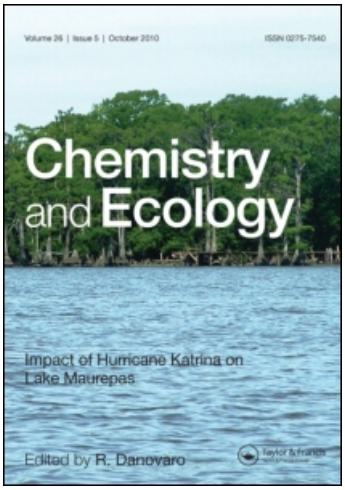

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Integrated biomonitoring assessment of the Lesina Lagoon (Southern Adriatic Coast, Italy): preliminary results

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Integrated biomonitoring assessment of the Lesina Lagoon (Southern Adriatic Coast, Italy): preliminary results

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An integrated biomonitoring program for marine and coastal ecosystems quality assessment combines the chemical characterization of a site with the evaluation of the possible structure alterations of its living communities. This can be considered an useful tool for better identifying the summarized effects of all the components interacting with the biota. Such an integrated procedure was carried out for the assessment of the quality of the Lesina Lagoon (Southern Adriatic Coast, Italy). The water parameters levels showed a high primary production (2–6 $\mu g\, l^{-1}$); the sediment and pore water toxicity bioassays recorded a low or moderate diffused toxicity. Besides, the benthic meiofauna community structure was characterized by prevalent Nematoda taxa with a homogeneous spatial distribution. On these basis, the Lesina Lagoon seems to be characterized by a prevalent organic pollution mainly related to agricultural and zootechnical activities which, due to the lagoon's conformation, presents a homogeneous spatial distribution.

Keywords: Coastal lagoon; Biomonitoring; Water parameters; Sediment; Toxicity bioassays; Meiofauna

1. Introduction

Coastal lagoons are currently ranked among the most endangered aquatic ecosystems as they are often directly and seriously affected by urban runoff, industrial effluents and domestic discharges. Furthermore, the large fluctuations in physical and chemical parameters may enhance the impact of both pollutant and nutrient inputs on the biota [1]. Due to the shallowness of the water column and to the low water exchange, sediment represents the main sink for many toxic substances, which can affect both benthic and pelagic organisms because of frequent resuspensions [2].

Monitoring such an ecosystem requires the assessment of tools that efficiently and reliably discern ecosystem changes in relation to environmental alterations [3–5].

Research trends in marine and coastal ecosystems quality assessment have demonstrated the need for an integrated approach which combines the chemical characterization of a site

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with the evaluation of the possible structure alterations of its living communities, in order to overcome the limits of each single investigation and to better identifing the summarized effects of all the components interacting with the biota [6–8].

The purpose of the present study was to verify the usefulness of a biomonitoring program which integrates different types of toxicological tools to obtain a broad and overall picture of a brackish ecosystem supposed to be rather scarcely impacted from anthropic activities.

The study was carried out at the Lesina Lagoon, along the Southern Adriatic Coast of Italy. The parameters used in the present study were (a) water column physico-chemical parameters; (b) sediment characterization; (c) sediment toxicity by use of the *Vibrio fischeri* bioluminescence test (Microtox®) and analysis of the level of the sea urchin *Paracentrotuslividus* fertilization and abnormal larval development; (d) benthic meiofauna community structure.

2. Materials and methods

2.1 *Study area*

The Lesina Lagoon (figure 1) is a brackish coastal basin located on the Adriatic Coast of Southern Italy (41°51′47″–41°54′40″ N and 15°18′48″–15°34′28″ E), extending along the Garganic Peninsula for 51 km^2. It is characterized by shallow water (0.7–1.5 m) and a weak hydrodynamism and is connected to the sea through two channels, Schiapparo and Acquarotta; the freshwater input mainly derives from the Pilla and Zanella springs and from the Lauro Canal, while the precipitation is quite scarce (400–700 mm per year). The catchment area (600 km^2) is mostly exploited for agriculture (grasses and greens crops); there are also fish and cattle breeding farms and three towns, with a total of about 30,000 inhabitants.

Twelve sampling sites (figure 1) were chosen according to the different natural and anthropic inputs: 1 and 9 (zootechnical facilities); 2 and 11 (channels to the sea); 4 and 10 (urban wastes, at the nearby sewage treatment plants); 7 and 12 (agriculture activities runoff); 3, 5, 6 and 8 (central transect). Samples were collected from April to November 2003.

2.2 *Water parameters*

Temperature, pH, salinity and dissolved oxygen were recorded monthly using a multiparametric probe (YSI 556MPS). Water samples for nutrients analyses were collected monthly by using a Niskin bottle, immediately transferred on ice, shipped to the laboratory and stored at −20 °C; analyses were carried out within a week. Nutrients (nitrite, nitrate, ammonia, phosphate) levels were evaluated using a Beckmann DU64 Spectrometer, according to the classical

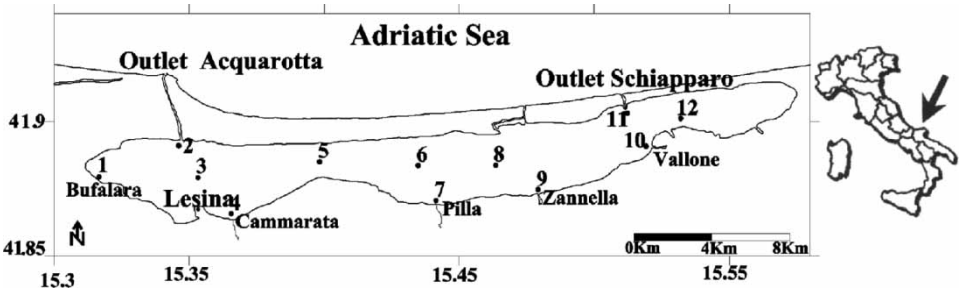

Figure 1. Sampling area and stations location (1–12).

methods reported in the literature [9, 10]. Chlorophyll *a* analyses were carried out according to Lorenzen and Jeffrey [11]. All analyses were carried out on three replicates.

2.3 *Sediment characterization*

Sediment samples were collected every 2 months by pole core (i.d. 4.5 cm, surface area 15 cm^2) down to a depth of 5 cm, placed in polyethylene flasks, immediately transferred on ice, shipped to the laboratory and stored at 4 °C; analyses were carried out within a week, on three replicates. Grain size characterization was performed on samples dried (60 °C), digested with a H_2O_2 solution and fractioned by wet sieving (63 μm mesh). Total organic matter (TOM) was determined on dried samples (65 °C for 24 h) by loss of ignition at 550 °C for 3 h in muffle furnace. The colonies of sulphur reductant bacteria were immediately evaluated after sampling, by incubation of prediluted sediment aliquots in AGAR SPS medium according to Davies *et al.* [12].

2.4 *Sediment toxicity bioassays*

Sediment samples for toxicity bioassays were collected, down to a depth of 5 cm by grab, twice a year (on spring and autumn), placed in polyethylene flasks, immediately transferred on ice, shipped to the laboratory and stored at 4 °C; bioassays were carried out within 48 h.

P. lividus embryotoxicity bioassay was performed on solid phase (SP) according to Pagano *et al.* [13]. Briefly, sediment aliquots (1% dry weight) were laid on wet discs of filter paper and allowed to settle on the bottom of polystyrene six-well microplates; sea urchin fertilized eggs were gently laid on the sediment samples, and after 48–72 h at 18 °C the percentage of normal plutei larvae was recorded.

P. lividus spermiotoxicity bioassay [2] was performed on pore water (PW), obtained by centrifugation (5000 rpm for 30 min at 4 °C) in an ALC 237R fuge. Briefly, sea urchin spermatozoa were diluted in PW at a ratio of 1:1000 and after 60 min at 18 °C, sperm aliquots were added to untreated egg solutions in seawater (150 ml^{-1}) at a ratio of 15000:1. After 30 min at 18 °C, the fertility percentages were evaluated. Both tests were performed in three replicates; sterilized sea water was the control. In addition to the one-way analysis of variance (ANOVA) statistical analysis, data were analyzed for statistical significance vs. the control by means of Student's *t*-test.

V. fischeri Microtox microbial luminescence bioassay was performed both on SP and on PW (three replicate tests for each sample), according to the Microbics 100% protocol [14]; each test consisted of one control and four serial dilutions of each sample; results were validated by using phenol as referent toxicant (EC_{50} 5 min = 18.4 mg/l, 95% confidence range).

2.5 *Meiofaunal analyses*

Sediment samples were collected in three replicates every 2 months by pole core (i.d. 3.5 cm, surface area 10 cm^2) down to a depth of 5 cm, placed in polyethylene flasks and immediately shipped in laboratory on ice. Sediment samples were fixed with 4% buffered formaldehyde in filtered seawater and sieved through 37 μm mesh nets and meiobenthic organisms were recovered according to Platt and Warwick [15], counted and classified (taxon) using a stereomicroscope. All nematodes were then identified (genus level) using light microscopy, according to Platt and Warwick [15].

2.6 *Statistical analyses*

ANOVA was used to analyze data from different sampling sites; when significant differences were found, Tukey's test was performed.

3. Results

3.1 *Water parameters*

Owing to the lagoon's shallowness and to the absence of water stratification [16], the collection of one sample of 40 cm depth (about the half of the height of the water column) was deemed necessary. Water temperature displayed a typical seasonal trend (from 14.2 °C in April to 30.2 °C in August), without differences in relation to the different sampling sites; pH values ranged from 7.7 to 9.3, without any significant difference in relation to the different sampling period and sites. Salinity distribution showed a barely significant ($F = 2.10$, $P < 0.05$) increasing trend from the western basin (stations 1–8) to the eastern basin (stations 9–12) and significantly differences among the sampling period ($F = 4.40$, $P < 0.01$), in relation to the rainfall and the water evaporation rates. The lowest salinity level (3 psu) was recorded in May at the station 10, while the highest one (33.3 psu) was recorded in August at the station 1. Also dissolved oxygen levels displayed significant differences among the sampling period ($F = 8.17$, $P < 0.01$), showing the lowest values, ranging 2.69–7.65 mg l^{-1}, in the summer period (June–August), and higher levels (8.28–12.65 mg l^{-1}), in the other sampling times (April–May and September–November). No differences in oxygen levels were recorded in relation to the sampling sites.

The nitrogen, phosphorus and chlorophyll *a* water levels strongly fluctuated with the sampling times, as shown in table 1. Mean values as well as the highest and lowest ones for each of the 12 sampling sites were reported.

The total dissolved inorganic nitrogen (DIN, $NO_3^- + NO_2^- + NH_4^+$) and dissolved inorganic phosphorus (PO_4^{3-}, DIP) displayed rather low levels and a high N:P atomic ratio. While for DIP levels no correlation was found among sampling sites, DIN levels displayed significant higher values ($F = 5.88$, $P < 0.01$) in sites 10 and 11. Chlorophyll *a* levels fluctuated greatly during sampling times, rarely were recorded consistent peaks (17.70 μg l^{-1} in September at

Table 1. Total DIN, phosphate (PO_4^{3-}, DIP) and chlorophyll *a* (CHL *a*) mean, lowest (min) and highest (max) values recorded at the different sampling sites.

	DIN(μg/l)			DIP(μg/l)			CHL *a* (μg/l)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
1	58.33	18.9	152.18	5.42	0.02	16.74	1.88	1.31	2.50
2	83.30	7.98	251.02	3.41	0.02	9.30	4.31	0.93	17.70
3	165.01	21.00	561.54	3.92	0.02	11.78	5.72	1.20	11.86
4	103.92	30.38	282.94	3.25	0.02	10.85	3.65	0.82	5.11
5	26.01	13.16	54.32	1.75	0.02	4.65	1.65	0.74	3.01
6	84.42	21.42	259.84	13.27	0.02	66.96	3.29	0.50	6.39
7	193.59	80.22	477.26	20.61	4.34	52.70	6.72	1.07	21.27
8	46.83	14.42	113.82	3.04	0.62	8.37	4.34	0.59	9.49
9	340.52	85.54	800.24	9.45	0.62	40.92	3.60	1.31	8.03
10	431.48	107.38	738.64	3.51	0.62	4.96	2.50	0.53	5.02
11	586.04	208.32	1080.0	3.05	0.62	6.82	1.62	0.56	2.19
12	95.83	20.58	259.42	2.11	0.62	4.96	2.60	1.01	4.47

Table 2. Water content and TOM of the sediment from the different sampling sites.

	Water content		TOM	
	%	±SD	mg g^{-1}	±SD
1	59.75	8.25	117.55	25.73
2	66.85	1.33	137.58	16.10
3	62.28	1.71	162.39	5.50
4	65.20	1.30	139.20	5.15
5	68.31	3.34	166.85	14.40
6	66.07	3.66	174.51	15.43
7	49.48	9.00	98.02	17.35
8	67.33	2.37	173.41	13.08
9	49.97	7.46	115.01	4.59
10	56.41	4.97	118.10	15.56
11	57.37	6.76	126.24	36.61
12	62.11	2.96	140.33	18.85

station 2 and 21.27 $\mu g\, l^{-1}$ in June at station 7). No correlation among chlorophyll *a* levels and sampling sites was recorded.

3.2 *Sediment characterization*

The granulometric determinations show the silt–clay (<63 μm) as the main sediment component fraction (91.83 + / − 4.16% of the total), whereas the remaining fraction mainly consists of shell fragments and other organogenic structures. As shown in table 2, the Lesina Lagoon sediments are characterized by a high water content, always ranging around 50%, and high TOM levels. TOM content was significantly higher ($F = 18.04$, $P < 0.01$) in the sites along the central transect, while no significant differences were found in the water content of the sediment in relation to the different sampling sites. The sulphur reductant bacteria colonies always ranged between 1×10^4 and 1×10^5 CFU g^{-1}, showing no correlation with the sampling site.

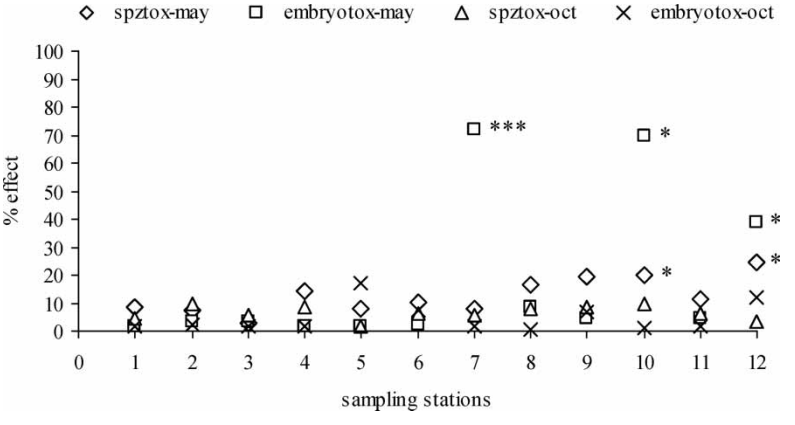

Figure 2. Effect percentages on fertilization rate (spztox) and on larval development (embryotox) recorded in spermiotoxicity and embryotoxicity tests, respectively, on sediment collected on spring and autumn at the 12 (1–12) sampling stations. The asterisks represent the significant difference *vs*. control (*$0.05 < P < 0.02$; ***$P < 0.001$).

Table 3. Toxicity levels recorded with Microtox PW and SP tests, expressed according to the toxicity scale of Onorati *et al.* [18].

	PW test		SP test	
	Spring	Autumn	Spring	Autumn
1	Very high	Very high	Moderate	Very high
2	Very high	Low	Very high	Very high
3	Very high	Moderate	Very high	Very high
4	High	Moderate	High	Very high
5	Very high	Low	Moderate	Low
6	Low	Moderate	High	Low
7	Absent	Moderate	Moderate	Low
8	Moderate	Low	Very high	Moderate
9	Absent	High	Moderate	Moderate
10	Moderate	Low	Very high	Moderate
11	High	Absent	Low	Low
12	Moderate	Low	Very high	Moderate

3.3 *Sediment toxicity bioassays*

Figure 2 shows the results of the sea urchin (*P. lividus)* toxicity bioassays. In the sediments collected in spring a significant detrimental effect on fertilization success (spermiotoxicity test on PW) respect to control sample was recorded for sites 10 and 12 ($0.02 < P < 0.05$) and for sites 9 and 11 ($P = 0.05$), whereas a significantly higher percentage of abnormal plutei larvae (embryotoxicity test on SP) was recorded for sites 7 ($0.001 < P < 0.01$), 10 ($0.02 < P < 0.05$) and 12 ($P = 0.05$). A significant correlation between sampling site and 'effect percentage' (percentage of unfertilized egg and of abnormal plutei, respectively) was recorded in the spermiotoxicity test for stations 9, 10 and 12 ($F = 12.72$, $P < 0.01$) and in the embryotoxicity test for stations 7, 10 and 12 ($F = 22.07$, $P < 0.01$). In sediments collected in autumn, no effect was recorded either on fertilization success and on larval development.

Table 3 shows the Microtox tests results. As the SP test is subjected to several interferences related to matrix variability such as loss of bacteria adhering to the finest sediment particles, the results of this test were normalized with the pelitic fraction of reference and expressed as ratio to reference, which equals toxic units (TU) of sample/TU of the reference sample [17]. Successively, in order to better displaying the different toxicity levels among sampling sites,

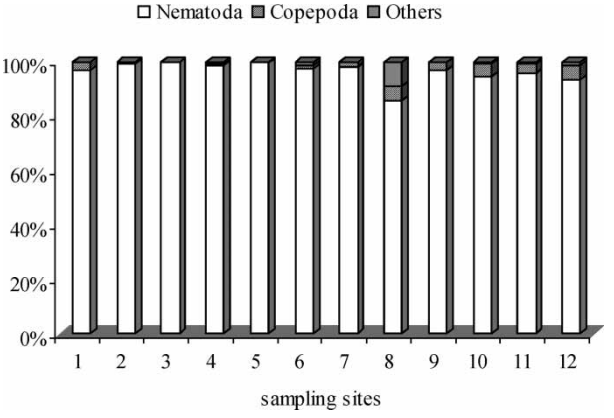

Figure 3. Meiofaunal community structure in the 12 sampling sites (data are expressed as percentage).

Table 4. Numerical abundance (numbers of individuals per cm^2) in the sediment from the different sampling sites.

	1	2	3	4	5	6	7	8	9	10	11	12
Copepoda	14.67	11.00	7.67	8.67	1.33	3.67	14.67	3.33	23.00	17.33	7.33	13.67
Nematoda	453.67	1196.00	1810.67	1839.67	520.00	327.67	783.00	56.67	737.33	337.33	193.00	224.00
Others	1.00	11.33	1.33	17.00	1.67	4.33	2.67	6.00	3.00	2.67	1.33	3.00
Total	469.33	1208.33	1819.00	1865.33	523.00	335.67	800.33	66.00	763.33	357.33	201.67	240.67

Note: others = Ostracoda, Turbellaria, Polychaeta, Kinorhyncha and Pryapulida.

the results of both tests were expressed according to a toxicity scale (Absent–Low–Moderate–High–Very high) [18]. The PWT levels in the sediments collected in spring showed a 'Very high' toxicity level only in stations 1, 2, 3 and 5, whereas no case of 'Very high' toxicity level was recorded in autumn. In the SPT, a 'Very high' toxicity level was recorded in the sediments collected in spring in stations 2, 3, 8, 10 and 12 and in sediments collected in autumn in stations 1, 2, 3 and 4.

Table 5. Identity and abundance (as percentage) of nematodes at the different sampling sites.

Genera	1	2	3	4	5	6	7	8	9	10	11	12
Anoplostoma	0.00	0.00	0.00	0.02	0.00	0.00	0.51	0.00	0.68	1.68	0.00	6.85
Thalassironus	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Syringolaimus	37.25	0.11	0.02	0.02	0.00	0.20	0.00	0.00	0.00	0.00	0.17	0.00
Viscosia	3.31	4.91	1.29	0.82	1.41	7.02	0.04	8.82	0.63	0.89	1.38	4.76
Metoncholaimus	0.07	1.95	0.18	0.05	0.00	0.31	0.00	0.00	0.14	0.00	0.00	0.00
Oncholaimus	0.15	0.59	0.00	0.00	0.00	0.31	0.00	0.00	0.14	0.00	0.00	0.00
Leptolaimus	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Axonolaimus	0.00	0.08	0.11	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aulolaimus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.60
Parodontophora	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00
Araeolaimus	0.15	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cyartonema	0.15	0.22	0.42	0.20	0.00	0.00	0.04	0.00	0.05	0.10	0.00	0.00
Aegialoalaimus	0.44	0.03	0.18	0.07	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Metalinhomoeus	0.59	0.00	0.35	0.16	0.26	0.00	7.54	0.00	0.23	0.89	0.00	3.13
Terschellingia	11.90	16.95	15.21	14.42	51.28	35.10	31.25	39.41	27.35	48.32	40.24	38.24
Xyalidae (family)	1.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Monhystera	0.96	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.45
Theristus	0.15	0.00	0.02	0.34	0.00	0.00	0.43	0.00	0.00	0.49	0.00	0.15
Daptonema	8.82	2.01	0.94	1.85	0.26	6.92	6.77	1.76	5.24	13.44	41.45	22.92
Linhystera	0.07	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cobbia	0.51	0.00	0.11	0.02	0.00	0.10	0.43	0.00	0.14	0.59	0.52	1.64
Sphaerolaimus	0.66	0.08	0.06	0.43	0.71	0.31	1.19	0.59	1.27	1.09	0.52	1.49
Spirinia	0.15	0.06	0.02	0.04	0.00	0.20	0.09	0.00	0.09	0.40	0.00	0.30
Chromaspirina	0.00	0.20	0.13	0.00	0.00	2.24	0.00	0.00	0.59	0.40	0.00	0.00
Molgolaimus	0.07	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00
Aponema	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sabatieria	13.96	69.40	79.78	80.30	44.68	37.74	47.64	41.17	58.00	25.10	11.57	13.54
Vasostoma	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00
Cyatholaimus	1.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00
Actinonema	2.13	0.11	0.11	0.07	0.00	0.00	0.00	0.00	0.09	0.40	0.35	0.00
Neochromadora	2.79	1.42	0.37	0.20	0.64	4.17	1.75	4.12	1.45	2.87	2.07	2.38
Chromadorella	1.47	0.03	0.00	0.00	0.00	0.61	0.13	0.59	0.09	0.10	0.00	0.00
Spilophorella	3.01	0.00	0.00	0.02	0.00	0.00	0.60	0.00	0.00	0.00	0.00	0.00
Ptycholaimellus	0.07	0.00	0.00	0.00	0.00	0.00	0.21	0.00	0.00	0.00	0.00	1.19
Chromadorita	0.15	0.00	0.07	0.00	0.13	0.10	0.04	0.00	0.14	0.00	0.00	0.00
Chromadorina	5.00	1.00	0.00	0.00	0.00	2.64	0.00	0.00	1.99	0.00	0.00	0.00
Unident	2.79	0.78	0.39	0.91	0.51	2.03	1.36	3.53	1.67	2.87	1.73	2.38

3.4 *Meiofaunal analyses*

Data of meiofaunal analyses are reported in figure 3 and tables 4 and 5. As shown in figure 3, Nematodes were by far the most dominant group in all the sampling sites, accounting for more than 90% of the total meiofaunal density, followed by Copepoda (0.2–5%). The total of other recovered meiofaunal taxa (Ostracoda, Polychaeta, Halacaroidea, Kinorhyncha, Priapulida and Turbellaria) never accounted for more than 6% of the total meiofaunal density. Even if Nematoda density (table 4) ranged from 56.67 (station 8) to 1839.67 (station 4) individuals per cm^2, no correlation was found with the different sampling site. Nematodes genera identified in the twelve sampling stations are reported in table 5; sediment of site 1 displayed the dominance of the *Syringolaimus* genus (37.25%), while all the other sampling sites displayed the dominance of the *Terschellingia* (from 14.42% in site 4 to 51.28% in site 5) and *Sabatieria* (from 11.57% in site 11 to 80.30% in site 4) genera.

4. Discussion

Owing to its shallowness, the hydrological condition of the Lesina Lagoon varies with time as a result of changes in freshwater inputs. As a result of the fact that tributaries of the lagoon are reliant on rainfall, the freshwater inputs are at their lowest level in the period June and September; in this period, in fact, the Lesina Lagoon showed its lowest water level and, as a consequence, the highest dissolved oxygen depletion and the highest salinity values were recorded, in agreement with those described for other coastal lagoons [19, 20]. Chlorophyll *a* levels (table 1) ranged from 2 to 6 $\mu g\, l^{-1}$, despite the fact that a few peaks over 10 $\mu g\, l^{-1}$ were recorded, suggesting that the Lesina Lagoon might be in a mesotrophic–eutrophic condition. Distribution of dissolved phosphorus and nitrogen in the lagoon water fluctuated highly with time and space. But if we compare the nitrogen annual means and the relative ranges (table 1) with those of other Mediterranean lagoons, they appear to be similar to those recorded in other Adriatic coastal lagoons such as Venice [21] and Sacca di Goro [22, 23], and lower than those recorded in areas which are strongly eutrophicated, such as the Rhone Delta area, France [24] and Thau Lagoon, France [19]. Moreover, if we consider the PO_4^{3-} level, it always appears lower than that recorded for the previously cited lagoons. Phosphorus therefore seems to play in the Lesina Lagoon a key role as a factor for limiting eutrophication [19].

Toxicity tests can be defined as a biological response to specific exposure conditions, and thus they may provide an indication of potential environmental hazard [7, 25]. Previous studies carried out on the sediments of the Lesina Lagoon showed detectable levels of heavy metals and pesticides (Cd 0.5–0.9 $\mu g\, g^{-1}$; Cu 25.5–2 $\mu g\, g^{-1}$; Zn 1155–255 $\mu g\, g^{-1}$ [26]; 3–10 $ng\, g^{-1}$ [27]; respectively) comparable to those reported in other similar ecosystems characterized by a moderate agricultural impact [2, 28–30]. The sea urchin *P. lividus* bioassays recorded no significant difference in the percentage of unfertilized eggs or abnormal plutei larvae vs. control in the sediment collected in autumn, whereas in sediment collected in spring, at stations 7, 10 and 12 the percentage of unfertilized eggs and of abnormal plutei larvae was significantly higher than that of the control. On the contrary, both Microtox SP test and PW test recorded, in all the sampling sites, medium–high levels of toxicity, higher in sediment collected in spring than in that collected in autumn. These results display that different levels of biotoxicity were recorded by using the two different biological tests (sea urchin *P. lividus* spermiotoxicity and embryotoxicity test vs. Microtox test); the Microtox test, in fact, seems to be more sensitive than the sea urchin *P. lividus* spermiotoxicity and embryotoxicity tests. Such a divergence in sensitivity among tests has been already reported for various test organisms [31, 32]; a bioassay response, in fact, is the result of the additive, synergistic or antagonist effects of pollutants,

which may affect the different test organisms and the different endpoints (mortality, fertility, larval development) in different ways [7, 8, 25]. Besides, both biological tests recorded a lower biotoxicity level in sediments sampled in autumn than in those collected in spring; this is in agreement with the results of other authors, who explain that sources of pollution may vary in quantity and quality from one period to another; and this is particularly true in this case, where agriculture is the main impacting factor. Moreover, temporal variation in environmental parameters (*i.e.* temperature, salinity, pH) can differently affect the availability of toxicants to test organisms [7, 31, 32]. Both tests on PW showed lower toxicity levels in comparison with the SP ones, suggesting that Lesina sediments might present lower levels of bioavailable water-soluble toxic substances [2]; on the other hand, it must also be taken into account that the higher toxicity levels recorded in the Microtox SP test could be partially due to confounding factors such as a high percentage of the pelitic fraction, presence of acid volatile sulphides and high organic matter content [17, 33, 34], which characterize the tested sediments.

Benthic meiofauna can be considered a useful indicator of environmental disturbance due to its short generation time, lack of larval dispersion and a life entirely spent in the sediment [35]. Studies carried out both in marine and coastal lagoon ecosystems showed that despite the fact that meiofaunal organisms, particularly Nematoda, are sensitive to hydrocarbons and different kinds of organic pollutants, their community structure is mainly influenced by the content of organic matter of the sediment. As nematodes are more tolerant of high levels of nitrogen compounds, their density reaches higher levels in areas exposed to nitrogen discharges such as aquaculture and agricultural wastes, whereas the density of copepods, foraminiferans and ostracods decreases in such conditions [36–38]. Our results are consistent with these observations; in fact a diffused, relatively high nitrogen level (table 1) and high sediment TOM content (table 2) corresponds with a meiofaunal community structure characterized by prevalent Nematoda taxa with a homogeneous spatial distribution.

5. Conclusions

An integrated analysis of these results allows us to point out some preliminary conclusions. The relatively high water nutrient and chlorophyll *a* levels suggest a high primary production where phosphorus is the limiting factor, but seem not to be correlated with the different anthropic inputs. Sea urchin *P. lividus* tests show low or absent sediment biotoxicity, again not correlated with the sampling sites and probably consistent with the low toxicant levels recorded in the previous chemical monitoring programs [26, 27]; Microtox tests display a higher sensitivity to our sediment samples in comparison with the sea urchin tests, but also in this case no correlation with the different sites is observed. Finally, the benthic meiofaunal community structure characterized by prevalent Nematoda taxa seems to be correlated with the homogeneously diffused high TOM content of the lagoon sediments. All these observations seem to suggest that the Lesina Lagoon is characterised by a prevalent organic pollution mainly related to agricultural and zootechnical activities which, due to the lagoon's shallowness and the absence of water stratification, presents a homogeneous spatial distribution.

The monitoring program is actually going on in order to better identify the temporal variations of all investigated parameters; moreover, the influence of phytoplankton and benthic macroalgae on the trophic status of the Lesina Lagoon is actually under investigation.

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