PHYSICAL DISPERSION AND DISAPPEARANCE OF BACTERIA IN THE *GOLFO DI PALERMO*: THE RESULTS OF TWO SURVEYS

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Abstract - This paper reports on some results of two surveys at sea carried out in the surroundings of a urban wastewater discharge on the coast of the *Golfo di Palermo*, western Sicily (Italy). At the time of the surveys (year 2005) the stretch of water lying before the central part of the capital town received the untreated wastewater originating from about 200 000 inhabitants, which was discharged on-shore without any prior treatment by the free-surface outfall of "Porta Felice main sewer".

This outfall has crucial importance in the water quality; indeed, the Municipality is steadily implementing a plan featuring an intercepting main sewer along the coast and some pumping stations to connect all the main sewers to the main wastewater treatment plant, located in the SE boundary area of the town. At the moment of the surveys, however, no mitigation measure had been applied yet and the quality of the Gulf was still largely affected by it.

Part of the Sanitation Plan was the characterization of the seawater; to this aim, in August and November, 2005, the *Università degli Studi di Palermo* - on behalf of the Municipality's *Ufficio del Centro Storico* - carried out two survey cruises in which the most important seawater quality features were investigated. What will be reported on herein is the part dealing with microbiological indicators, taking the salinity field as background.

Introduction: outfall configuration and discharge data. The survey cruises.

Until year 2005 – when the surveys were made – the free-surface outfall of the "Porta Felice main sewer" (Figure 1) discharged on-shore the wastewater originating from 200 000 inhabitants of the historic quarters of the town of Palermo. The surrounding area is relevant for tourism and residential activities and is located at the mouth of the trade harbour. Such conditions increase the importance of the expected benefit of the measures for improved urban drainage and sanitation, planned by the Municipality.

An uninterrupted circle of mountains shields the Gulf from the winds blowing from W-SW sectors; more, winds from E - NE are not beneficial. Therefore, currents and waves are often driven in a pattern that severely reduces the pollutants dispersion in the sea. Unfavourable sea currents patterns can establish themselves such as in the maps of Fig. 2.

Figure 1 - The coast site investigated.

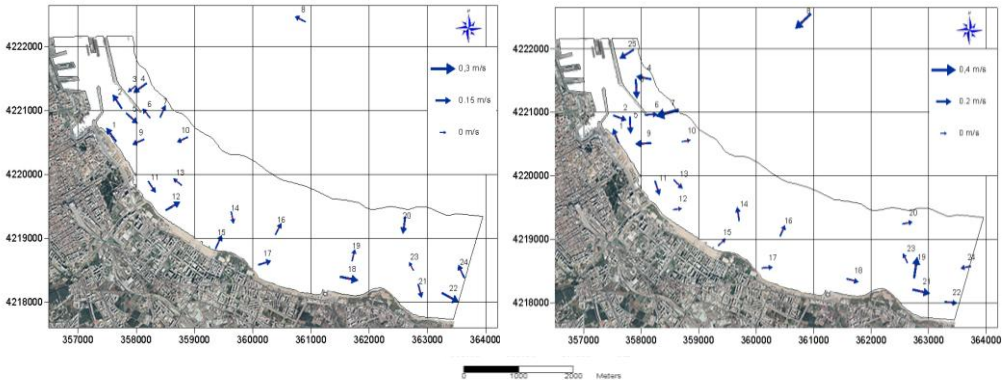

Figure 2 – Sea currents in the *Golfo di Palermo*, measured at 0,50 m below sea surface during the cruises of August (left) and November (right), 2005. Speeds do not exceed 0.2 m/s anywhere. *(Current meter VALEPORT mod. 106)*

The cruises were based on 25 measuring stations making up an array distributed in the stretch of water in front of the city (about 1 km^2 wide) and were carried out in wind calm days in early August and late November, 2005. One of the stations (ID–8) was positioned over 2 nautic miles away from the coast, in order to obtain a *blank sample* to which relate all measures.

Figure 3 shows the distribution of the measuring stations. The stations in which only chemical-physical parameters were investigated *in situ* are marked with "M" as *Misura* – Measure; the stations in which, furthermore, a sample was also taken for laboratory analysis are marked with "CM", where C stands for *Campionamento* - Sampling. All of them lie on transects (the orange segments) purposely drawn as significant.

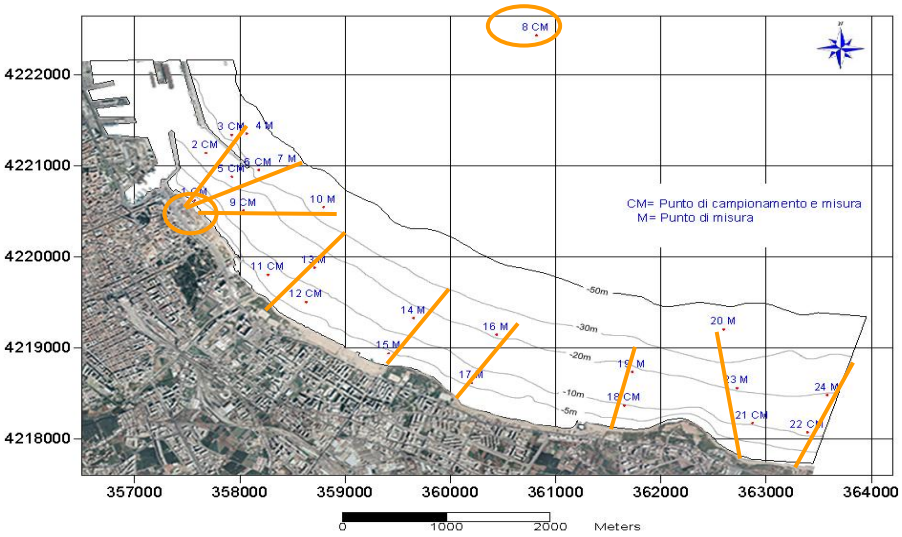

Figure 3- Distribution of the measuring stations. The *Porta Felice* on-shore outfall and the farther offshore sampling station, ID-8, are circled in yellow.
(Ship bearing: GPS MAGELLAN Meridian Platinum)

Two multi-parametric probes (an IDRONAUT Ocean Seven 301 and a DKK mod. WQC—24) were used to measure temperature, dissolved oxygen (DO), conductivity, salinity, turbidity, redox potential both at surface (50 cm below sea level) and at 2 meters depth. In 14 stations out of 25 a water sample was also taken for each depth intended for laboratory analyses aiming to the most important bacteria and the nutrients.

Figure 4 shows the water temperature profiles in August (left) and in late November (right). The effect of heat loss towards atmosphere and of wave motion on thermocline disruption in November is quite evident, as well as the mild temperatures still holding.

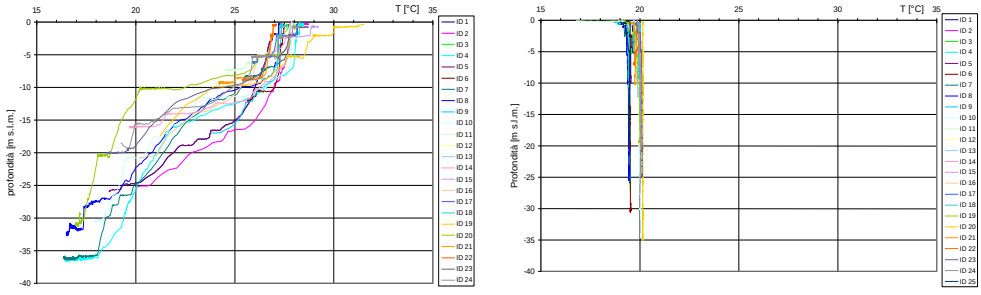

Fig. 4- Thermal profiles versus depth at sea, in Summer (left) and in Autumn cruise (right).

The necessity obviously arose of organizing the great amount of information given by the field campaigns, and also to represent the collected data in a format easy to understand. To this aim the software SURFER® (Golden Software Inc., Golden, CO, USA) was applied and made possible to generate a set of maps representing the distribution of each parameter at each depth, in the stretch of water investigated during each cruise.

Materials and methods for sampling and examination

The samples for microbiological examination were collected - as customary - with disposable sterile wide-necked bottles of 500 ml at depth about 0,5 m below sea level and then stored in the boat's refrigerator. The samples were brought to the laboratory in an insulated bag and kept at 4 °C until the analysis, which were started within 6-8 hours.

In each sample four parameters were analyzed, namely total coliforms (TC), faecal coliforms (FC), *Escherichia coli*, and enterococci (EC). They were selected in agreement with Italian rules on sea water quality and with the recommendations of WHO, in which *Escherichia coli* and EC are regarded as exhibiting a higher correlation with outbreaks of swimming-associated gastroenteritis than FC.

About *Escherichia coli*, it has long been believed that it can only survive for short time in the environment (4 - 6 weeks), so is almost universally used as an indicator of recent water fecal contamination. On the other hand, EC - typical microorganisms of faecal origin included in the larger group of fecal streptococci - show prolonged survival in adverse environmental conditions, especially in marine waters. This property makes them suitable as long-range tracers of wastewater discharges, and was confirmed in these cruises.

The microbiological analysis were performed using the method of membrane filtration (MF) for Colony – Forming Units (CFU). Water samples, after repeated stirring, were filtered through cellulose nitrate sterile membranes (diam. 47 mm, pore diam. 0.45 □m). The membranes were then transferred to different selective agar media. Two replicates of water samples (50 ; 10; 1; 0.1; 0.01; and 0.001 ml, according with the species, the sampling site and the turbidity of the sample) were filtered.

Total coliforms- Endo Agar LES was used to count coliform bacteria in water by MF. The coliform bacteria produce red colonies with a metallic (golden / green) sheen within 24 ±2 hours incubation at 36 ±2°C. In case of suspect colonies confirmatory tests were made with miniaturized biochemical systems (API 20 E, bioMérieux).

Faecal coliforms - For FC, m-FC with Agar Rosolic Acid was used in cultivating and counting within 24±2 hours incubation at 44.5±2°C. Colonies of FC usually are various shades of blue. Faecal coliforms (i.e., those found in the feces of warm-blooded animals) are differentiated from those coming from environmental sources by their ability to grow at high temperatures. Again, in case of suspect colonies confirmatory tests were made.

***Escherichia coli* -** TBX Agar (Tryptone Bile X-glucuronide medium) was used to count *E. coli* in water by MF within 18-24 hours of incubation at 44°C. The blue and green-blue colonies were counted as presumptive *E. coli.* In case of suspect colonies, confirmatory tests were made as above.

Enterococci - Slanetz-Bartley Medium Agar was used. When incubated at 44 – 45 °C, all red or brown colonies are confirmed as faecal Streptococci. The membranes that had given a positive presumptive result were transferred to a dish with Bile Esculin Azide Agar,

and the plates were incubated at 44 ±0.5°C for 2 hours. After incubation typical colonies (brown-black surrounding medium) were counted as intestinal enterococci.

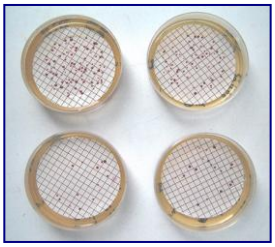

Fig. 5 - Enterococci colonies on Slanetz Bartley Agar.

Some results of the two surveys.

The thematic maps in Figure 6 show in a quantitative pictorial way the results of the microbiological tests performed on samples from the summer survey, put in log scale. Just 2 sampling points were not included in the correlation: ID 8, where all the bacterial counts performed gave zero, confirming the role of "negative control" assigned to it in the research plan; and the extra sample ID-1-bis (visibly, a heavily contaminated one) taken in the bay near ID 1, whose values were in the order of 10^5 for all the four species.

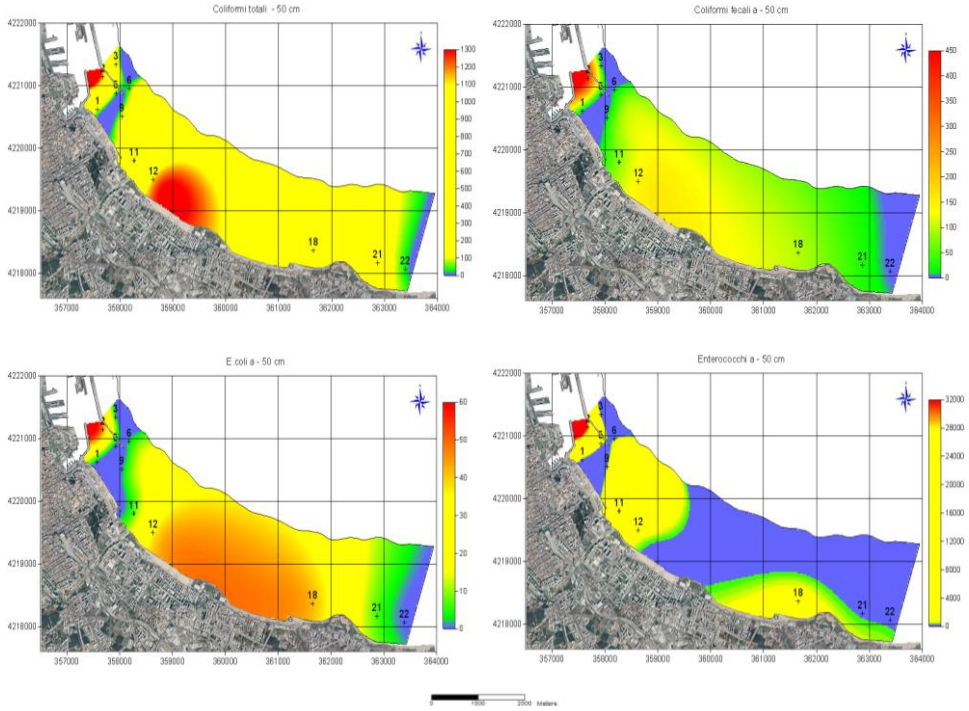

Figure 6 – Thematic maps of microorganisms concentrations found in the summer survey.

The general agreement is good, except for an unexplained low number of enterococci in ID-12, where all other three indicators were high. How predictable, the most heavily contaminated samples were those taken at the trade harbour entrance (site ID 2) and those on either side of the mouth of the "*Oreto*" river (ID 11 and 12). It is interesting that microbiological contamination of the sea is negligible in front of the outfall of the WWTP "*Acqua dei Corsari*" (ID 22), where the values of coliform bacteria 500 m off the coast fell largely into the limits prescribed by the regulations.

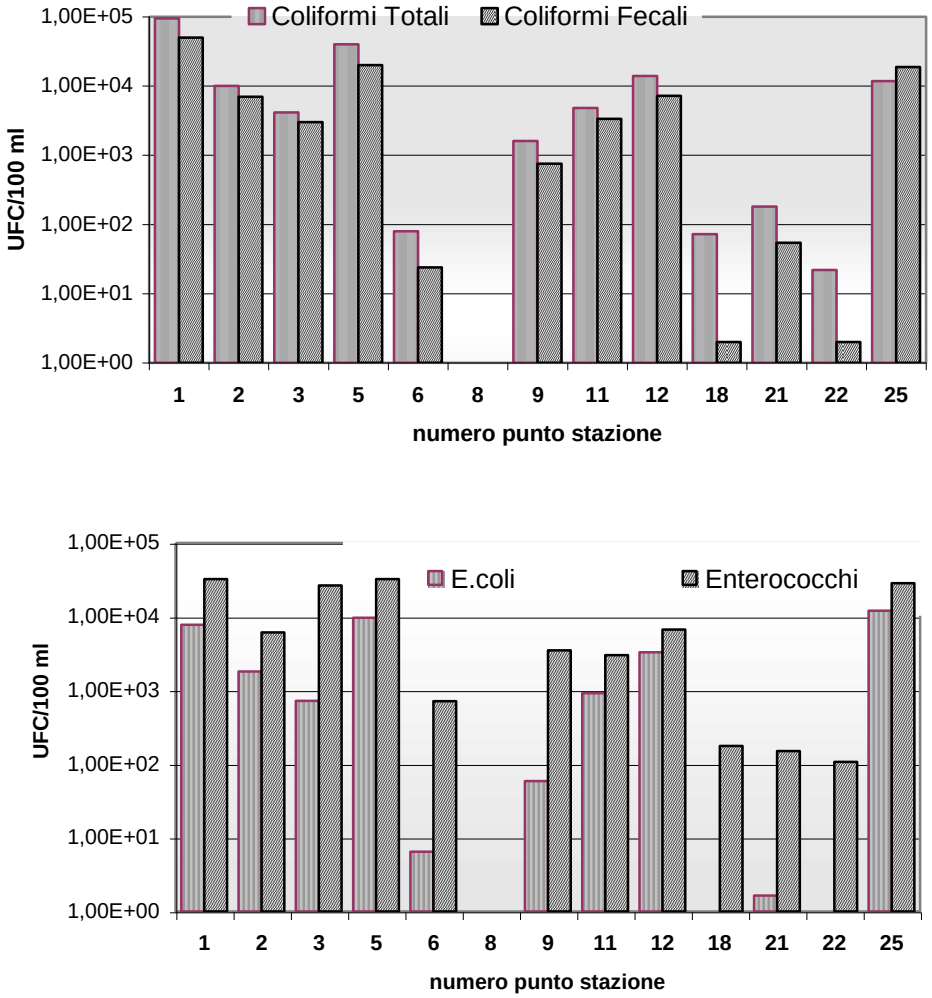

Fig. 7 – Example of Total and Faecal coliforms (top) and *E. coli* and enterococci (bottom); autumn cruise.

While *E. coli* concentrations were always recorded at values lower than 50 CFU/100ml, those of enterococci were always higher, indicating the greater capacity of survival of them. These results are in agreement the studies that demonstrate the importance of enterococci as most suitable indicators of fecal contamination at sea.

For easy comparison the count results in autumn cruise were also drawn - as specimens - in the histograms of Figure 7 (preceding).

Table 1- Coefficients of linear correlations of 3 bacterial species to total coliforms, TC.

	August 2005			**November 2005**		
Log CF =	1,07	x Log TC	-0,98	1,29	x Log TC	-1,37
Log E. coli =	1,04	x Log TC	-0,83	1,33	x Log TC	-2,1
Log EC =	1,53	x Log TC	-1,22	0,74	x Log TC	+1,04

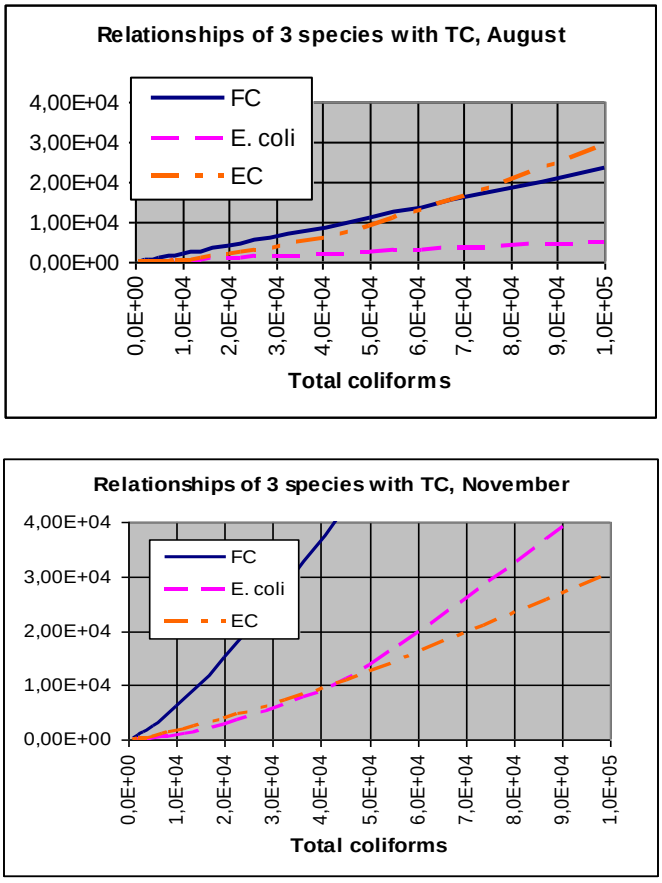

Fig. 8 – The 3 selected bacterial species versus total coliforms in the two cruises.

A linear correlation was also attempted between the 4 bacterial strains and species counted in the samples. Correlation coefficients found were fair to good (R = 0.45 to 0.90); the expressions are arranged in Table 1 and are exponential, as graphs in Figure 8 show.

Discussion

Bacterial concentrations measured in autumn cruise were generally higher by 1 log than those in summer, reaching almost 10^5 CFU for coliforms and enterococci in several sampling points. This may be explained with some concurrent facts:

1- in this season the town is fully populated; also, more frequent rainfall increases flows in sewers, which leads to regular flushing and particulate matter conveying;

2- lower temperatures (although still mild: 18 – 20 °C) and weaker solar radiation cause slower microbial decay at sea. This is especially favourable to *E. coli;*

3- turbulence of the water in the autumn campaign was greater than that of summer campaign: it is likely therefore that there occurred a re-suspension of sediments and micro-organisms attached onto them. Even in this case, however, the concentrations of faecal coliforms in the stretch of sea in front of the "*Acqua dei Corsari"* municipal WWTP, around the outlet point of its near-shore discharge pipe, remained low.

The next step in the interpretation of these results will be attempting to correlate the ratios between the four bacterial species counted with either salinity or turbidity measured in the corresponding stations at sea.

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References

IRSA-CNR - Metodi analitici per le acque, Sezione 6000 and Sezione 7000. APAT Manuali e Linee Guida 29/2003,Rome, Italy (**in Italian).**