Editorial

Microbes and their use as Indicators of Pollution

Gabriella Caruso*

Institute for Coastal Marine Environment (IAMC), Italian National Research Council (CNR), Spianata S. Raineri, 86, 98122 Messina, Italy

Two thirds of the Earth is covered by water and about 99.7% of water found on this planet is contained in the oceans and seas. Marine ecosystems are an important resource not only for the extraordinary variety of life they host but also for the multiple activities that occur in them (i.e. recreational and productive activities like tourism, aquaculture and fisheries; maritime shipping and traffic; exploitation of energy resources like oil and gas extraction; pipelines and submarine cables location; uses for military activities, and others). The anthropic impacts on the marine environment are particularly evident in coastal areas, which are the most vulnerable to pressures [1], and are exposed to urban, agriculture runoff and industrial discharges - with their load of organic and inorganic particles, xenobiotics and other contaminants, oil spill, heavy metals, etc - with consequent impairment of water quality and alterations of ecosystem functioning. The awareness of the increasing threats caused by the anthropic pressure over marine ecosystem explains the growing interest towards the protection of marine environments, which has recently been shown by the issue of the European Marine Strategy Framework Directive (2008/56/EC) [2].

This Directive deals with the set up of monitoring plans and the adoption of measures for the achievement of Good Environmental Status (GES) by 2020. The GES is the environmental status of marine waters under which the ecological diversity and viability of the seas and oceans are preserved, in order to keep them clean, healthy and productive; the use of the marine environment must be in a sustainable way, safeguarding its potential for uses and activities for present and future generations. The definition of GES varies according to the different sub-regions (i.e. for the Mediterranean Sea, the Western Mediterranean Sea, the Adriatic Sea and the Ionian and Central Mediterranean Sea). Specific criteria and indicators are set to achieve qualitative and quantitative targets, acting on the pressures and in order to reduce the impacts. The Marine Strategy is based on an integrated "ecosystem" approach and aims at becoming the environmental pillar of the future Maritime Policy of the European Union. Preservation of the Mediterranean waters from contamination is particularly needed due to the central role of this basin for most of the European, African and Middle East Countries.

The use of natural resources must match with the maintenance of optimal productivity levels and health status. To achieve such an objective, it is needed to identify some sustainability limits of marine ecosystems, which must be respected in all human activities.

Safeguard and protection of areas at risk must rely on the continuous observation of parameters describing the health status of each marine ecosystem. In this context, monitoring of biological indicators is suggested, as they play a fundamental role in the description and prediction of environmental changes. Particularly, the use of marine microorganisms is recommended to assess the quality of marine ecosystems, in parallel with physical and chemical parameters, due to their ubiquitous distribution in seawaters, high biomass turnover and quick response to environmental variations [3]. Bacteria or their antigens, genes or metabolic pathways can be used as suitable markers of contamination [4].

This note focuses on some "in situ" applications developed at the IAMC Messina which are suggested to give insights on two different features of marine contamination:

a) The determination of bacterial indicators of faecal pollution through the set up and application of rapid analytical methods.

b) The assessment of microbial metabolic activities and of the changes in their rates in response to the presence of contaminants or organic inputs (i.e. organic polymers as substrates undergoing microbial decomposition).

This last topic (letter b) investigates the variations in some microbial enzymatic activity rates, such as leucine aminopeptidase and alkaline phosphatase, induced by the presence of organic (allochthonous or autochthnous) inputs or contaminants (i.e. hydrocarbons), in order to propose their suitability as markers of ecological quality and potential indicators for early warning of eutrophication and oil spill events.

Focusing on the point a) the microbiological quality of coastal waters is usually estimated by determination of faecal indicator bacteria. The development of rapid protocols for the detection and enumeration of faecal pollution indicators within the bacterial community inhabiting natural waters has been the objective of the research performed during the last decades at IAMC-CNR in Messina; this topic is of great interest for human health preservation from hygienico-sanitary risks. Microscopic counts give a quantitative estimate of the whole microbial community, which do not provide detailed information of its specific composition. Immunological methods (like the fluorescent antibody method) offer a labour- and cost-effective approach to detect and identify target bacterial cells directly "in situ", according to their morphological and antigenic properties. This method has found several applications in marine and brackish environments, also using automatic devices [5,6] for the search of selected species of microorganisms, which holds a hygienico-sanitary significance, like faecal pollution indicators, Escherichia coli [7-12] or Enterococcus faecium [13] or bacteria that are pathogens to humans (Salmonella spp. [14]) or animals (Vibrio anguillarum and Photobacterium damselae subsp. piscicida [15]). It is also well known that a consistent percentage of bacteria may overcome adverse environmental conditions entering a "viable but not culturable" state, VBNC), which is recognised as a survival strategy; fluorescent antibody method can be combined with enzymatic assays or viability stains to assess the pathogen viability [16]. Microscopic E. coli counts can be used to fed and validate provisional models to follow the fate of fecal pollution indicators at sea [17,18].

Concerning the point b) microbes and their metabolic activity

*Corresponding author: Gabriella Caruso, Institute for Coastal Marine Environment (IAMC), Italian National Research Council (CNR), Spianata S. Raineri, 86, 98122 Messina, Italy, E-mail: gabriella.caruso@iamc.cnr.it

can also be used as sensitive markers of environmental perturbations. Thanks to the spectra of hydrolytic enzymes they are equipped with, microorganisms are able to catalyse the hydrolysis of organic polymers, playing a key role in the biogeochemical cycles and regulating the matter and energy fluxes within the marine ecosystems [19,20]. As the microbial community is able to diversify its metabolic spectra in relation to the different nature and composition of organic polymers available in the waters, the determination of enzyme activities has been suggested as a simple tool to characterise the ecological status of transitional areas [21,22] as well as to assess soil health [23]. Enzymatic activities involved in organic matter transformation are functional attributes of the microbial community, that are responsive to environmental changes; the detection of significant relationships between some enzymatic activities (leucine aminopeptidase, alkaline phosphatase and beta-glucosidase) and abiotic or biotic indicators suggests the use of enzymatic activity measurements as additional parameters to characterise the quality status of natural ecosystems, with particular reference to their trophic conditions. Significant correlations found between leucine aminopeptidase and alkaline phosphatase activity rates and Particulate Organic Carbon or Chlorophyll-a as descriptors of the trophic state and productivity of aquatic ecosystems indicate both these enzymes as suitable and quick markers to discriminate different water bodies according to their trophic state. Microbial enzyme activities could be proposed as sentinels to detect the presence of organic inputs [24-26] as well as those derived from fish farming [27]. Monitoring microbial enzymatic activity rates could be particularly important in coastal areas, where high organic matter available supports bacterial viability and stimulates enzyme synthesis; here, all enzymes act synergically to degrade new organic compounds produced by photosynthesis..

Microbial metabolism of organic polymers could be successfully applied also to follow the evolution of the microbial community in terms of structure (specific composition) and functions (degradation patterns) [28]. Cappello et al. [29], in microcosms artificially added with hydrocarbons in order to simulate oil spill occurrence, found significant enhancements in the rates of leucine aminopeptidase and alkaline phosphatase after exposure to oil.

Increased leucine aminopeptidase and beta-glucosidase activity rates have been measured in oil-contaminated surface seawater from the Deepwater Horizon oil spill during a bioaugmentation/biostimulation experiment [30]. Although not specifically related to oil degradation, the determination of such microbial enzymatic activities active in the decomposition of organic polymers such as proteins, polysaccharides and organic phosphates, is equally important because it gives insights on the effects induced by oil and bacterial amendment on the bacterial community able to breakdown these organic substrates in aquatic environments.

On the other hand, organic compounds can also be transformed by microbial degradation and decomposition [31] and the rates of microbial activities are conventionally estimated for soil quality assessment [32]. Alkaline phosphatase inhibition assays have been proposed as early warning signals of toxicity due to heavy metals and phenolics in freshwater environments [33]. Besides AP, other enzymatic activities (like leucine aminopeptidase, sulfatase, chitinase) are useful probes for assessing ecological damages due to organic halogens in sediments contaminated by industrial discharges [34]. In coastal areas heavily affected by anthropic inputs, Misic et al. [35,36] reported the occurrence of high enzymatic activity rates on organic polymers.

In conclusion, other than common ecological quality indicators (macrophytes, diatoms, macrobenthos and fish) also the microbial community deserves to be investigated due to its functional role. The above reported examples are only some methodological approaches based on the use of microbes and their associated molecules as bio-indicators in the marine environment monitoring. Both the protocols of fluorescent antibody method and enzymatic activity assays represent suitable tools to assess the anthropic pressure on marine ecosystems; they can be provided to the scientific community and to local Administrators or stakeholders as rapid methods for environmental controls in alternative to the conventional ones.

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