The "SAM" integrated system for coastal monitoring

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Abstract

Funded by the Italian Ministry for Scientific Research, the multidisciplinary research programme "SAM" (Advanced Systems for automatic monitoring of the marine coastal pollution) aims at the installation of an integrated coastal monitoring network and at the development of new instrumentation and rapid analytical procedures to be applied in nowcasting and water quality assessment.

The new strategies here described (analysis methods and automatic devices) provide reliable and useful tools for the control of seawater pollution and, more in general, for the management and sustainable use of coastal zones. Flexibility in the assembly of the technical instrumentation makes the SAM systems a versatile equipment for environmental surveillance.

Research of new methods for the rapid estimation of faecal pollution microbial indicators has been addressed towards the optimisation of an immunofluorescence staining assay and the definition of an analytical procedure for the determination on liquid medium of the beta-glucuronidase activity of *Escherichia coli* involving the use of a fluorogenic substrate. The immunofluorescence and enzymatic protocols proposed suggest simple, rapid and sensitive alternative ways for the specific detection of $E. coli$ in natural waters.

The application of the protocols developed to the analysis of field samples collected in March-April 2001 has highlighted the advantages of the two methods, both of them providing near real-time results that correlate significantly with conventional plate counts.

1 Introduction

The assessment of the anthropic impact over the coastal environment represents the prerequisite for any action aimed at protecting the water quality from

microbiological and chemical contamination. Due to the many origins and forms of water pollution, more knowledge is needed in order to establish appropriate remediation plans. Coastal environments are mainly affected by faecal contaminants which arrive indirectly via rivers or directly from the discharge of urban wastes into the sea. Unfortunately, the limited self-depuration capacity of aquatic environments has widely been exploited by man, so that it has become more and more urgent to preserve natural resources from irreversible damages.

Progress made in the field of the technologies for environmental monitoring allows the use of advanced methodologies and systems for the evaluation of the occurrence and extent of pollution phenomena.

The SAM "Advanced Systems for Coastal Waters Monitoring" project, funded by the Italian Ministry for Scientific Research, aims to set up an integrated coastal monitoring network and to develop new analytical protocols and devices to be used as a tool for coastal management. In particular, one of the key topics of research is the increased vigilance against microbiological contamination through the development of rapid methods for the accurate estimation of microorganisms indicators of water quality as powerful and efficient alternatives to traditional culture methods. The first objective of the study focused on the establishment of a network comprising coastal buoys and a little boat for on-line monitoring. The second research approach of the programme was the evaluation of rapid methods (immunofluorescence and enzymatic fluorogenic assay) for microbiological monitoring of faecal pollution in seawaters.

2 Materials and methods

2.1 The monitoring network

The advanced technology systems used in the SAM program are based on the know-how obtained in the past decade during the CNR Strategical Project for automatic pollution monitoring in southern Italy seas, and PRISMA2, now further developed and updated in order to build an integrated approach for coastal water quality monitoring [1, 2, 3].

The network comprises fixed monitoring platforms and a small boat equipped for continuous water monitoring. Two kinds of platforms are used, both about 8 square meters width: a triangular shaped, deriving from the refitting of a platform that was installed in Messina Straits since 1996, and a rectangular shaped, obtained redesigning and resizing a prototype produced by CEOM (a Company of the ENI Group) (Figure 1).
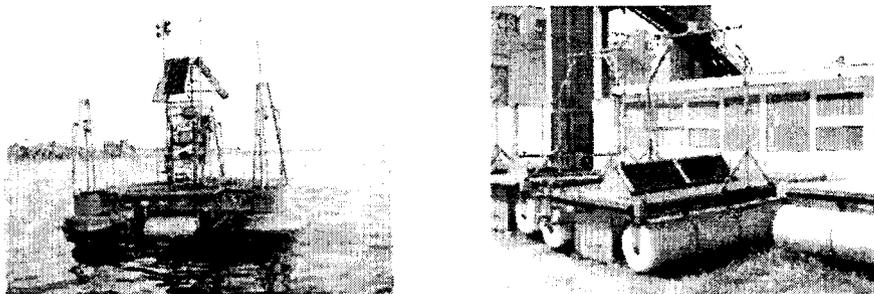
Figure 1:   The Messina platform in its mooring (left) and one of the other
platforms during its fitting (right)

The platforms host the following instrumentation:
- Data Acquisition and Communication System
- Meteo Station equipped with temperature, pressure, solar radiance, wind direction and speed sensors
- Idromar system for pumping water samples from 5 depths, equipped with IM50 CTDO turbidimeter-fluorometer
- Systea colorimetric nutrient probe analyzer
- Custom designed water multi-sampler for microbiological laboratory analysis
- 5 SBE 39T in situ probes at the same sampling depths

The Messina platform is equipped also with a Nortek Aquadopp 600 ADCP and a subsurface IM50 CTDO probes.

Electronic devices used, based on PC104 bus, have showed good efficiency and low power consumption; several models of cellular GSM modems were tested, selecting a dual band device capable of data transmission via SMS. This operating mode, in conjunction with services of data routing toward e-mail addresses, enables to locate the Data Acquisition Centre wherever preferred, with great functional and economic advantages. The system is schematized in Figure 2.
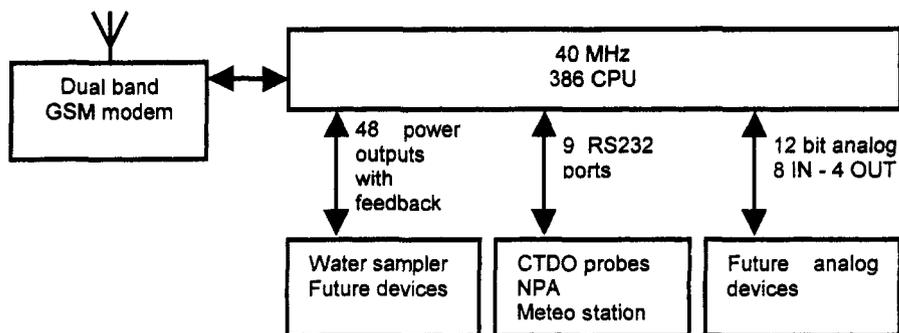

Figure 2: The Acquisition and Communication System block scheme

The software, written in Microsoft Professional Compiled BASIC v 7.1, enables to fully control the platform instruments both in local and in remote mode using a special set of macro commands. An embedded "sequence manager" starts each hour a programmed sequence that can be different for each time of the day and is remotely reprogrammable.

The pumping system was designed by Idromar for use on platforms; it pumps sea water from five depths into a measurement chamber, where an IM50 CTDO probe with fluorometer and turbidimeter sensor is fitted. The water can be extracted to feed the NPA probe and the automatic water sampler. At the end of the measurement cycles the chamber is washed with fresh water.

The autonomous water sampler was designed and built for use on platforms; it can fill eight 200 ml bottles, adding also a fixative to prevent degeneration of the sample for further laboratory analysis; after the sampling, the fluid circuit is washed with fresh water. It is possible to remotely program and control all sampling events.
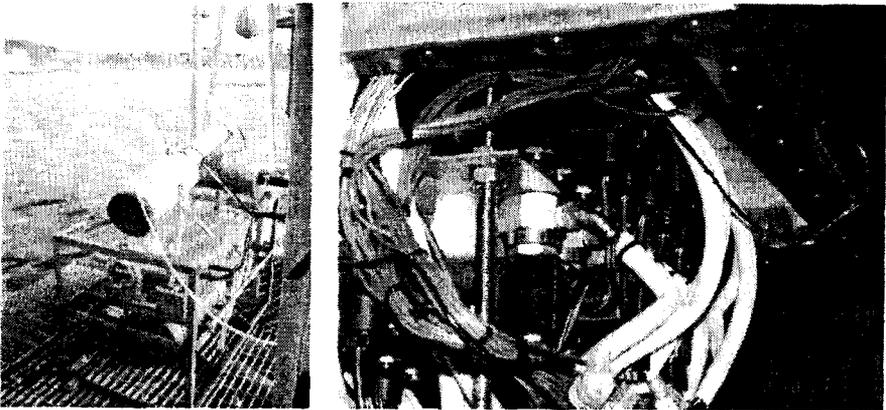

Figure 3: The pumping system with the probe on its top (left) and a close-up view of the water sampler collectors and valves (right)

The Nutrient Probe Analyzer (NPA) was designed by Systea for automatic nutrient monitoring; the instrument measures in few minutes ammonia, nitrate, nitrite and ortophosphate using a colorimetric method; in the near future, each NPA will be equipped with an external buffer tank, in order to speed up the measurements at the different depths, so enabling to investigate zones having fast variations in the water column.

The mooring sites chosen for the platforms are the Straits of Messina, the Gulf of Milazzo , Palermo, Siracusa, and Mazara del Vallo.

The research boat (15 meters overall length) is equipped with sampling and measuring instruments for biological, coastal oceanography and monitoring researches and contains one "wet" and one "dry" laboratory. It is fitted with:

- SBE 32C rosette with 12 8 liters bottles and SBE 911plus CTDO probe equipped with altimeter and SCUFA fluorometer and turbidimeter
- System for continuous monitoring on surface waters, comprising a SBE 19 plus CTDO probe, SCUFA integrated fluorometer-turbidimeter, Systea nutrient analyzer Micromac fast NP3
- 2000 Kg Hydraulic crane hosting an hydraulic winch with 300 m of 6 mm armoured cable and 200 m of 8 mm iron rope

Data collected are included in a database in a format complying with those stated at international level to facilitate oceanographic data exchange. Software procedure will enable integration of data over space and time in order to establish a monitoring network with expanding potentialities.

2.2 The microbiological methods

A total of 32 surface samples were collected during oceanographic surveys performed in March-April 2001 in three coastal areas (Gulf of Palermo, Gioia Tauro, Straits of Messina) differently impacted by anthropic inputs.

For microscopic counts, samples were fixed and treated according to the immunofluorescence (IF) assay previously tested in our laboratory [4]. Preliminary laboratory experiments were also performed in order to optimise the conditions of the assay in terms of concentration of reagents and times of incubation. Briefly, 100ml volumes of water were filtered through a 0,22μm Nuclepore black membrane and further treated with a 1:40 dilution (in phosphate buffered saline, PBS) of Murex *E.coli* agglutinating sera (mix of 2+3+4 pools), specific for enteropathogenic and enteroinvasive *E.coli* serotypes. After labeling with a 1:80 dilution of goat anti-rabbit IgG conjugated with fluorescein isothiocyanate (FITC), filters were counted under a Zeiss Axioplan epifluorescence microscope equipped with filter set BP 490, FT 510 and LP 520. Cells specifically labelled by fluorescent antibodies appeared to be rod-shaped and with green outlines.

Another experimental approach followed for the indirect estimation of the presence of *E.coli* in seawaters was an enzymatic assay involving the use of the fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide for the detection of beta-glucuronidase. This enzyme is specifically present in *E.coli* and *Shigella* species and its measure is a function of the amount of the fluorescent end product (methylumbelliferone) released by the substrate hydrolysed after incubation with the sample. A protocol [5] was especially developed and applied to the analysis of the samples above collected; it relied on the addition of increasing amounts (from 10 to 50μM) of fluorogenic substrate to 10ml subsamples and on the spectrofluorometric measurement of the fluorescence released after 3 hours of incubation at 44°C. Through calibration with known concentrations of the standard methylumbelliferone (MU), the fluorescence values were converted into the potential rate of hydrolysis of the substrate by beta glucuronidase and

expressed in terms of maximum velocity of hydrolysis (Vmax, in nM of MU released per liter and per hour).

Immunofluorescence and enzymatic data were compared with the values of faecal coliforms (FC) as estimated by membrane filtration and counting on m-FC (Difco) medium.

3 Results

As a sample of the capabilities of the system, measurements taken at 1 hour intervals from the 5 SBE probes of the Messina buoy are reported in Figure 4; it is easy to see a good similarity among the depths in detecting the arrival of different sea water masses (characterised by different temperatures), in correspondence of the tidal phases, with some unhomogeneities recorded near the surface layer, probably due to the arrival of water from a prospicient urban sewage.
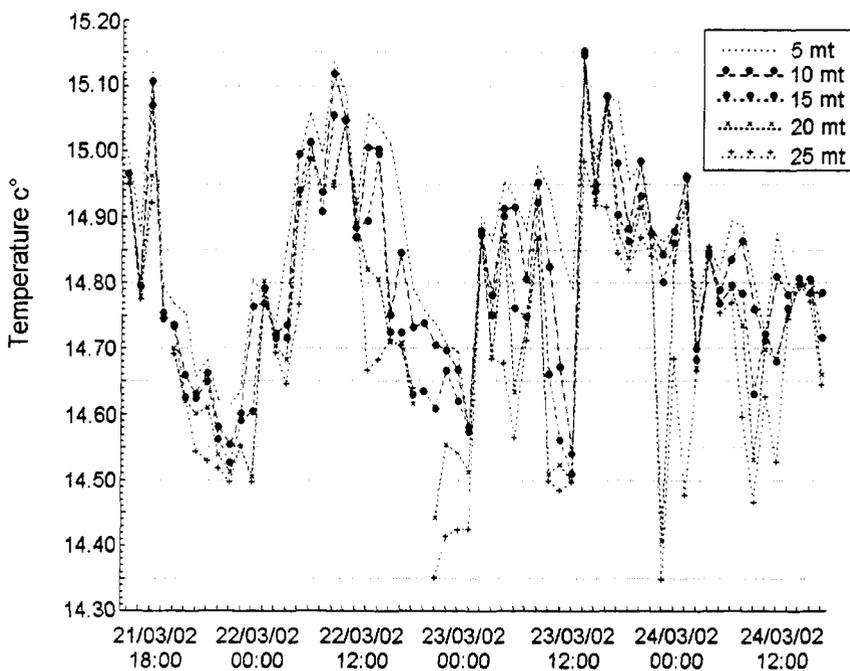

Figure 4: Water temperature measured at five depths by the buoy "Messina" during three days of its test phase

For what concerns the laboratory methods, some preliminary microbiological data obtained during the sites characterization phase are now presented. Results of the analyses (Figure 5) have shown the presence of a widespread contamination in the areas analysed, with average values of faecal coliforms (FC) and *E.coli* (IF) equal to 6.6×10^2 CFU 100 ml^{-1} and 2.6×10^4 cells 100 ml^{-1} respectively.

Among the three areas examined, low levels of pollution were detected in Gioia Tauro and Palermo Gulf, on average in the order of 10^2-10^4 cells 100ml^{-1}. The Straits of Messina suffered of a heavier microbial pollution, ascribable to urban wastes. In the greater percentage of the stations sampled, *E.coli* concentrations overcame the standard values prescribed by Italian laws for recreational purposes, reaching a maximum of 5.2×10^3 CFU and 2.3×10^5 cells 100ml^{-1}.
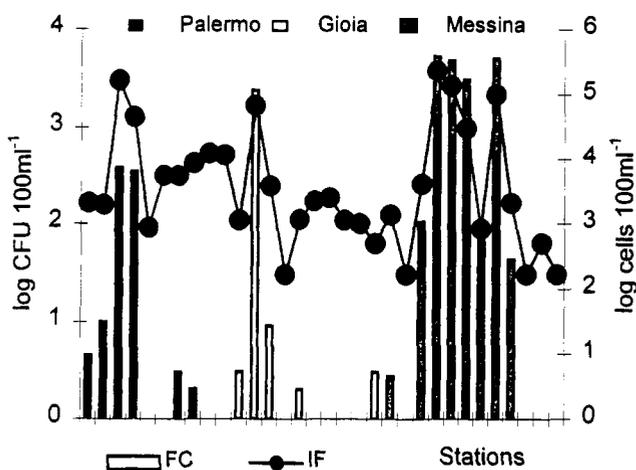

Figure 5: Immunofluorescence (IF) and plate (FC) counts obtained on samples examined. Grids distinguish samples collected from different sites

The discrepancy of about two order of magnitude between microscopic and culture counts is consistent with the ability of the immunofluorescence method to estimate also viable non-cultivable bacteria which could be not otherwise evaluated. However, the analysis by linear regression ($R^2 = 0.6228$) confirmed the actual agreement of data obtained with the two methods.

The immunofluorescence results proved this technique to be rapid and convenient for the direct determination of *E. coli* in filtered water samples by epifluorescence microscopy; the method provides quantitative information about the distribution of specific markers in the natural environments. Also observation may be repeated by keeping filters at –20°C. Statistical Student's test (t=0.011, n=32) confirmed the high degree of agreement between values obtained by

immunofluorescence and reference methods; however, due the high amount of debris in natural waters, the step of concentration of samples by filtration may limit the sample volume to be analysed.

Also enzymatic data are comparable to conventional culture counts (t=0.003, n=32), encouraging the application of the method in the early detection of heavy and mean pollution episodes. Using both methods, specific responses are available in near real-time (2-4 hours), with sensitivity thresholds of 10^2 cells per 100 ml of water.

Comparison by linear regression between the $\log_{10}$ transformed enzymatic data versus the $\log_{10}$ transformed immunofluorescence values and enzymatic data versus faecal coliform values (Figure 6) revealed that enzymatic values correlated with IF counts ($R^2=0.561$, n=32) more significantly than with faecal coliform counts ($R^2=0.272$, n=32) suggesting the higher selectivity of the enzymatic assay for *E.coli* rather than for faecal coliform group on the whole.
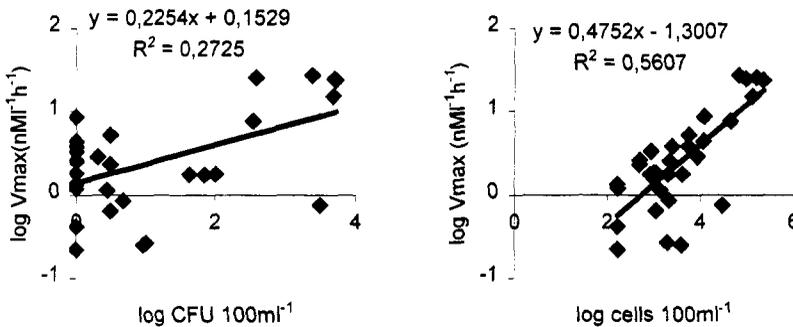

Figure 6: Plots of enzymatic activity values versus plate counts (left) and
enzymatic activity versus immunofluorescence counts (right)

4 Discussion

The potential of integrated systems for environmental monitoring constitutes an attractive alternative to current approaches of identifying and recovering human impacted marine sites. The two main research lines under execution at the Istituto Sperimentale Talassografico of Messina are mainly based on the set up of new technologies and analytical methods for the study of coastal water quality levels.

As far as the first monitoring line is concerned, the availability of new technological devices has allowed to design and built a new generation of data acquisition and transmission equipments, which matches low costs with good performances in terms of power consumption, size and reliability. The instrumentation developed overcomes the well-known limitations of traditional equipments in the long term acquisition of time series of environmental data.

The second research line concerns the assessment of the presence of faecal bacteria, as microbiological markers of contamination. As conventional methods are inadequate to provide rapid information on the microbiological quality of seawaters, new techniques are currently under development to be applied to routine seawater analysis which allow water-borne diseases control through the day-to-day pollution monitoring. Combined with basic environmental parameters, microbial data can provide insight into the factors affecting faecal-indicator organism concentrations in coastal waters. The methods proposed in this paper allow the detection of sewage sources with precision and rapidity; their results correlate significantly with conventional plate counts.

Due to the actual interest on coastal pollution and preservation of environmental health, and taking into account the potential of automatic systems for monitoring and recovery of polluted marine environments, the research approaches proposed here meet on social, politic, and healthy common problems so that they are promising for future developments and open new perspectives.

The instruments and systems here described are the first developed in Italy for automatic and real time monitoring of marine waters. The SAM system offers multivariate potential applications, from pure research to the environmental management; it provides an effective management tool in assessing compliance of waters with microbiological quality standards. The final user of the system could have scientific objectives (oceanographers, modellers), as well as environmental management ones (local authorities, fisheries, sea-farmings, touristic activities). The availability of advanced instrumentation and methodologies is particularly useful in emergency situations, when the efficacy of remediation measures depends on time required for environmental assessment.

The network will represent the most flexible and integrated monitoring system available and operating at this time along the Italian coasts. Work for technical implementation of new equipments is constantly in progress to add new sensors and techniques for more easy and complete understanding of the environmental processes.

Acknowledgements

References

[1] Zappalà, G., Crisafi, E., Caruso, G., Azzaro, F., Magazzù, G., Coastal monitoring by an advanced technology platform. *Proc. of Oceanology International 98 - The Global Ocean,* I, pp. 69-84, 1998.

[2] Zappalà, G., Alberotanza, L., Crisafi, E., Assessment of environmental conditions using automatic monitoring systems, *Proc. of MTS-IEEE Conference Oceans '99 - Riding the Crest into the 21st Century,* II, pp. 796-800, 1999.

[3] Zappalà, G., Advanced technologies equipments for environmental monitoring in coastal areas. *Science Technology Synergy for Research in the Marine Environment: Challenges for the XXI Century*, eds. L. Beranzoli, P. Favali & G. Smriglio, Elsevier: Amsterdam, 2002.

[4] Zaccone, R., Crisafi, E. & Caruso, G., Evaluation of fecal pollution in coastal Italian waters by immunofluorescence. *Aquatic Microbial Ecology*, **9(1)**, pp. 79-85, 1995.

[5] Caruso, G., Zaccone, R., Crisafi, E., Monticelli, L., Rapid detection of *Escherichia coli* in coastal waters by use of the fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide: preliminary results. *Rapports et procès-verbaux des Réunions de la CIESM*, **35**, pp. 342-343, 1998.