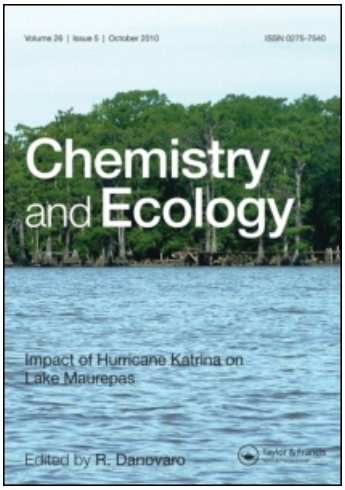

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Luminescent bacteria as indicators of marine water quality: Preliminary results from the Campania coastal waters

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Luminescent bacteria as indicators of marine water quality: Preliminary results from the Campania coastal waters

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This study reports preliminary results on the concentration of marine heterotrophic bacteria (Apparent Bacterial Concentration, ABC), and their luminous fraction (Apparent Luminous Bacterial Concentration, ALBC), here expressed as percentage of luminous bacteria (LB%), in coastal waters along the Campania shoreline. The results show that the Campania coastal area includes sectors characterised by different levels of water quality, following a geographical gradient from north to south. LB% appears related to inputs of pollution, showing low values in contaminated areas (<4%), and higher values in unimpacted waters (8–38%). In most cases, low values of this parameter were associated with high concentrations of faecal coliforms, suggesting that the LB% index is particularly sensitive to organic pollution. The pattern of water quality as estimated by LB% is in a strong agreement with previous results obtained by applying, in the same areas, other kinds of approaches, such as trophic indexes and algal bioassays. However, the main limit of LB% is that it should be used in the frame of a site-dependent approach: it cannot be used to identify absolute conditions of 'good' and 'bad' water quality, but just to highlight the extent of the 'deviation from integrity' characterizing each site in relation to the whole study area.

Keywords: apparent bacterial concentration (ABC); apparent luminous bacterial concentration (ALBC); faecal coliforms; water quality; Campania coastal waters

1. Introduction

In Italy, public concern about the environmental quality of seawater essentially deals with the possible negative effects on human health, via the exposure to pathogenic agents while bathing or the ingestion of contaminated seafood.

With the exception of a few acts aimed at the protection of aquatic environments, such as Law 979/82 [1] ('*Sea Defence'*) and Law 349/91 [2] ('*Outline Law on Marine Protected Areas*'), most laws are based on aesthetical and sanitary criteria (e.g. D.p.r. 263/97, [3]), without considering that any change or damage to aquatic ecosystems could indirectly cause problems to humans, as in the case of contamination of seafood products [4].

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One of the best ways to assess water quality is the use of bioindicators, which permits to obtain rapid responses on the effects of pollutants in marine ecosystems [5].

Previous studies have used heterotrophic marine bacteria and their luminescent fraction to characterise the quality of aquatic environments, basing on their peculiarity to rapidly change their concentration in response to inputs of pollution [6–9]. However, the use of the luminescent fraction as a bioindicator still remains controversial [6,10], despite the fact that toxic substances undoubtedly produce an inhibition of light-emitting biochemical processes [4], and that organic pollutants increase the number of total bacteria; both effects causing a decrease of the luminescent fraction.

The main aim of this study was to test the effectiveness of the use of the 'Apparent Bacterial Concentration' (ABC) and its luminous fraction, the 'Apparent Luminous Bacterial Concentration' (ALBC), here expressed as the percentage of luminous bacteria over the total (LB%), as an indicator of the quality of coastal waters along the Campania shoreline. The term 'apparent' indicates that data may underestimate the real bacterial concentration of the samples, because only a small fraction of total indigenous bacteria is capable of developing colonies [8].

The results obtained by applying the LB%-based approach were also compared with the concentration of faecal coliforms.

2. Materials and methods

2.1. *Study-area and sampling*

Twenty–five stations were selected along the Campania shoreline, in the Southern Tyrrhenian Sea (Figure 1); they were located at a maximum distance of 500 m from the coast and had a maximum water depth of 10 m.

Sampling was carried out in both summer (May–October 2003) and winter (November 2003–February 2004), and sampling sites were chosen as follows:

- Nine along the 'Litorale Domizio': five stations between the Garigliano and Volturno river mouths (sts. VG 1÷5, on 12 November 2003), and four between Regi Lagni and the Volturno outfalls (sts. RV 1÷4, on 28 May and 5 November 2003);
- Four in proximity of Licola (sts. LI 1÷4, on 3 December 2003);
- Four in the Miseno lagoon (sts. MI 1÷4, on 17 June 2003);
- One near the Pozzuoli promenade (st. PO, on 8 May, 14 October, 9 December 2003, 12 and 27 January and 3 and 18 February 2004);
- Two along the coastal area of Torre Annunziata (sts. TA 1,2, on 13 January 2004);
- Five along the Salerno shoreline (sts. SA 1÷5, on 12 May 2003).

2.2. *Estimate of ALBC and ABC and faecal coliforms*

Sampling of marine waters was carried out with sterile glass bottles, 15 cm below the surface. Two replicates were sampled at each site, stored in refrigerated containers (4°C) until arriving in Lab and processed after 3–6 h from collection [4].

For each replicate, aliquots of 1, 5 and 50 ml of the samples were filtered on sterile, 0.45 μm pore size cellulose ester membranes, 47 mm in diameter (Millipore). Filters were plated on Seawater Complete (SWC) Agar Medium [11], prepared with 750 ml of seawater, 250 ml of distilled water, 3 ml of glycerol, 5 g of peptone, 0.5 g of yeast extract, 1 g of sodium carbonate (or 2.7 g of sodium carbonate decahydrate) and 200 g of agar, and sterilised in an autoclave at 121°C and 100 kPa for about 15 min.

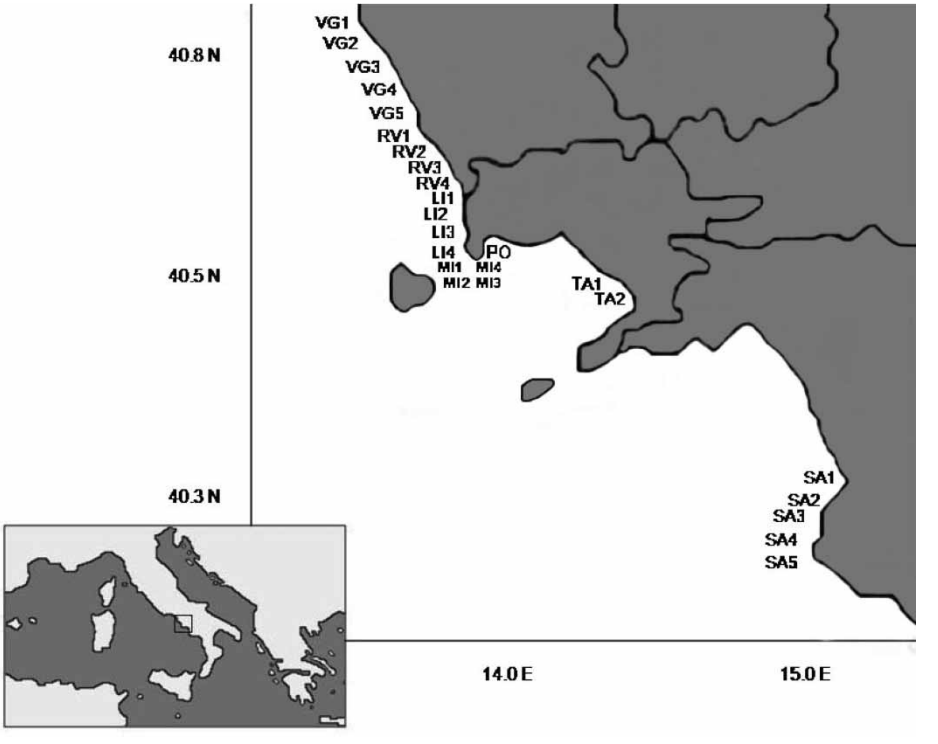

Figure 1. Sampling sites along the Campania shoreline.

Plates were incubated for 48 h at 18°C and colonies were then counted:

- in the dark, to obtain the ALBC;
- in the light, to obtain the ABC.

Both were expressed in colony forming units (CFU).

Plates with more than 300 CFUs were not considered as statistically significant, as it is the maximum number of colonies supported by the filters [8]. Hence, to process samples collected in areas with high levels of faecal contamination, a dilution with distilled water was made.

100 ml filtered water samples were cultured on membrane Faecal Coliforms (mFC) Agar medium at 44°C to obtain the concentration of faecal coliforms; blue colonies were counted after 24 h.

2.3. *Bacterial identification*

Tests of bacterial identification were carried out in the station of Pozzuoli. Some luminous colonies were isolated from the plates and resuspended in a solution of deionised water and tryptone; the samples obtained in this way were filtered and incubated at 18°C, both on standard SWC and on agar mediums modified with the addition of five different sources of carbon (maltose, mannitol, proline, lactate, propionate) in order to identify the bacterial strains present at this site [12]. The dilution of the samples with freshwater could induce bacterial cells to burst, and, consequently, to underestimate the real bacterial abundance of the samples. However, this is not relevant to bacterial identification, since the tests are aimed at qualitatively identifying the presence of a bacterial strain, by comparing its ability to develop CFUs on SWC and on modified SWCs.

3. Results

The pattern of LB% and the corresponding values of ABC are presented in Figure 2.

The Apparent Bacterial Concentration varied between 2 and 463 CFU/ml; the lowest concentrations (<10 CFU/ml) were found in the coastal area of Salerno, the highest (200–460 CFU/ml) in the area between Regi Lagni and the Volturno outfalls and in the Miseno lagoon. LB percentages were higher in summer, when they could reach values of 38% in the Gulf of Salerno.

Sites with low LB% – especially the Miseno lagoon (MI), and, sometimes, the northern sites (VG, RV) – were generally characterised by high concentrations of faecal coliforms (200–1600 CFU/100 ml, Figure 3), suggesting that the bad quality of these areas was essentially due to urban discharges. The concentration of faecal coliforms varied between 0 and 700 CFU/100 ml in winter and could reach 1680 CFU/100 ml in summer. With the exception of the Miseno lagoon and few stations along the Litorale Domizio, the concentration of faecal coliforms rarely exceeded the limit of 250 CFU/ 100 ml established by the *Directive 2006/7/EC* [13], but about an half of the stations showed values considered as critical for bathing according to the Italian law 979/82 [1].

Following Nocciolini et al. [10], the data set obtained in this study may be read in relation to the average value of the LB% by a simple standardisation, which consists in calculating the ratio (X-m)/S.D., where 'X' indicates the experimental value, 'm' the arithmetical mean, and 'SD' the standard deviation. The standardisation of data was applied only on samples collected from November to February (Table 1), since the bacterial abundance (and, consequently, the percentage of luminous bacteria) is associated to seasonal variations and shows a major stability in winter [7]. Standardised data were then grouped into discrete 'classes', on the basis of their distance from the mean: class '0' indicates mean ± SD/2; class '+1' indicates (m + SD) ± SD/2; class '−1' indicates (m − SD) ± SD/2; class '+2' indicates (m + 2 SD) ± SD/2; class '−2' indicates (m − 2 SD) ± SD/2.

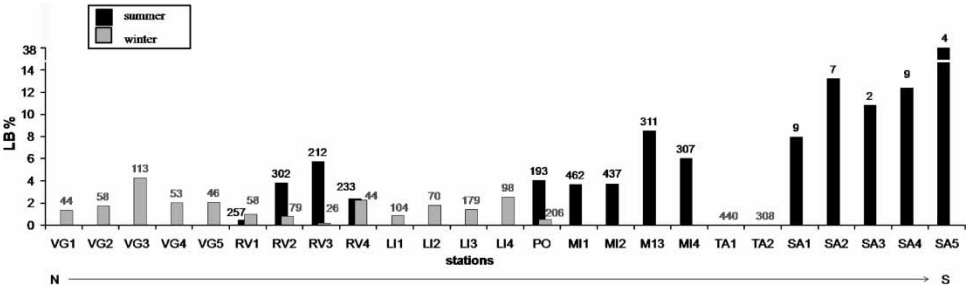

Figure 2. Pattern of LB% in summer and winter; values on each bar represent the ABC (expressed in CFU/ml) in the corresponding stations.

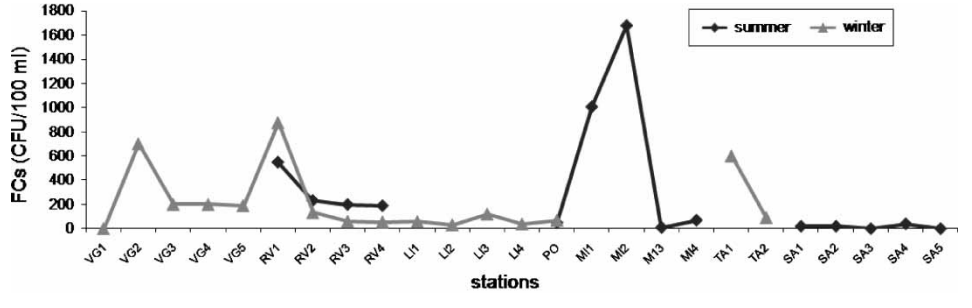

Figure 3. Concentrations of faecal coliforms in the sampling sites.

Table 1. Standardised data for sampling sites of the Campania shoreline.

Classes of Standardised LB%	Stations belonging to each class
−2	RV 1 ÷ 3 PO TA 1,2
−1	VG 1,2,4,5 RV4 LI 1 ÷ 3
0	LI 4
+1	VG 3
+2	–

Table 2. Results of the specific bacterial identification (according to Reichelt & Baumann, 1973 [12]).

Test	Luminous colony-forming units (CFU/ml)
SWC	1400
SWC+maltose	1895
SWC+mannitol	1440
SWC+proline	1420
SWC+lactate	412
SWC+propionate	475

In this study, LB% has been considered the main criterion that allows us to discriminate sampling sites in relation to their different pollution levels, while the other parameters (ABC, FCs) provide additional information, which contribute to characterise the sites in terms of water quality. Faecal coliforms, for example, represent one of the main organic pollution tracers, and are used as indicators of urban pollution in European and Italian regulations [13–15].

Table 1 shows that most stations belong to the classes corresponding to the worst levels of water quality, since they were characterised by very low percentages of the luminous microbial component. In particular, coastal waters between Regi Lagni and Volturno, and near Pozzuoli and Torre Annunziata appear as characterised by the worst water quality of the whole Campania region, as highlighted by the low values of the luminous fraction, and, sometimes, by their complete absence.

The tests of bacterial identification carried out on the samples of Pozzuoli highlighted that the development of CFUs was enhanced by the presence of maltose, mannitol and proline and inhibited by the addition of lactate and propionate (Table 2), hence suggesting the presence of *Vibrio fischeri* at that site. Although this result cannot be generalised to the whole study area, it is worth mentioning that *Vibrio fischeri* is one of the most abundant luminescent species in the world [16], and does not seem to be affected by significant variations of concentration during the year [7].

4. Discussion

With the exception of the sampling sites of Pozzuoli and Torre Annunziata (where the luminous component was completely absent) LB% followed a clear spatial gradient along the Campania shoreline (Figure 2), showing low values in the northern sector (Litorale Domizio, Licola) and high percentages in the southern area (Salerno).

Generally, LB% decreased with increasing total bacterial abundance. The low concentrations of the northern sector of the coast are most likely related to inputs from the Garigliano River and the

Regi Lagni outlets, which are vectors of organic and industrial pollutants, while the total absence of luminescent bacteria in front of Torre Annunziata depends on the river Sarno, which seriously contaminates the adjacent coastal waters, and can be held responsible for 70% of the marine pollution from Torre Annunziata to Sorrento [17]. The strong antrophogenic impact associated to this river was demonstrated by Albanese et al. [18].

These authors related the high concentrations of pollutants measured in sediments to the wide extent of local industrial and agricultural activities. Other studies pointed out to a strong genotoxic action of the mixture of pollutants present in the Sarno River. This mixture was held responsible for biological damages to organisms, as showed by the results of micronuclei-test and single cell gel electrophoresis (the Comet assay), testing DNA migration in an electrophoretic field using erythrocytes of the species *Gambusia holbrooki*. The frequency of mutations was significantly higher in individuals collected from the Sarno River than in the remaining part of the surveyed area [19].

The poor quality of coastal waters near the Pozzuoli promenade may be related to inputs from shipping activities (hydrocarbons and trace metals), as this zone is not far from the harbour. In particular, the Pozzuoli marine area shows the higher concentrations of copper and nickel than the whole coastal waters of Campania [20]. Moreover, an unauthorised outflow, located between the harbour and the promenade, was working at the time of our sampling, and possibly contributed to produce the decrease of LB%.

Conversely, Salerno appears as the less contaminated area, since the LB% was much higher if compared to the whole studied area: it varied between 8 and 38%, while it rarely exceeded 4% in the remaining part of the surveyed area.

Although it was not possible to establish a clear correlation between the luminous fraction and FCs, it is interesting to stress that low percentages of luminous colonies were often associated to high concentrations of faecal coliforms. As opposed to LB%, which decreases in polluted areas, the total bacterial component (ABC), and, in particular, faecal coliforms (indicators of organic contamination, [15]), increase strongly in sites characterised by high levels of disturbance. In the presence of urban seawage, luminous bacteria increase to a lesser extent than the number of total bacteria (ABC), leading to a lower proportion of LB% in waters with high levels of organic pollution [8]. Previous studies, carried out in the Tuscany region, show that, in coastal areas contaminated by civil outflows, the ABC increased of three orders of magnitude, causing a strong reduction of the luminous component [4].

Data standardisation (Table 1) confirms that Torre Annunziata (TA), the Pozzuoli promenade (PO) and the area between Regi Lagni and the Volturno outfalls (RV) are the areas characterised by the highest levels of pollution along the Campania coast. These results strictly agree with previous data, obtained in the same areas, by applying trophic indexes and ecotoxicological bioassays. Tests based on the measure of the 'Algal Growth Potential' (AGP) showed conditions of high eutrophication near the Garigliano and Volturno Rivers, while values of AGP typical of oligotrophic waters were found in the area of Salerno [21].

Similarly, values of the TRIX index highlighted that the southern sector of the Campania coastal zone was characterised by an 'excellent' trophic state, while the northern area included sites characterised by 'poor' to 'good' trophic states [22]. Both approaches confirm the gradient of water quality (improving from north to south), highlighted in our study by using LB%.

The parameter LB% has shown to be a useful tool to discriminate different levels of disturbance in the study area. The application of this index has permitted us to draw a 'zonation' of areas characterised by a marked difference on quality levels of the coastal waters. Unfortunately, it is not possible, at present, to provide a range of values of LB% allowing the identification of situations of 'integrity' in marine coastal waters: this index may be used only to quantify the 'relative' quality of each site in relation to the whole studied area. Further experiments are needed to check the possibility to identify 'absolute' values of LB%, typical of polluted and unpolluted waters.

For a correct application, we believe that LB% should be used in the frame of a 'site-dependent' approach and avoiding excessively homogeneous environments in order to measure the 'deviation from integrity' of each site in relation to the whole study area [10].

Furthermore, we suggest the use of the LB%-based approach only in winter, since the values of ABC, and, consequently, of LB%, may be affected in summer by frequent fluctuations. According to Sbrilli et al. [23], in the summer period the abundance of total bacteria may be high both in 'clean' and in polluted waters; the abundance of luminous colonies (ALBC) is more influenced by seasonal variations, reaching a peak in summer, but, as for ABC, it does not change in relation to the level of organic pollution; only toxic substances may cause the inhibition of the light emission, leading to a decrease of its values [6]. As a consequence, during the summer LB% may appear similar in areas with different degrees of disturbance. On the contrary, in the winter a good stability of ABC and its luminous fraction was observed. This method, easy and not expensive [8], may be considered as an additional tool to provide useful information, during the winter, about the 'relative quality' of marine coastal areas.

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