the 1979 summer season.

A statistical analysis of the variances of the distributions associated with each value of the abscisa reveals the existence of conditions close to homocedasticity in most cases. Consequently, a regression by the least squares method was carried out without correction for lack of strict homocedasticity conditions.

Table III summarizes the values of the standard deviation associated to each of the overall data samples, for the three microbial indicators considered. Also included in Table III is the standard deviation inherent to the WHO criteria, the MOPU standards, and the EEC standards.

Table III. — Overall analysis of the standard deviation of the microbiological quality of coastal waters. Linear regression by the least squares method.

MED-VII Pilot Zone	Season	Microorganism	Number of points	Confidence Interval of the Standard Deviation (α = 0.05)
Malaga	Spring	TC	210	1.73 ± 0.12
Malaga	Spring	FC	196	1.73 ± 0.12
Malaga	Spring	FS	191	2.20 ± 0.20
Malaga	Summer	TC	433	1.75 ± 0.08
Malaga	Summer	FC	412	1.85 ± 0.12
Malaga	Summer	FS	458	1.56 ± 0.07
Tarragona	Summer	TC	304	1.44 ± 0.07
Tarragona	Summer	FC	194	1.49 ± 0.12
Tarragona	Summer	FS	244	1.47 ± 0.07
WHO criteria, 1974		FC		1.80
MOPU standards, 1977		FC		1.26
EEC standards, 1975		TC and FC		3.74
$s = \ln XX84 - \ln XX50 = \ln XX50 - \ln XX16$				

Tableau IV — Confidence intervals of the standard deviations of the microbiological water quality at a sampling station. Normal distribution model.

Microbial Indicator	Spring			Summer		
	Stations	Data per Station	Confidence Interval of « s » (α = 0.05)	Stations	Data per Station	Confidence Interval of « s » (α = 0.05)
Malaga						
TC	39	8	2.10 ± 0.60	47	12	2.20 ± 1.20
FC	22	8	1.70 ± 0.60	47	12	2.20 ± 1.90
	16	8	2.65 ± 1.10			
FS	39	8	2.95 ± 1.90	47	12	1.85 ± 1.00
Tarragona						
TC	23	6	1.75 ± 1.80	27	14	1.50 ± 1.00
FC	9	6	2.50 ± 3.20	27	14	1.55 ± 1.20
FS	19	6	1.85 ± 1.60	27	14	1.75 ± 0.80

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

From the results summarized in Table III the following conclusions can be drawn :

1. Microbiological quality of coastal waters, in terms of total coliforms, fecal coliforms, and fecal streptococci, follow satisfactorily a lognormal frequency distribution, within a given coastal area.

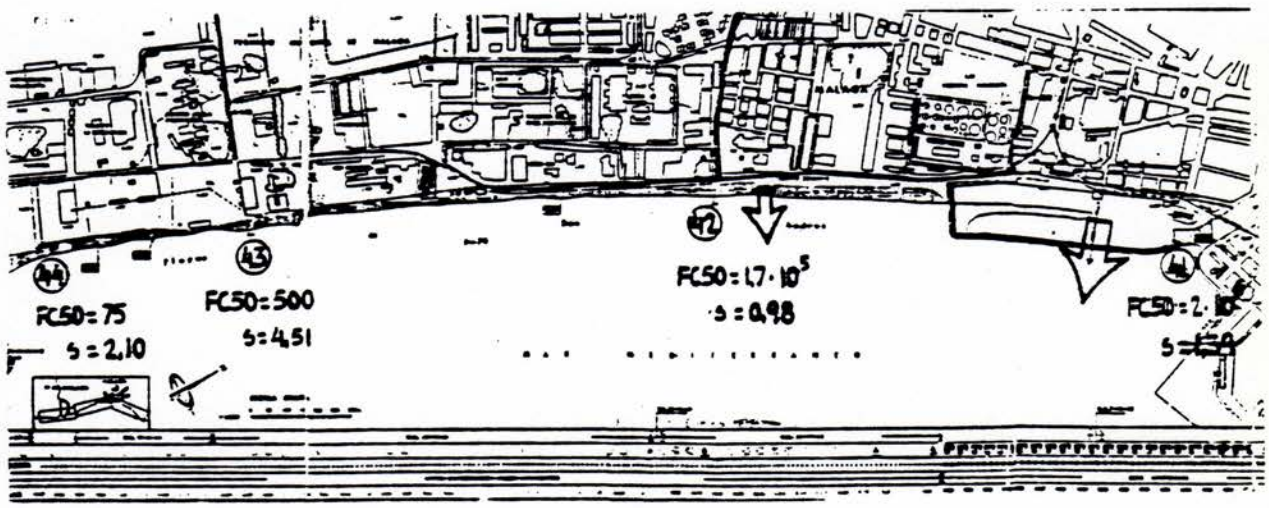

Figure 3 - Effects of wastewater disposal location on the microbiological quality of coastal waters, measured in terms of the median fecal Coliforms concentration and its associated standard deviation. Sampling stations at the coast of Malaga, Summer 1979.

2. The « natural variation » of these microbial indicators, measured in terms of their overall standard deviation, varies from 1.6 to 2.2 in the coast of Malaga, and is close to 1.5 in the coast of Tarragona.

3. The above values are much closer to the variation inherent to the WHO criteria than to either that of the MOPU or the EEC standards.

4. The standard deviation inherent to the EEC standards, for total coliforms and fecal coliforms, are notably higher than those experimentally observed. This observation, together with aspects previously described, explains the highly restrictive character of the EEC coastal water quality standards.

2. Statistical distribution of standard deviations

A precise estimate of the overall standard deviation has been obtained in the previous section because of the large data sample available, from 200 to 400 microbial concentrations for a given coastal area. However, when the data sample is much smaller, its standard deviation will be expected to vary within a much large confidence interval. The practical question arising then is to determine whether ther is a significant difference between several standard deviation estimates, with the same order of magnitude, and coming from different sampling stations.

The theoretical background considered to study this point has been the following :

1. To assume that the microbial concentration observed at a sampling station, during a given season, belongs to a single population, normally distributed with parameters (μ, δ^2).

2. To consider that the standard deviation « s » of samples of size « n », taken from the previous population at different sampling stations, approximately follows a normal distribution with parameters (δ, $\delta^2/2(n-1)$) (HALD, 1952).

Figure 2 illustrates the excellent agreement between the experimental values and the proposed normal model. Similar results obtained for the two other microbial indicators studied (MUJERIEGO *and coworkers*, 1980) indicate that the standard deviation of the microbiological quality of coastal waters, during a sampling season, approximately follows a normal distribution, throughout the whole coastal area.

Table IV summarizes the confidence intervals of the standard deviation, derived by the normal distribution model, for each microbial indicator. The considerable amplitude of these intervals is a direct consequence of the relatively small number of data values available from each sampling station.

Figure 2 also illustrates the desagreement frequently observed, at the tails of the normal distribution, between the experimental data and the proposed model. An analysis of variance reveals that rejection of the « s » values identified in Figure 2 results in a sample of standard deviations considered to be homogeneous by the Barlett's test. Consequently, the remaining « s » values are not significantly different from each other, and can be considered as originated from a single normal population (μ, δ^2).

Table V summarizes the statistical parameters of the fecal coliforms concentration at the sampling stations identified in Figure 2. Also included in Table V are some physical characteristics of these stations. An analysis of Table V reveals that : **1.** standard deviation values close to 1 are consistently associated with sampling stations either of excellent microbiological water quality or in the vecinity of a major sewage discharge, and **2.** standard deviation values above 3 are consistently associated with sampling stations located at the boundary zone of major sewage discharges. Figure 3 illustrates the case involving stations 42 and 43 from the Pilot Zone of Malaga.

Results from a similar analysis carried out for total coliforms and fecal streptococci, on the coast of Malaga during the 1979 summer season, reveals that any standard deviation value outside the approximate interval 1 to 3 is most likely associated with a singular water sampling station. Values below approximately 1 would correspond to either very clean or extremely unsatisfactory coastal waters. Values above approximately 3 would correspond to coastal areas under the influence of nearby sources of pollution, with either a highly variable rate of discharge or a systematic interference from coastal water circulation patterns.

Table V — Statistical parameters of fecal Coliforms concentrations at selected sampling stations of the coast of Malaga. Summer 1979.

Sampling Station	Fecal coliforms, FC/100 ml			Notes
	FC50	FC90	s	
21	18	47	0.75	No discharge in proximity
84	10	35	0.95	No discharge in proximity
42	$1.7\ 10^5$	$6.0\ 10^5$	0.98	800 m from Guadalmedina sewer (0.5 m^3/s)
36	2	7	1.00	Small protected beach
81	3	13	1.10	No discharge in proximity
56	470	62 000	3.75	1 000 m from broken sewage outfall (approximately O.05 m^3/s)
51	$2.5\ 10^4$	$5.5\ 10^6$	4.16	Guadalhorce River mouth (approx. 0.1. m^3/s)
43	500	$1.6\ 10^5$	4.51	2 500 m from Guadalmedina sewer (0.5. m^3/s)

$s = \ln FC84 - \ln FC50 = \ln FC50 - \ln FC16$

Conclusions

From the results obtained during the programme of study carried out in 1979 at the MED-VII Pilot Zones of Malaga and Tarragona, the following conclusions can be drawn :

1. Microbiological quality of coastal waters, in terms of total coliforms, fecal coliforms, and fecal streptococci, can be adequately interpreted by a lognormal probability distribution model.

2. Correct compliance with any statistically expressed water quality standard requires comparison of two probability distributions, and not only two pairs of frequencies.

3. Consistent application of presently accepted EEC coastal water quality standards classifies the vast majority of sampling stations as unsatisfactory.

4. The standard deviation of the three microbial indicator concentrations approaches quite closely that implied by the WHO coastal water quality criteria, while notably disagrees with that inherent to the EEC standards.

5. The standard deviation of the concentration of a microbial indicator, at a sampling station, is a useful and sensitive parameter for detecting discontinuous sources of pollution.

6. A standard deviation estimate, derived from a set of 12-14 microbial indicator concentrations, which lies outside the 1 to 3 interval can be likely associated to a singular water sampling station.

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