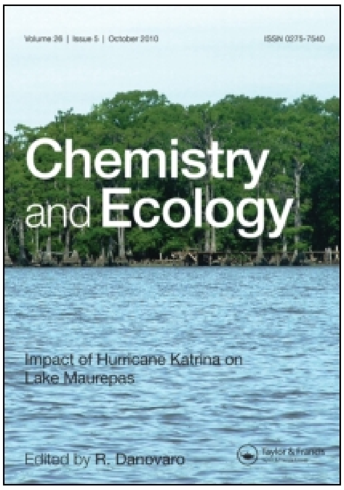

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Study of the water quality close to urban sewers in eastern Ligurian coast by means of bioluminescence tests and conventional analyses

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Five municipal sewers with small flow rates (Manarola, Riomaggiore, Portovenere, Tellaro, Bocca di Magra) were studied using the Microtox® test and a bacterial luminescence bioassay in order to evaluate the water quality in these areas of the eastern Ligurian coast. The work was performed including chemical analysis, microbiological and ecotoxicological assays. Analyses were carried out on both the effluents and the water body close to the pipe discharge. None of the measured chemical parameters exceeded the permitted limits, both in the effluents and in the seawater samples, even if some slightly critical situations were highlighted by the Microtox® test on the effluents. On the other hand, the Microtox® appeared to be less effective than another ecotoxicological assay based on the luminescent fraction of epibacteria for the evaluation of seawater quality. The good water quality of the considered areas was assessed although a slightly worse condition was encountered in Bocca di Magra.

Keywords: Water quality; Microtox®; Luminescent bacteria; Ligurian sea

1. Introduction

The disposal of wastewater is considered to be one of the main problems for the management of the Italian coasts, particularly in Liguria where the most part of the population lives in the coastal zone, which has great importance for tourism. Water quality laws provide for the determination of a certain number of chemical and biological parameters. According to the Council Directive 91/271/EEC concerning Urban Waste Water Treatment the chemical quality of effluents is usually assessed by means of global parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD) and total suspended solids (TSS) [1]. Local laws and guidelines generally indicate more specific parameters such as nutrients, heavy metals, total surfactants, etc.

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Total coliforms, fecal coliforms, *Escherichia coli* and enterococci are the biological parameters most commonly used in water quality and health risk assessments. Although they are usually not pathogenic, these indicator bacteria are used because they are much easier and less costly to detect and enumerate than the pathogens themselves [2].

The water quality control based on conventional chemical and biological analysis alone does not allow the evaluation of the global toxic effects since toxicity is a biological response. Biological systems may retain synergistic or antagonistic effects, which are not easily assessed without specific tools for the evaluation of the negative effect on living organisms. Kraak *et al.* [3] reported that the toxic effect of a mixture cannot be predicted from the effects of individual toxicants. Therefore a correct evaluation of the water quality should be carried out, taking into account chemical-biological analysis and ecotoxicological assays.

In 1982, Bulich introduced a practical method for monitoring the toxicity of aquatic samples, the Microtox® test, based on the inhibition of light emission of the bacterium *Photobacterium phosphoreum*, now known as *Vibrio fischeri* [4]. The assay proved to be an interesting alternative to conventional tests [5]; among the microbiological methods, the Microtox® is considered one of the most sensitive, rapid and reliable [6] and is still used in water pollution tests [7, 8].

Marine bacteria represent a significant part of the microplankton biomass and provide important food resources to planktonic food webs [9]. Considering their ecological role and their sensitivity to the presence of organic matter and toxic substances, heterotrophic bacteria with their luminescent component can be employed as indicators of coastal water quality [10–13]. Information obtained with this indicator has to be correlated with environmental parameters such as dissolved organic matter (DOM), water temperature, predation, etc., which could modify bacterial abundance [14].

The aim of the present study was the application of the Microtox® test and of a bacterial luminescent bioassay to evaluate seawater quality close to household drains of some seaside locations in the eastern Ligurian coast (figure 1). The results obtained with these ecotoxicological assays were discussed taking into account conventional analyses and the permitted limits of the regional law.

Five municipal sewers with small flow rates were examined: Manarola, Riomaggiore, Portovenere, Tellaro, and Bocca di Magra. To our knowledge, no recent data is available in the literature for the marine area where these effluents discharge. Chemical analysis, microbiological and ecotoxicological assays were carried out on both the effluents and the water body close to the pipe discharge.

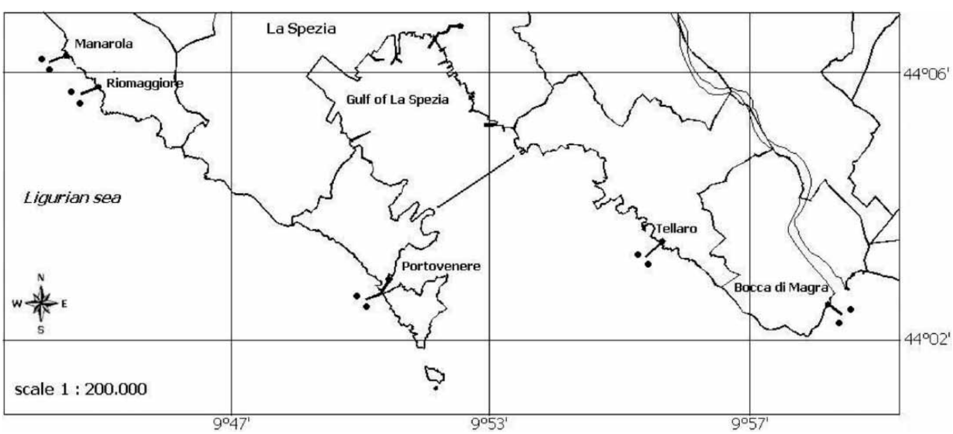

Figure 1. Map of the sampling area.

2. Material and methods

2.1 *Study area*

Effluents of the five drains studied in this work convey to the sea by submarine pipelines and only two of them include a sewage disposal. The terminal part of the pipes is situated in fairly deep waters, thanks to the characteristics of the Ligurian coasts: Manarola discharges at a depth of 18 m, Riomaggiore 20 m, Portovenere 28 m and Tellaro 17 m. Only Bocca di Magra discharges at a depth of 8 m because of the low and sandy coasts near the river mouth. The five drainpipes present a seasonal variability with capacities ranging from 170 and 600 m^3/day and serving 800–2900 inhabitants. Effluents were sampled monthly from January to May 2003. Two points near each sewage disposal were selected to collect samples in surface waters and near the bottom.

Seawater was sampled in August 2002, January, April and May 2003.

2.2 *Effluents*

Effluent waters were stored in 2 litre polypropylene bottles for the chemical analysis and in dark glass bottles for the Microtox® test.

Water temperature, conductivity and pH were measured *in situ* using a multiparametric probe. Biological oxygen demand (BOD_5) was measured by means of an OXITOP® respirometer from WTW® [15]. Total suspended solids (TSS) were collected by filtration of 500 ml of water through dried and pre-weighted 0.45 mm membrane filter. Filters were dried in the oven at 105 °C until constant weight was achieved. The difference between the dry weight of membrane filters before and after filtration was expressed in mg/l [16].

F^-, Cl^-, NO_3^-, NO_2^-, SO_4^{2-}, PO_4^{3-} concentrations were measured by an ionic chromatograph, Dionex DX-100 [17].

Chemical oxygen demand (COD) [18], total phosphorous (total P) [16], NH_4^+ and total surfactants [15] were analysed with an UV–VIS spectrophotometer, VARIAN CARY 1.

Total hydrocarbons [18] were determined by an IR spectrophotometer, BIO-RAD FTS 3000 Excalibur.

Heavy metal concentrations were determined using a PERKIN-ELMER Optima 2000 DV plasma emission spectrometer (ICP-OES) [15].

The bioassays were performed using Microtox® Analyzer Model 500 at 490 nm. The test consists of measuring the light emitted by the marine bacteria *Vibrio fischeri* in samples of different dilution. Light emission was determined after 5, 15 and 30 minutes of incubation and compared to an aqueous control (2% NaCl solution). The freeze-dried bioluminescent bacteria *Vibrio fischeri* were supplied by Azur Environmental (Carlsbard, CA, USA).

Effluent salinity was corrected by adding a 22% NaCl solution to obtain a final salinity of 2%.

2.3 *Seawater samples*

Seawater samples were collected by a Niskin bottle and stored in 2 litre polypropylene bottles for the chemical analysis and in dark glass bottles for the Microtox® test. Samples for the hydrocarbon analysis were stored in 1 litre dark glass bottles.

Spectrophotometric methods were used for the determination of NH_4^+, NO_3^-, NO_2^-, PO_4^{3-}, total P [16], anionic surfactants [15], COD and hydrocarbons [18].

The following microbiological parameters were measured: total coliforms, fecal coliform, fecal streptococci and *Escherichia coli*. Seawater samples were stored at 4 °C and analysed within 8–10 hours of collection. 100 ml of water sample was filtered using sterile 0.45 mm cellulose ester membranes (Millipore). The filter was plated on media agar; the plate was incubated at different temperatures depending on the biological indicator; and finally the typical colonies were counted and the result was expressed in colony forming units per 100 millilitres (CFU/100 ml).

In order to quantify heterotrophic marine bacteria, samples of marine water were stored in sterile 500 ml screw-cap polypropylene containers. The samples were filtered within 6 hours of collection using round sterile, 0.45 mm cellulose ester membranes (Millipore®). The filters were plated on Marine Agar and incubated at 18.0 ± 0.2 °C for 48 hours; the colonies were counted in the dark for the luminous component and in the light for the total one. Therefore two parameters expressed as CFU/ml were obtained. Sample volume was chosen to obtain a minimum of 15 colonies in each filter.

Measurements with Microtox® were performed using synthetic seawater as a control.

3. Results and discussion

3.1 *Effluents*

Results of chemical analyses, reported as mean values of the samples collected in January, February, March, April and May 2003 are presented in table 1.

Inorganic nitrogen compounds present similar values in the five effluents; concentrations of NH_4^+ are decidedly higher than the other dissolved inorganic nitrogen forms. This trend is typical in urban drains where the continuous inputs due to the human activities change the natural balance among the different nitrogen forms. On the other hand, the NH_4^+ average value of the five effluents (31.6 mg/l) is rather high and especially the value of Portovenere (36.5 mg/l) is quite close to the permitted limit of 40 mg/l (L.R. 43/95) [19]. Therefore it will be important to verify whether the natural balance is re-established in seawater.

Table 1. Effluent mean values of the measured parameters.

	Manarola	Riomaggiore	Portovenere	Tellaro	Bocca di Magra	Average
T (°C)	15.5	15.5	14.8	14.2	13.8	14.8
pH	7.7	7.7	7.8	7.5	7.5	7.6
Conductivity (mS/cm)	658	654	1366	1243	1462	1076
BOD_5 (mg/l)	100	152	258	325	257	219
COD (mg/l)	261	263	425	543	436	386
TSS (mg/l)	43	47	127	84	75	75
NH_4^+ (mg/l)	27.4	28.8	36.5	34.6	30.7	31.6
NO_3^- (mg/l)	1.0	0.4	2.0	1.0	0.6	1.0
NO_2^- (mg/l)	0.4	0.1	<DL	<DL	<DL	0.1
Total P (mg/l)	1.7	1.6	10.9	1.9	2.1	3.6
SO_4^{2-} (mg/l)	93.6	125.3	168.8	262.2	97.4	149.5
Cl^- (mg/l)	56.3	57.9	222.5	76.4	221.6	126.9
F^- (mg/l)	0.4	0.1	2.6	0.5	0.1	0.7
Total surfactants (mg/l)	1.6	1.7	1.6	6.4	4.8	3.2
Fe (mg/l)	0.13	0.13	0.12	0.15	0.23	0.15
Mn (mg/l)	0.03	0.04	0.07	0.03	0.06	0.05
Cu (mg/l)	0.01	0.03	0.01	0.02	0.01	0.02
Zn (mg/l)	0.08	0.07	0.14	0.05	0.06	0.08

The mean value of SO_4^{2-} is 149.5 mg/l with a predominant contribution of Tellaro effluent (262.2 mg/l).

The highest value of total phosphorous, approximately one order of magnitude higher than other sites, was measured at Portovenere (10.9 mg/l).

Tellaro, Bocca di Magra, Portovenere show higher values of conductivity, BOD_5, COD and TSS than Riomaggiore and Manarola.

The TSS are in general quite low: a maximum value of 386 mg/l was observed in January at Portovenere.

Total surfactants show the highest values at Tellaro and Bocca di Magra (6.4 mg/l and 4.8 mg/l respectively); these chemicals do not represent a problem, considering the permitted limit of 15 mg/l (L.R. 43/95).

The presence of the following heavy metals was investigated: Cr, Cd, Ni, Mn, Pb, Cu, Fe and Zn. The measured concentrations were quite low and in any case within the permitted limits. Cd, Cr, Ni, Pb were always below the detection limit (0.01 mg/l) while Fe and Zn were in the range 0.12–0.23 mg/l and 0.05–0.14 mg/l respectively.

Conventionally a global evaluation of effluents based on chemical analysis uses the definition of "water strength" [20]. The strength of sewage is mainly dependent on the level of BOD, TSS and COD in the sewage. There are three classes: high strength, medium strength and low strength. Taking into account this definition, the five effluents studied can be considered as medium strength waters. Besides, comparing each chemical parameter with the values reported in the Ligurian regional law (L.R. 43/95), it can be observed that they are below the permitted limits.

Such results are not sufficient to guarantee the absence of toxic effect because, as already discussed, living organisms react to external changes with complex mechanisms which also depend on synergistic or antagonistic effects.

Toxicity assays were then performed on the water samples from the five drains using the Microtox® . Results after 5, 15 and 30 minutes of exposure are shown in figure 2.

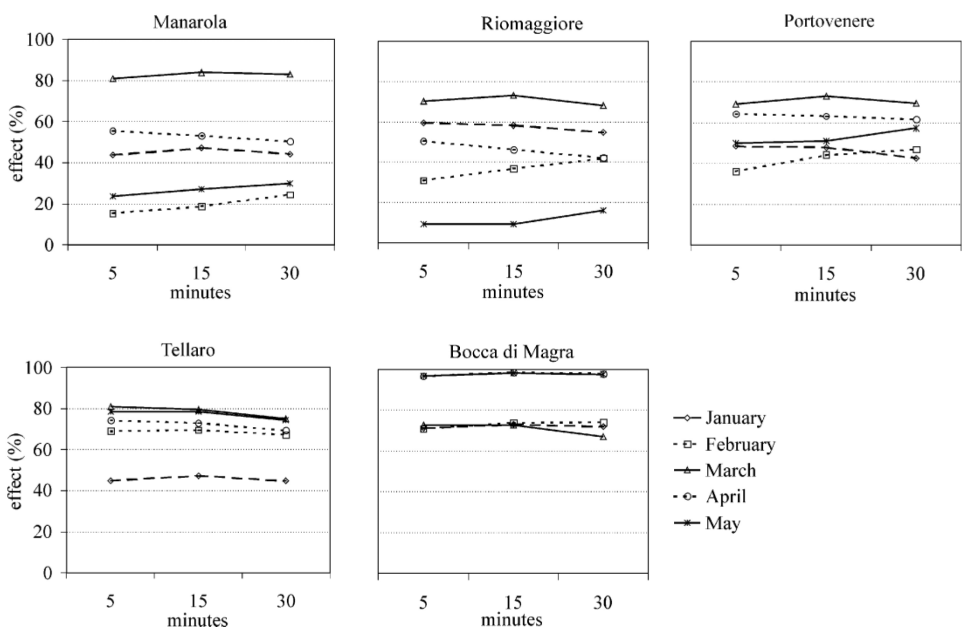

Figure 2. Microtox® response to effluents after 5, 15, 30 minutes.

The most toxic effluents were Tellaro and Bocca di Magra. Riomaggiore and Manarola showed the lowest inhibition values (<60%), except for the percentage measured in March.

In order to investigate the incubation time influence on acute toxicity, the trend of effect percentage was considered. According to some authors [8, 21] the decrease of the curve-inhibition time (at 5, 15 and 30 minutes) could be the consequence of the presence of organic compounds. This behaviour can be observed in the effluents of Riomaggiore and Portovenere in January and April and in the effluent of Manarola in April.

An increase of the effect percentage in the first 15 minutes followed by a decrease from 15 to 30 minutes can be due to the different speed of the toxic action. This trend is shown in Riomaggiore and Portovenere in March.

The Microtox® results were also expressed by means of EC50 and toxic units (TU = 100/EC50). EC50 is the concentration of the sample that reduces light emission to 50% compared to the control. In order to assess the real impact of effluents on the receiving water body the total Toxic Charge (TC = TU × wastewater flow rate) was calculated [22]. According to EPA [23], this parameter was expressed in MEU/day (million effective units/day). This normalization allows the comparison among drainpipes having different flow and toxicity. The described parameters are shown in table 2.

When the value of EC50 was higher than 100, indicating a null or very low concentration of toxic substances, TU and TC were not calculated. Most measurements performed in Manarola,

Table 2. Toxicity parameters (after 30 minutes), flow rate and Toxic Charge of the five effluents.

	Effect (%)	EC50	TU	Flow rate (m^3/day)	TC (MEU/day)
Manarola					
January	44.3	/	/	150	/
February	24.5	/	/	150	/
March	83.3	20.8	4.8	170	817
April	50.4	/	/	170	/
May	29.8	/	/	170	/
Riomaggiore					
January	54.7	81.7	1.2	260	318
February	41.9	/	/	260	/
March	68.2	43.9	2.3	380	865
April	42.2	/	/	380	/
May	16.1	/	/	380	/
Portovenere					
January	42.6	48.0	2.1	200	417
February	46.8	/	/	200	/
March	69.6	41.7	2.4	600	1439
April	61.5	68.7	1.5	600	873
May	57.3	/	/	600	/
Tellaro					
January	44.7	/	/	250	/
February	67.2	22.5	4.5	250	1114
March	75.3	19.0	5.3	250	1315
April	69.4	27.5	3.6	250	911
May	97.5	12.5	8.0	250	2000
Bocca di Magra					
January	71.8	9.8	10.2	260	2661
February	74.0	21.9	4.6	260	1188
March	67.0	42.1	2.4	260	617
April	97.8	10.6	9.4	260	2455
May	74.3	5.5	18.4	260	4772

/ represents values not reported because of the low toxicity (EC50 > 100%).

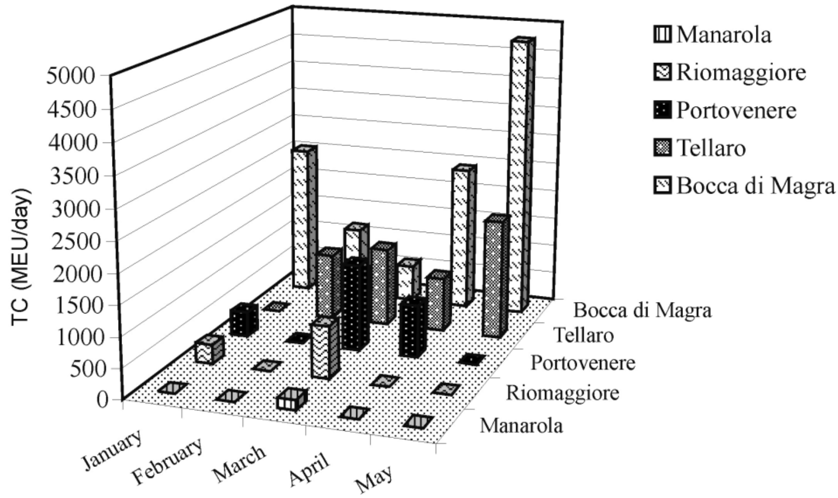

Figure 3. Toxic Charge values in the five effluents.

Riomaggiore and Portovenere reflect this case. In Tellaro TU values were calculated each month except for January, with a maximum of 8 in May. Bocca di Magra generally exhibits the highest TU values, again with a maximum in May.

Toxic Charge values (figure 3) are quite similar to the toxicity results (TU) because the flow rates of the five considered effluents are comparable. All the five sewers are small, consequently their impact on the receiving body is probably low, although some values of effect percentage are rather high.

In order to highlight the chemical parameters which mostly affect the toxic activity, correlation curves were drawn by plotting the effect percentage versus each chemical parameter, considering all data from the five effluents. Although a high correlation ($p < 0.05$) between toxicity test results and the concentration of BOD_5 ($r = 0.39$), COD ($r = 0.42$), and surfactants ($r = 0.47$) was found; the chemical parameter which seems to influence mainly the Microtox® results is NH_4^+. In fact, as shown in figure 4, its concentration presented a very high correlation with effect percentage ($p < 0.01$; $r = 0.65$). Microtox® appears to be particularly sensitive to ammonia which, as already pointed out while discussing chemical results, presents concentration values very close to the permitted limits. This bioassay seems to work effectively in a concentration range very close to the values of the regional law, thus giving evidence to critical situations.

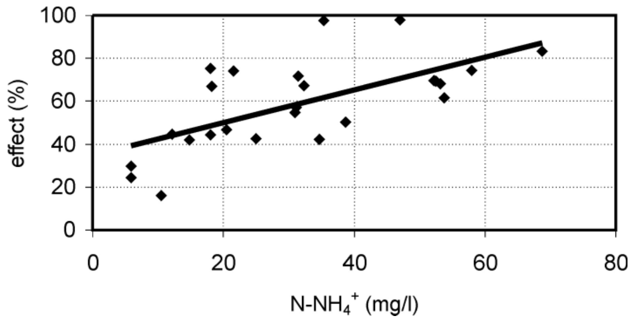

Figure 4. Correlation of the toxicity (effect percentage after 30 min) with NH_4^+ concentration.

Interesting considerations can be shown comparing Microtox® test data with the permitted limits of the chemical parameters of the Ligurian regional law (L.R. 43/95).

Four different cases can be observed:

(A): chemical data below permitted limits and negative Microtox® result (effect % $< 50\%$)
(B): chemical data above permitted limits and positive Microtox® result (effect % $> 50\%$)
(C): chemical data below permitted limits and positive Microtox® result (effect % $> 50\%$)
(D): chemical data above permitted limits and negative Microtox® result (effect % $< 50\%$).

For the complete set of 25 samples we observed 9 cases of (B), 7 cases of (C), 6 cases of (A) and 3 cases of (D), as shown in figure 5.

Chemical results were in accordance with those of the bioluminescence test for 60% of the samples (cases A + B). It is particularly interesting to remark the cases (C) for which the bioassay gave evidence to critical situations. In fact relation (C) highlights the usefulness of Microtox® bioassay as a complementary technique to chemical analysis: these samples proved to be toxic even if their chemical values were below the permitted limits. As discussed before, synergistic effects of different chemicals can cause toxicity in living organisms whose biological response can be simulated by bacteria used in bioassays.

Cases (D), in which chemical parameters were above the permitted limits with negative Microtox® results, could be explained supposing that *Vibrio fischeri* is not sensitive to the substances contained in those effluents. Some authors claim that the harmful effects of water drains can best be determined through the use of a battery of tests involving two or more bioassays utilising different species [24]; in fact a single species of bacteria can be quite resistant to some kinds of toxicants [8].

3.2 *Seawater*

Chemical parameters were firstly examined. No significant differences between the two sampling points of each drain were observed. Fluctuations between the four samplings (August, January, and April, May) did not show remarkable trends. Therefore, considering also the large number of obtained data, only mean values of the various parameters are summarized in table 3.

The considered parameters showed generally quite low concentration values, suggesting that no particular environmental risks are present. The concentrations of nutrients are rather low in both surface and bottom waters and fall in the typical range reported for oligotrophic water, with the nitrogen reduced forms significantly lower than NO_3^-. Ammonia, whose values were quite high in the effluents, shows concentration levels characteristic of the Ligurian Sea, due to the dilution capacity of the receiving water body.

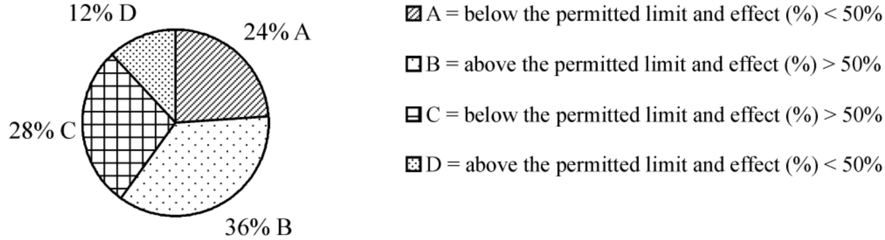

Figure 5. Case record of Microtox® and chemical analysis results compared to the regional law (L.R. 43/95).

Table 3. Average concentrations of various parameters in surface (S) and bottom waters (B).

	NH_4^+ (mg/l)		NO_2^- (mg/l)		NO_3^- (mg/l)		PO_4^{3-} (mg/l)		Total P (mg/l)		COD (mg/l)		Conductivity (mS/cm)		pH		Salinity (‰)		Surfactants (mg/l)	
	S	B	S	B	S	B	S	B	S	B	S	B	S	B	S	B	S	B	S	B
Manarola	0.011	0.009	0.05	0.10	1.2	0.9	0.3	0.2	0.09	0.08	32.1	31.1	57.7	58.0	8.3	8.2	37.9	38.2	0.3	0.3
Riomaggiore	$<$DL	0.006	0.09	0.04	0.6	0.8	0.3	0.3	0.08	0.09	35.7	31.6	57.6	51.2	8.3	8.3	37.9	38.2	0.3	0.2
Portovenere	0.009	0.005	0.10	0.04	0.9	1.0	0.5	0.3	0.16	0.10	36.3	29.9	57.4	58.3	8.3	8.3	37.7	38.2	0.4	0.3
Tellaro	0.007	0.012	0.08	$<$DL	0.8	0.8	0.1	0.2	$<$DL	0.06	24.6	32.1	57.2	58.1	8.3	8.3	37.5	38.1	0.2	0.2
Bocca di Magra	0.006	0.006	0.11	0.04	1.1	0.8	0.1	0.1	$<$DL	$<$DL	25.0	25.4	52.6	56.4	8.3	8.3	34.1	36.9	0.2	0.3
Average	0.007	0.008	0.09	0.05	0.9	0.9	0.2	0.2	0.08	0.07	30.7	30.0	56.5	56.4	8.3	8.3	37.0	37.9	0.3	0.3

The Bocca di Magra drain exhibits the lowest salinity (36.9‰ and 34.1‰ in bottom and surface samples respectively) due to the contribution of freshwater from the river. The other areas show similar results, with higher salinity in the bottom samples. Other chemical parameters, not reported in table 3, were measured. Total hydrocarbons concentration was always below the detection limit (0.010 mg/l), with just the exception of Manarola in August that showed a concentration of 0.055 mg/l. Different heavy metals were also measured (Cd, Cr, Pb, Cu, Zn, Fe, Mn, Co): their concentration was generally below the detection limits (0.010 mg/l) apart from those of zinc and iron. The concentrations in the five sites ranged between 10–200 μg/l for Fe and between 13–100 μg/l for Zn apart from occasional values (440 μg/l of Fe at Tellaro for bottom waters in January, 172 μg/l of Zn at Manarola for surface waters).

In table 4 data related to total coliforms, fecal coliforms, fecal streptococci and *Escherichia coli* are reported: their measured values are always below the permitted limits. In four out of five sites bottom waters exhibit higher values of microbiological parameters than surface waters. This was expectable since the wastewater flow undergoes a significant dilution moving towards the surface, which causes the reduction in bacterial load. A reverse trend was observed for all parameters in Bocca di Magra, with a higher concentration of microorganisms in surface waters than in bottom waters. In this area the river contribution has to be taken into account: the organic matter brought by the Magra river affects the composition of seawaters in surface samples.

The Microtox® test performed on the seawater samples did not reveal toxic conditions, but only evidence of light stimulation or inhibition in comparison to the control. Mean values of effect percentage ranged between −10% and 10%. As predicted in the considerations regarding Microtox® results of the effluents, the big dilution capacity of the receiving water body does not allow to reveal critical conditions although in August, Manarola and Riomaggiore are characterized by effect percentages of 12 ÷ 18%; these values are slightly lower than the level for which the sample would be considered toxic (effect percentage = 20%).

On the contrary, the effects of biostimulation are probably connected to the optimal concentration of nutrients obtained with the effluent dilution. The suitability of bacterial luminescence test as another bioindicator to assess seawater quality in the considered area was verified. A simplified detection method [11, 25, 26] based on the epibacteria component able to develop colonies on agar media was used. Two parameters, expressed as colony forming units per millilitre of water (CFU/ml), were measured: Apparent Bacterial Concentration (ABC) and its luminous component, defined as Apparent Luminous Bacterial Concentration (ALBC). Then, the percentage of luminescent bacteria (%LB) was calculated as the ratio (ALBC/ABC) ∗ 100.

This bioluminescence assay is simple, sensitive and provides rapid and clear information. High concentration of particulate organic matter leads to an increase of bacterial abundance, while low bacterial concentration may indicate both "clean" water and the presence of toxic substances. For this reason, sensitivity of bioluminescent bacteria to toxic substances can

Table 4. Average values of fecal contamination indicators in seawater samples (expressed in CFU/100 ml).

	Total coliform		Fecal coliform		*Escherichia coli*		Fecal streptococci	
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
Manarola	29	101	3	21	1	11	1	8
Riomaggiore	15	56	10	32	7	26	6	17
Portovenere	8	17	1	7	1	6	4	10
Tellaro	15	38	5	18	2	14	21	16
Bocca di Magra	98	42	41	22	10	10	34	28
Average	33	51	12	20	4	13	13	16

be helpful in the interpretation of the obtained results. High bacterial concentrations, with reduced luminescent proportion, indicate an increase in respiration rate due to organic pollution, while low bacterial concentrations with a reduced or absent luminescent component are related to the actual presence of toxic substances. In particular the ABC indicator may vary of two order of magnitude or more in the presence of high quantities of organic particulate matter from domestic sewage, while luminescent bacteria (ALBC) increase less (one order of magnitude); as a result, the %LB parameter shows low values [12]. A systematic sampling for a longer period is desirable for the correct use of this indicator. Interesting remarks, although preliminary, can be made considering data reported in figure 6.

Manarola, Riomaggiore and Portovenere stations show similar ABC values while Tellaro and Bocca di Magra show higher concentrations, ranging from 46.5 to 87.3 CFU/ml. In particular, Bocca di Magra has the highest ABC values (in January a peak of 199.5 CFU/ml

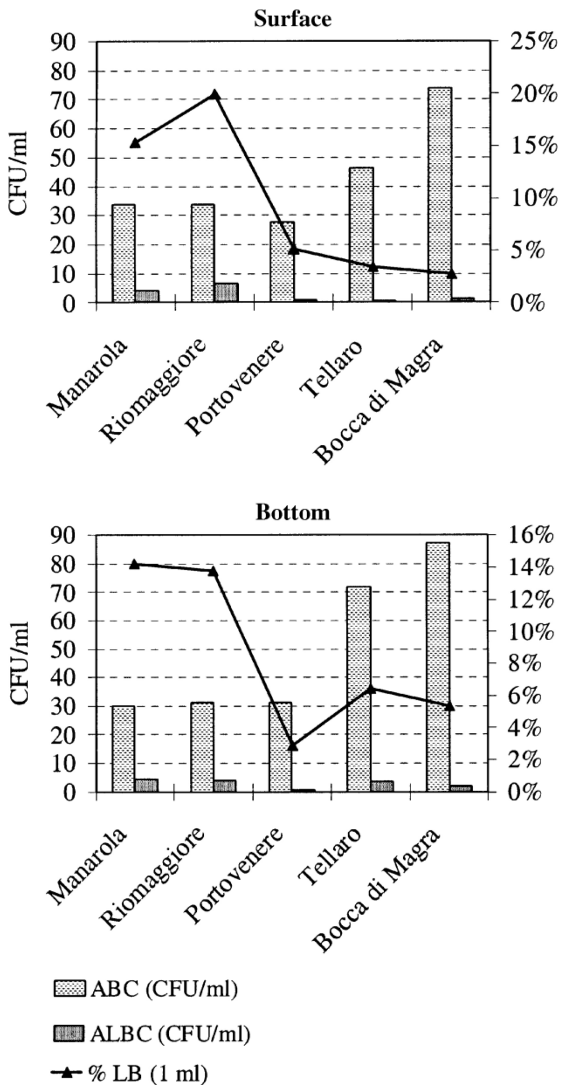

Figure 6. Average values of ABC, ALBC, %LB in the five stations.

Table 5. The analysis of variance (ANOVA).

Source	F calculated	F critical value ($p = 0.05$)	
Between groups	4.14	2.54	significant
Bocca di Magra vs others	12.02	4.02	significant
Tellaro vs others	1.41	4.02	not significant
Portovenere vs others	2.99	4.02	not significant
Riomaggiore vs others	2.07	4.02	not significant
Manarola vs others	2.21	4.02	not significant
Manarola, Riomaggiore, Portovenere	0.02	3.16	not significant

was observed in bottom waters). These results, coupled with the relatively low percentage of luminescent bacteria (%LB) highlight the consistent organic contribution brought by the Magra river.

The highest ALBC values were observed in Manarola and Riomaggiore; at the same time the %LB values were high, suggesting a better seawater quality for these two areas.

A comparison between these results and data obtained in a non anthropized area by other authors (Sbrilli, personal communication) was made. The area is called Punta Rossa and is located 500 meters from the southern shore of Elba Island, a distance from the coast comparable to that of our sampling points. Punta Rossa seawater is far from pollution sources and not influenced by river contributions.

In Punta Rossa the annual values of the considered indicators varied as follows: ABC 1.0–12.2 CFU/ml, ALBC 0.1–0.9 CFU/ml, %LB 0.3–20.0%.

Comparing these values with those shown in figure 6, it is evident that ABC values of Punta Rossa are decidedly lower. The difference in some months reached one order of magnitude; for instance, during the month of May we found 50.5 CFU/ml in Portovenere against 4.0 CFU/ml in the reference area. Anyway it should be taken into account that a heavily anthropized area could reach 1000–5000 CFU/ml [12].

On the other hand, when comparing the %LB values it can be observed that they are quite similar (in the five areas %LB ranged from 1.1% to 20.3%). These data indicate that bioluminescence is efficiently carried on by the luminous fraction of bacteria and consequently suggest a good water quality.

In order to point out the variability among the 5 sampling areas, all data of the apparent bacterial concentration (ABC) obtained during the studied period, in the two sampling points of each site and at the two different depths, were subjected to the analysis of variance (ANOVA).

Obtained results are reported in table 5.

A significant difference among all the five stations is highlighted ($p < 0.05$). Particularly the Bocca di Magra area is significantly different from all the others. As previously observed, this is probably due to the organic load of the Magra river. The result obtained with ANOVA for Bocca di Magra is in agreement with the indications coming from the chemical analysis of seawater samples. The values of ABC and ALBC substantially confirm the slightly critical nature of the area close to the drain of Bocca di Magra.

4. Conclusions

The multidisciplinary approach of this study, performed by means of chemical, microbiological and ecotoxicological analysis, showed the complementary nature of the results achieved with the different methods. The Microtox® test confirmed to be a very useful screen tool for the evaluation of drain toxicity, whereas it did not allow a clear assessment of the toxicity of the receiving water body. The use of another bioluminescence test proved to be effective for the aim of this study; low costs and ease of the measurement make luminescent bacteria a valid bioindicator for the evaluation of seawater quality. Results obtained by means of chemical and biological analysis were in most cases below the permitted limits. Through the integrated evaluation of the different parameters, the good water quality of the considered areas was assessed; only in Bocca di Magra the bioluminescent bacteria indicator suggests a slightly worse condition of the area. Further studies will be performed taking into account other effluents in the same area and systematically monitoring the water quality of the far eastern Ligurian coasts for longer periods.

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