The Effect of Floods on Sediment Contamination in a Microtidal Coastal Lagoon: The Lagoon of Lesina, Italy

Article *in* Archives of Environmental Contamination and Toxicology · May 2014

DOI: 10.1007/s00244-014-0037-3 · Source: PubMed

CITATIONS
14

READS
98

6 authors, including:

Raffaele D'Adamo
National Research Council - Institute of Marine Science
112 PUBLICATIONS **629** CITATIONS
SEE PROFILE

Antonietta Specchiulli
Italian National Research Council
121 PUBLICATIONS **459** CITATIONS
SEE PROFILE

Daniele Cassin
Italian National Research Council
40 PUBLICATIONS **605** CITATIONS
SEE PROFILE

Margherita Botter
Italian National Research Council
12 PUBLICATIONS **196** CITATIONS
SEE PROFILE

Arch Environ Contam Toxicol (2014) 67:297–309
DOI 10.1007/s00244-014-0037-3

The Effect of Floods on Sediment Contamination in a Microtidal Coastal Lagoon: The Lagoon of Lesina, Italy

Raffaele D'Adamo · Antonietta Specchiulli · Daniele Cassin · Margherita Botter · Roberto Zonta · Adele Fabbrocini

Received: 14 November 2013 / Accepted: 16 April 2014 / Published online: 27 May 2014

Abstract The effects on the microtidal lagoon of Lesina of runoff and the discharge of water and material from agricultural activities were investigated combining chemical analyses of pollutants [11 metals and 16 priority polycyclic aromatic compounds (PAHs)], determination of organic matter and grain size, and performance of innovative ecotoxicological tests. For metals, enrichment factors >3 for arsenic, nickel, and copper (Cu) were observed in the eastern zone of the lagoon, which is affected by nearby urban activities with discharge of water and domestic waste and by agricultural input with waters rich in fertilizers. Cu was correlated with no other metal, and its high concentrations ($\leq$77 μg g^{-1}) may result from the use of Cu-based fungicides in vineyards. Total PAHs (2,230 ± 3,150 ng g^{-1}) displayed a wide range of concentrations with hot spots near freshwater inputs from the part of the catchment area exploited for wheat crops. Pyrolitic contamination also emerged, with higher-mass PAH congeners, such as asphalt, bitumen or coal, usually present in higher fractions as the dominant components. Ecotoxicological evaluations recorded moderate to high toxicity levels; the innovative MOT test bioassay showed good discriminatory ability because it identified a lagoon area whose inputs mainly depend on agricultural activities and which is impacted by metals rather than PAHs. Floods during periods of heavy rain and the discharge of water and material from agricultural activities may impact vulnerable systems, such as the lagoon of Lesina, where the presence of hot spots with remarkably high pollution values was observed.

R. D'Adamo · A. Specchiulli · D. Cassin · A. Fabbrocini (✉)
Sect. of Lesina (FG), Institute of Marine Science, UOS of Lesina (FG), National Research Council, Via Pola, 4, 71010 Lesina, FG, Italy
e-mail: adele.fabbrocini@fg.ismar.cnr.it

M. Botter · R. Zonta
Sect. of Venice, Institute of Marine Science, National Research Council, Castello 2737/F, 30122 Venice, Italy

Coastal marine environments, including lagoons, are sensitive areas that receive inputs from various anthropogenic sources (e.g., urban, agricultural, recreational) sometimes causing massive accumulation of pollutants (Liß and Ahlf 1997; Libralato et al. 2008). A broad class of environmental chemical pollutants, including trace elements and organic compounds, pose risks to the ecological structure of coastal marine environments and human health (Storelli et al. 2007).

Shallow lagoons are characterized by relatively long residence times due to limited exchange with the sea. Intense use of the surrounding land for industrial, agricultural, and livestock purposes causes alteration of water quality and habitats (anoxic crises, changes in community structure, changes in type of bottom and vegetation, etc.). In addition, especially in areas with a large watershed, excessive discharges of water and material due to external causes, including climatic events (storms, torrential rains, rapid runoff), are often the main factors responsible for further degradation and contamination of the aquatic environment (Zonta et al. 2005; Guerra et al. 2009; Specchiulli et al. 2011). The resulting transport of sediment into lagoons involves a series of processes that distribute fine-grained sediment between various sources and sinks. During the transport process, sediments are often extensively modified, recycled, and reworked by storms, wind waves, and tidal mechanisms. The final result is that the lagoons primarily function as a sink for particle-bound pollutants (Eyles and Meriano 2010). Fine sediment deposits can later be remobilized by locally generated waves.

Lesina Lagoon, one of the most economically important lagoons in southern Italy, is an example of an ecosystem

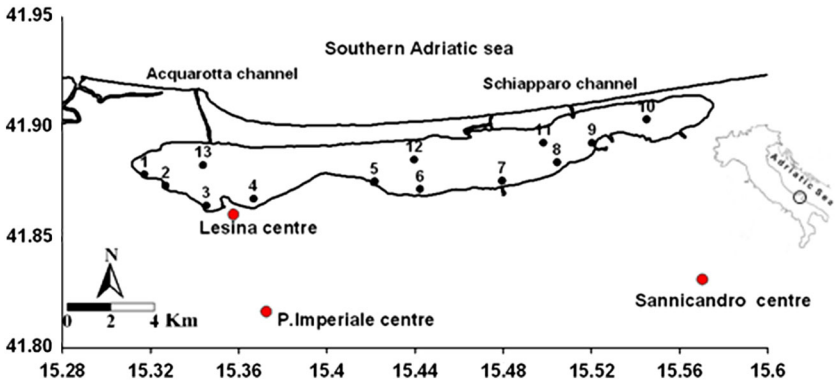

Fig. 1 Lagoon of Lesina with locations of sampling sites

that has had anthropogenic impact in recent years. From the late 1990s to 2005, local farmers shifted their agricultural land nearer to the edges of waterways due to lack of rainfall, thus causing a decrease in channel cross sections. After 2005, the climate became rainy again (>1,000 mm year^{-1}) with sudden filling of the catchment area channels. Between 2008 and 2009, at the farmers' request, the channels were dredged to ensure the free flow of waters and prevent fields from flooding, and the resulting material was dumped along the edges. Since that time, exceptional rainfall events have occurred in autumn (as much as 100 mm of rainfall during 24 h in October 2010), which caused the transport to the lagoon of a large amount of water and sediments. Thus, dredged channels have become the main source of sediments and potentially associated pollutants for the Lesina Lagoon. Hence, the main aim of this study was to assess chemical contamination in the lagoon after a flood event and its possible adverse effects on the biota. Chemical and biological approaches were used, providing sufficiently specific information to allow the chemical perturbation to be identified and its potential ecological relevance to be assessed. The results of this study should provide a more general comprehension of its effects on fragile ecosystems such as this one. In detail, this work focused on (1) the evaluation of pollutant level alteration in lagoon sediments after an exceptional climate event (a flood occurred in October 2010) by comparing chemical and ecotoxicological data before and after the event, and (2) the evaluation of discriminatory ability with respect to the lagoon sediments of both standardized (FEC test, Microtox test) and innovative (MOT test) ecotoxicological tests.

Materials and Methods

Study Area

Lesina Lagoon (41.88° N, 15.45° E), situated on the southern Adriatic coast of Italy (Fig. 1), is a shallow (0.7–1.5 m) microtidal water body approximately 22 km long with a total area of approximately 51 km^2 and a catchment area of approximately 600 km^2. It is separated from the sea by a sand bar approximately 18 km long and 1 km wide, to which it is connected by two narrow tidal channels.

The main freshwater inputs occur along the southern zone of the lagoon from two rivers (Lauro and Zannella) collecting wastewaters from most of the catchment area, six intermittent streams, and two drainage pumping stations (Lauro and Pilla).

Much of the annual freshwater budget is discharged into the southeastern basin accounting for the east–west salinity gradient, which is more pronounced during summer (Manini et al. 2003; Roselli et al. 2009; Ferrarin et al. 2014). Precipitation is approximately 400–1,000 mm year^{-1} following a strong seasonal pattern with low levels in summer and most rainfall in autumn to winter (Consorzio di Bonifica di Capitanata, Foggia, Italy; http://www.consorzio.fg.it). The lagoon of Lesina is a nursery area for numerous fish and crustacean species of commercial value, and fishing is an important activity for the local economy. Currently approximately 30,000 people reside within its immediate catchment area, but during the summer season the number doubles due to the presence of tourists. Moreover, the catchment area is overexploited for agricultural activities, which entail crop rotation two–three times per year. In addition, many fish and livestock farms discharge into the lagoon. The industrialization of local agriculture, with its consequent increase in commercial activities and urban development, has caused stress to the lagoon and its adjacent areas.

Sampling Design

Thirteen sites were chosen for the purposes of the study: nine of them near freshwater inputs and four in the central area of the lagoon (Fig. 1); their main characteristics are listed in Table 1.

Triplicate superficial sediment samples (top 5 cm) were collected from the sites on 20 November 2010 using a modified Van der Horst core sampler with a polyethylene

Table 1 Main characteristics of sampling sites

Sampling sites	Longitude (E)	Latitude (N)	Site characterization	Sediment characteristics	
				Mud (%)	OM (%)
1	15°18′55.69″	41°52′35.91″	Wastewater discharge of zoo-technical facilities	54	5
2	15°19′30.25″	41°52′ 7.52″	At the mouth of streams collecting agricultural runoff	94	6
3	15°20′37.23″	41°51′44.98″	Close to the urban centre and wastewater discharge of small productive facilities	89	6
4	15°21′54.32″	41°51′56.06″	Near the main municipal sewage in the western part of the lagoon	99	6
5	15°25′12.01″	41°52′24.51″	Near the pumping station	73	9
6	15°26′25.99″	41°52′12.16″	At the mouth of streams collecting agricultural runoff	89	5
7	15°28′40.01″	41°52′25.54″	Wastewater discharge of zoo-technical facilities	98	7
8	15°30′09.96″	41°52′55.52″	Near the main municipal sewage in the eastern part of the lagoon	95	7
9	15°31′06.89″	41°53′27.40″	At the mouth of streams collecting agricultural runoff	93	9
10	15°32′36.19″	41°54′05.89″	Inside the eastern area at the border of a rich zone of phragmites	45	9
11	15°29′47.39″	41°53′27.50″	Near the entrance of the channel communicating with the sea	83	11
12	15°26′16.33″	41 52′59.97″	Located along the north-central zone of the lagoon far from local input	86	13
13	15°20′31.68″	41°52′51.03″	Located along the north-central zone of the lagoon far from local input	97	9

OM organic matter

(PE) liner 1 m long and 4 cm in diameter. Samples were collected during the rainy season when rivers and streams usually discharge large quantities of terrestrial and runoff material into the lagoon. Two superficial sediment samples were stored at −30 °C in glass bottles [for polycyclic aromatic compounds (PAHs)] and in PE bottles (for metals) with Teflon-lined caps. Before analyses, samples were unfrozen, sieved to <0.2 cm after removing stones, and fully homogenized. Data for metals (μg g^{-1}) and PAHs (ng g^{-1}) are reported on a dry-weight (dw) basis.

The third superficial sediment sample was collected for the ecotoxicological tests and kept at +4 °C in PE bottles with Teflon-lined caps. Porewaters (PWs) were extracted within 4 h of sampling as described later in the text.

In addition, sediment grain size and PW ammonia levels were evaluated. Grain size analyses were performed by wet-sieving mud (<62.5 μm), sand (62.5–500 μm), and gravel (>500 μm) fractions. PW ammonia levels were evaluated in accordance with American Public Health Association methods (Grasshoff et al. 1983).

Major and Trace-Metal Analyses

Each sediment sample was gently squeezed to break down aggregates and screened through a PE sieve to remove particles >1 mm. Part of the screened sediment was dried in an oven at 105 °C until reaching constant weight to measure water content (Percival and Lindsay 1997). The dried sediment was ground to powder using an agate pestle and mortar before analyzing its major and trace metal content.

The dried and ground part of each sample (approximately 0.4 g dw) was extracted with 8 ml of HNO_3 (69 %) in a microwave oven (Multiwave 3000; Anton Paar, Graz, Austria). The extracted samples were left to cool at room temperature and then filtered through a 0.45-μm nitrocellulose membrane filter. The extract collected in a volumetric flask was then diluted to 40 ml with MQ water (United States Environmental Protection Agency, USEPA 1994a).

The concentrations of the metals [aluminum (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn)] were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES; Optima 2100DV; Perkin Elmer, USA; USEPA 1994b). Mercury (Hg) analyses were performed by cold vapour atomic absorption spectrophotometry AAS; Analyst 100; Perkin Elmer; USEPA 1976).

The blank sample and the certified reference material (BCR-277r estuarine sediment, Community Bureau of Reference) were extracted and analyzed with each batch of 15 samples in accordance with method 6010C (USEPA 2007a). Good recoveries were obtained ranging between 80 % (Zn) and 100 % (Hg). The analytical variation,

determined using five replicates of homogenized samples, was <10 % for all metals. Calibration for ICP-AES and AAS analysis was performed with prepared external standards by way of the standard curve approach. Full calibration was performed after every set of 48 samples. The method detection limit for metal analysis was defined as three times the SD of 10 replicate blank measurements and ranged between 0.01 (Hg) and 15 (Al) μg g^{-1}.

PAH Analysis

Sediment samples were thoroughly mixed, sieved through a 1-mm mesh to remove any debris, and subsequently air dried in the dark at room temperature for 48 h on hexane-rinsed Al foil. The dry samples were finely ground in an agate mortar. Extraction was performed using a Microwave Sample Preparation System (Multiwave 3000; Anton Paar) in accordance with USEPA recommendations (2007b; Method 3546). Two grams of dried sediments were accurately weighed into lined microwave extraction vessels. This was followed by the addition of a 25 ml of 1:1 acetone–hexane solvent mixture. At the end of the oven program, vessels were cooled to room temperature, and the extracts were filtered and rinsed with the same solvent mixture. Sulphur compounds were removed by soaking the extracts with activated Cu powder. Purification and fractionation were performed by eluting extracts through chromatography glass columns packed with silica gel/alumina/Florisil (4 + 4 + 1 g). The first fraction, containing most polar compounds, was eluted with 25 ml of *n*-hexane, whereas the second fraction, containing the PAHs, was eluted with 30 ml of 8:2 *n*-hexane–methylene chloride solvent mixture (Fossato et al. 1996, 1998) and then concentrated with a rotary evaporator (Rotavapor-R Buchi, CH) for analysis.

The concentrations of 16 USEPA priority pollutant PAHs [naphthalene, acenaphthene (Ace), acenaphthylene, fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (Ft), pyrene (Py), benzo(*a*)anthracene (B[*a*]A), chrysene (Chy), benzo(*b*)fluoranthene (B[*b*]Ft), benzo(*k*)fluoranthene (B[*k*]Ft), benzo(*a*)pyrene (B[*a*]Py), dibenzo(*a*,*h*)anthracene (diB[*a*,*h*]A), benzo(*g*–*i*)perylene, and indenopyrene)] was determined by high performance liquid chromatography (PE 200; USA) coupled to a programmed fluorescence detector (HP 1046A; USA). The column used was a reverse-phase Supelcosil LC-PAH (L = 150 mm id = 3 mm, 5 μm). Linear gradient elution was executed with acetonitrile–water mixture as the mobile phase at a flow rate of 0.8 ml min^{-1}. The mobile phase started with 40 % acetonitrile held constant for 4 min, increased to 100 % in 11 min, and then held constant for another 10 min. The column compartment was thermostated at 40 °C.

PAHs were identified by matching retention times and quantified with reference to calibration curves established for each compound by analyzing five external standards. The average R^2 of the calibration curves was >0.99, and the RSD of the calibration factors was always <20 %. Average PAH recoveries were in the range 80–108 %. The method detection limits (measured using the calibration curve method) ranged between 0.05 and 0.1 ng g^{-1}. Blanks were run for the entire procedure. Validation of recovery and accuracy was based on IAEA-417 (2001) sediment sample certified reference materials purchased from the International Atomic Agency, Wien, Austria.

Ecotoxicological Tests

Bioassays were performed on the PW samples immediately after extraction. PW was extracted by centrifuging (4,300×g for 30 min at 4 °C) each sediment sample in capped PE centrifuge tubes within 4 h after sampling. The salinity of the PW samples was measured and adjusted to 35 ± 0.5 ‰ when necessary.

Pools of eggs and sperm were obtained from adult sea urchins (*Paracentrotus lividus*, Lmk 1816) and reared to sexual maturation under controlled conditions as described in Fabbrocini and D'Adamo (2010, 2011). Fertilization and motility tests on sea urchin gametes were performed as described in Fabbrocini et al. (2010).

For the sea urchin fertilization test (FEC test), aliquots of 0.1 ml of semen (1.5 × 10^7 sperm cells) were diluted in 10 ml of PW and incubated for 60 min at 18 °C. At the end of the incubation period, untreated eggs were added (sperm-to-egg ratio 15,000:1) and incubated in 10-ml polystyrene multiwell dishes (30 min at 18 °C in the dark). Samples were preserved in concentrated buffered formalin, and the percentage of fertilized eggs was scored by observing 200 eggs/sample. Artificial sea water (ASW; American Society for Testing and Materials, ASTM 2004) was used for the negative control and Cu (in scaled concentrations from 12 to 96 μg g^{-1}) was used as the reference toxicant for the positive controls.

For the sea urchin motility test (MOT test), dry sperm were diluted in PW at a ratio of 1:1,000 and incubated for 60 min at 18 °C. After incubation, sperm motility was evaluated by a computerised motion analysis system, the Sperm Class Analyzer (Microptic, s.l., Barcelona, Spain) whose acquisition parameters were set as described in Fabbrocini et al. (2010). Each recorded field consisted of a mean of three replicates each analyzing from 250 to 500 sperm tracks.

The following motion parameters were assessed: (1) percentage of rapid sperm (percentage of sperm with curvilinear velocity, VCL >100 μm s^{-1}), (2) VCL (μm s^{-1}), (3) straight-line velocity [VSL (μm s^{-1})], and (4) average path velocity [VAP (μm s^{-1})].

ASW was used as a negative control, whereas cadmium as Cd(II) solution (Baker Italy, Milan), in scaled concentrations from 0.1 to 10 mg g^{-1}, was used for the positive control.

For the *Vibrio fischeri* (Microtox) test, lyophilised *V. fischeri* bacteria (NRRL B-11177) and all Microtox reagents were obtained from SDI Europe, Hampshire, UK. Every fresh vial of bacteria is certified to have an EC_{50} value for phenol falling in the acceptability range (13.0–26.0 mg l^{-1}). Aqueous extracts were prepared as described previously and tested using the Comparison Test for Marine and Estuarine Samples (Azur Environmental Ltd. 1998). Data were expressed as effect percentages in terms of decrease in bioluminescence after 5 and 15 min of exposure. Bioluminescence responses were measured using a Microtox Model 500 analyser (SDI Europe, Hampshire, UK). Because negligible differences in toxicity were observed between the two different exposure durations, 5- and 15-min data were grouped together and used for all processing.

Statistical Analyses

Descriptive data analyses (referencing the mean, median, minimum, maximum, SD, and variability coefficient) were performed to highlight the spatial variability of pollutants and to associate possible hot-spot areas with anthropogenic inputs. Pearson correlation analysis was performed to clarify the relationships between abiotic variables, and values were considered significant at $p < 0.01$.

As for ecotoxicological data, the responses of each end point (motion parameters and fertilization rate in *P. lividus* and decreased bioluminescence in *V. fischeri*) were corrected for effects in controls by applying Abbott's formula (ASTM 2004), thus obtaining the effect percentage. For fertilization rate and velocity parameter end points, some negative values were returned for effect percentages at sites 2, 4–6; as they were never >10 % greater than control levels and given the generally low toxic responses recorded in these sites, we considered them a consequence of the biological variability of controls rather than an overcompensation response to stress (Chapman 2002), and they were recorded as zero (say 0.0001) for statistical purposes.

Data for Cd and Cu were expressed as EC_{50} values with 95 % confidence limits calculated using the Trimmed Spearman–Karber statistical method (ASTM 2004). The differences among sites in the effect percentages after exposure to sediments were analyzed by one-way analysis of variance (ANOVA); when significant effects were observed, Duncan's post hoc test was performed. p values <0.01 were considered to be significant. Before analysis, data were arcsine or square root transformed and tested for normality using Cochran's test and for homogeneity of variance using Shapiro–Wilk test.

Last, principal component analysis (PCA) was used to determine the relationships between chemical data and toxicological tests and to identify possible hot spots in the lagoon, thus underlying factors that explain the pattern of correlation within the observed variables. All statistical analyses were performed with the software STATISTICA 6.0 (StatSoft, Tulsa, Oklahoma, USA).

Results

Metal Content

Major and trace metal concentrations, together with the main descriptive statistics for the top layer of sediments in the lagoon of Lesina, are listed in Table 2 together with the limits laid down by Italian legislation concerning sediments in coastal marine environments (DM 260/2010 in response to Decision N.2455/2001/EC of the European Parliament and of the Council of 20 November 2001) and National Oceanic and Atmospheric Administration, NOAA (1999) guideline values (Long et al. 1995). The Italian regulatory limits are seen to be exceeded in some sites for some metals. For Hg, the observed concentrations were always lower than the limit of 0.3 μg g^{-1}, with the highest value (0.12 μg g^{-1}) measured at site 2, which is located in the western part of the basin near the most densely populated area near the lagoon. Twelve of 13 sites had Cd in excess of the legal limit (reaching approximately 0.70 μg g^{-1} at sites 2, 9, and 11). Sites located in both the western and eastern areas, along the southern edge of the lagoon, had excessive levels of Cr (reaching 60 μg g^{-1} at sites 7 and 8) and As (reaching 20 μg g^{-1} at sites 4 and 8). A few sites (4, 7, and 8) had excessive levels of Pb [36 μg g^{-1} (site 7)], whereas Ni was above the limit of 30 μg g^{-1} at 8 of 13 sites (maximum 48 μg g^{-1} at site 8). Comparison with NOAA sediment guidelines (Long et al. 1995) (Fig. 2a) shows that no metal had values higher than the effect-range median (ERM). Three metals (Ni, Cu, and As) had concentrations between the effect range low (ERL) and ERM, a range within which adverse effects may occasionally occur, whereas Hg, Cr, Pb, Cd, and Zn were all below the ERL, which is used to estimate conditions in which adverse effects will rarely occur. The potential risk posed by Ni and Cu was confirmed by the enrichment factors (EFs) calculated as reported in Bloundi et al. (2009). EFs of approximately 4 were observed for Cu at sites 7 and 8, and EFs >3 were observed for Ni at sites 9 and 11.

PAHs

Total PAH concentrations in the sediment ranged from 56 to 10,484 ng g^{-1} (Table 2) with four–five-ring PAHs being

Table 2 Main descriptive statistics for metals (μg g^{-1}), single PAH congeners (ng g^{-1}), and ΣPAHs (ng g^{-1})

	Mean	Median	Min.	Max.	SD	DM 260/2010	ERL/ERM
Major and trace metals							
Hg	0.05	0.05	0.005	0.12	0.03	0.3	0.15/0.71
Cr	44.90	48.60	11.70	63.80	15.30	50	81/370
Cu	35.10	31.50	8.50	76.70	18.70	–	34/270
Ni	31.80	31.10	9.30	48.40	11.20	30	21/52
Pb	26.10	26.70	10.60	36.10	6.80	30	47/218
As	13.20	13.00	2.50	21.00	5.10	12	8.2/70.0
Cd	0.61	0.52	0.27	1.13	0.23	0.3	1.2/9.6
Zn	87.00	90.00	32.00	116.00	23.00	–	150/410
Mn	826	865	448	1,139	182	–	–
Fe	32,894	34,087	7,992	47,601	11,732	–	–
Al	52,118	54,887	10,986	78,140	18,718	–	–
PAHs							
Ace	7.25	6.41	0.005	37.44	9.71		16/500
Fl	4.91	0.005	0.005	27.07	8.61		19/540
Phe	148.90	55.72	13.99	801.30	234.20		240/1,500
Ant	61.61	12.62	1.82	351.50	100.29	45	85.3/1,100
Fluoranthene	500.00	119.00	10.61	2,233.00	727.36	110	600/5,100
Py	346.40	76.82	8.47	1,849.80	540.34		665/2,600
B[*a*]A	185.50	48.44	0.01	856.70	257.96		261/1,600
Chy	231.20	58.17	1.24	1,138.50	331.44		384/2,800
B[*b*]Ft	216.80	52.44	5.21	941.70	286.65	40	–
B[*k*]Ft	91.63	19.93	1.17	430.40	128.55	20	–
B[*a*]Py	186.40	29.17	0.43	872.80	265.08	30	430/1,600
diB[*a*,*h*]A	15.01	0.005	0.01	86.16	26.74		63.4/260
Benzo(*g*–*i*)perylene	136.20	25.53	2.17	456.50	165.15	55	–
Indeno(1,2–3-cd)pyrene	98.38	27.73	0.005	400.90	132.79	70	–
ΣPAHs	2,230	525	56	10,484	3,153	800	4,022/44,792

Comparison with Italian regulatory guidelines for sediments in coastal marine environments (DM 260/2010) and ERL and ERM values specified in NOAA sediment guidelines (Long et al. 1995)

the most important components. The highest PAH concentrations were detected in sediments from sites 3 (close to the town of Lesina), 5 (near the Pilla drainage pumping station), and 12 (central lagoon) ranging from 4,695 to 10,484 ng g^{-1}. With respect to Italian guideline values for sediments (DM 260/2010; Table 2), total PAHs are >800 ng g^{-1} in 6 of 13 sites (site 1 = 1,117 ng g^{-1}, site 3 = 10,484 ng g^{-1}, site 5 = 5,895 ng g^{-1}, site 10 = 3,493 ng g^{-1}, site 11 = 4,695 ng g^{-1}, and site 12 = 1,807 ng g^{-1}). Specifically, high molecular congeners, such as B[*a*]A, B[*k*]Ft, and B[*a*]Py, show high values in approximately half the sites with maximum levels reached in the sediments of sites 3 and 5. Anthropogenic impact from micro-organic pollution at sites 3 and 5 emerges from comparison of total PAHs as well as each congener with ERL and ERM values (Fig. 2b). The sources of the PAHs were investigated with reference to the molecular ratios of specific hydrocarbons as reported in other studies (Baumard et al. 1998; Cardellicchio et al. 2007). Specifically, Phe/Ant and Fl/Py were used to discriminate between petrogenic and pyrolytic contamination. High-temperature processes, such as the combustion of organic matter (OM), generate PAHs characterized by Phe/Ant ratios <10 and Fl/Py ratios >1, whereas the slow maturation of OM during catagenesis leads to Phe/Ant values >15. Nevertheless, for both ratios, the limits separating the two processes are not precise, and reliable identification of the PAH source requires reference to both indices. In the data set as a whole, the Phe/Ant ratios were <10 (1.5–7.5), and the Fl/Py ratios were >1 (1.1–1.8).

Ecotoxicological Tests

The computer-assessed motility parameters and the fertilization capability of gametes from the *P. lividus* specimens used in the assays, recorded before exposure to the tested matrices, fell in the acceptability range (Fabbrocini and D'Adamo 2011).

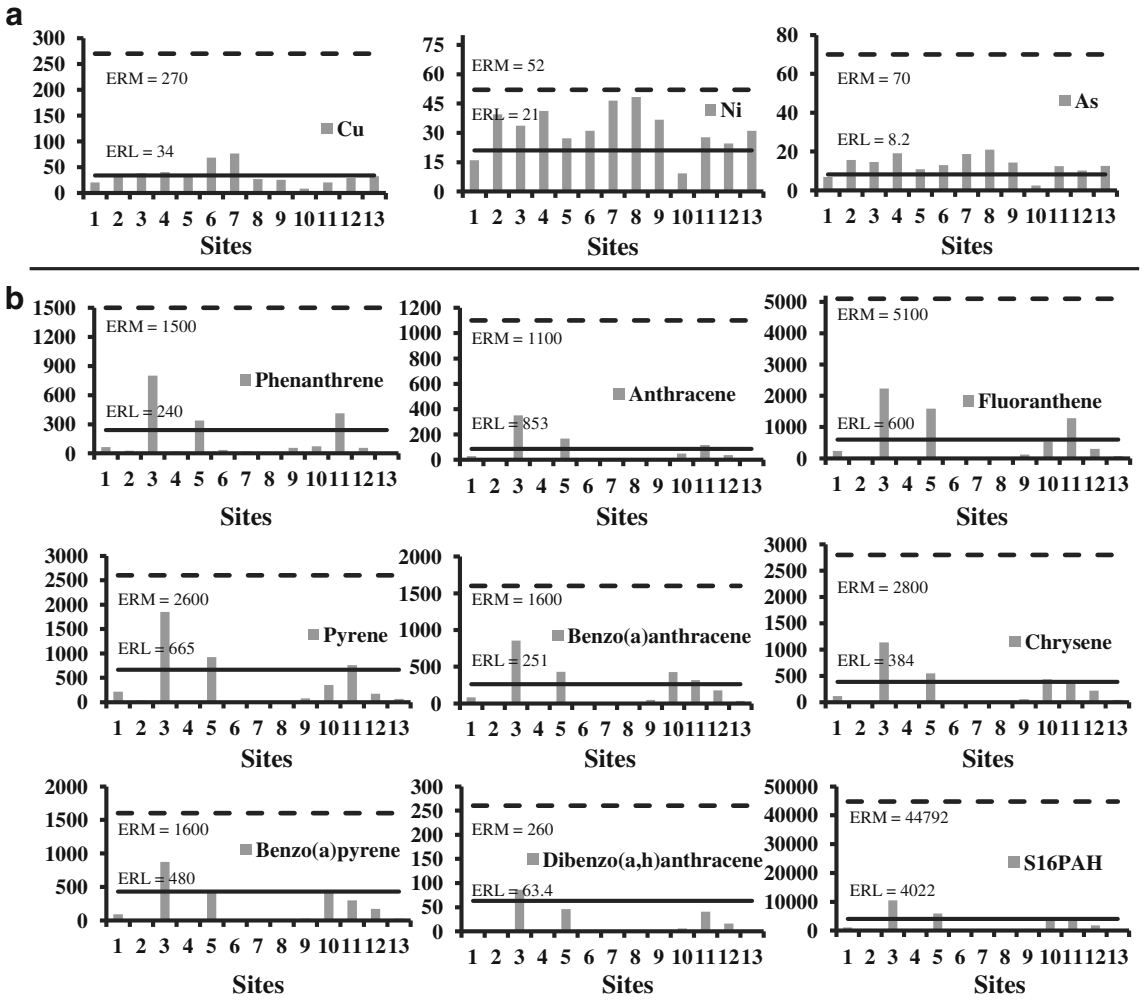

Fig. 2 **a** Metals (μg g^{-1}dw) and **b** PAHs (ng g^{-1} dw) compared with ERL and ERM values given in Long et al. (1995)

The effect of the tested PWs on *P. lividus* fertilization rates (FEC test) is shown in Fig. 3e. Significant differences among the 13 sampling sites were recorded by ANOVA ($F_{1,12} = 74.99$). Toxicity was found to be absent or negligible (effect percentage <10 %) in samples from sites 1 to 6, whereas those from sites from 7 to 13 returned effect percentages of 30–80 %. The highest toxicity was recorded for site 11; Duncan's post hoc test showed that it did not differ significantly from sites 9, 12, and 13 but was significantly higher than all of the other sites. No significant differences were recorded among sites 7, 8 and 10, whereas site 7 showed significantly lower toxicity than the "intermediate toxicity" at sites 9, 12, and 13. The effect of the tested PWs on the sperm motility parameters (MOT test) is shown in Fig. 3a–d.

For all four end points, ANOVA returned significant differences among the 13 sampling sites ($F_{1,12} = 202.47$, $F_{1,12} = 120.75$, $F_{1,12} = 77.44$, and $F_{1,12} = 91.83$ for RAP, VCL, VSL and VAP, respectively). Duncan's post hoc test showed that the highest toxicity levels were recorded at sites 1 and 8, with effect percentages of 80–90 %, and were similar to each other and significantly higher than those of all of the other sites. At sites 7 and 9, the effect percentages ranged from 50 to 70 % without significant differences; the values were significantly lower than those at sites 1 and 8 but again higher than the others. Last, effect percentages of 20–40 % were recorded at sites 10–13, which were significantly higher than those at sites 2–6, for which the lowest effect percentages (≤10 %) were reported.

As expected, there was a high positive correlation ($0.89 < R^2 < 0.98$) among the 4 end points tested (RAP, VCL, VSL, VAP). In contrast, no correlation was found with data from the FEC test. The EC_{50} values recorded for the reference toxicant (Cd) on the four motility end points fell in the same range previously recorded for this test (Fabbrocini et al. 2010).

The effect on *V. fischeri* (Microtox) of the tested PWs is shown in Fig. 3f. The reported data represent the averages of the 5- and 15-min readings because no differences were recorded between the two times. Strong inhibition of luminescence was recorded for all sampling sites, although

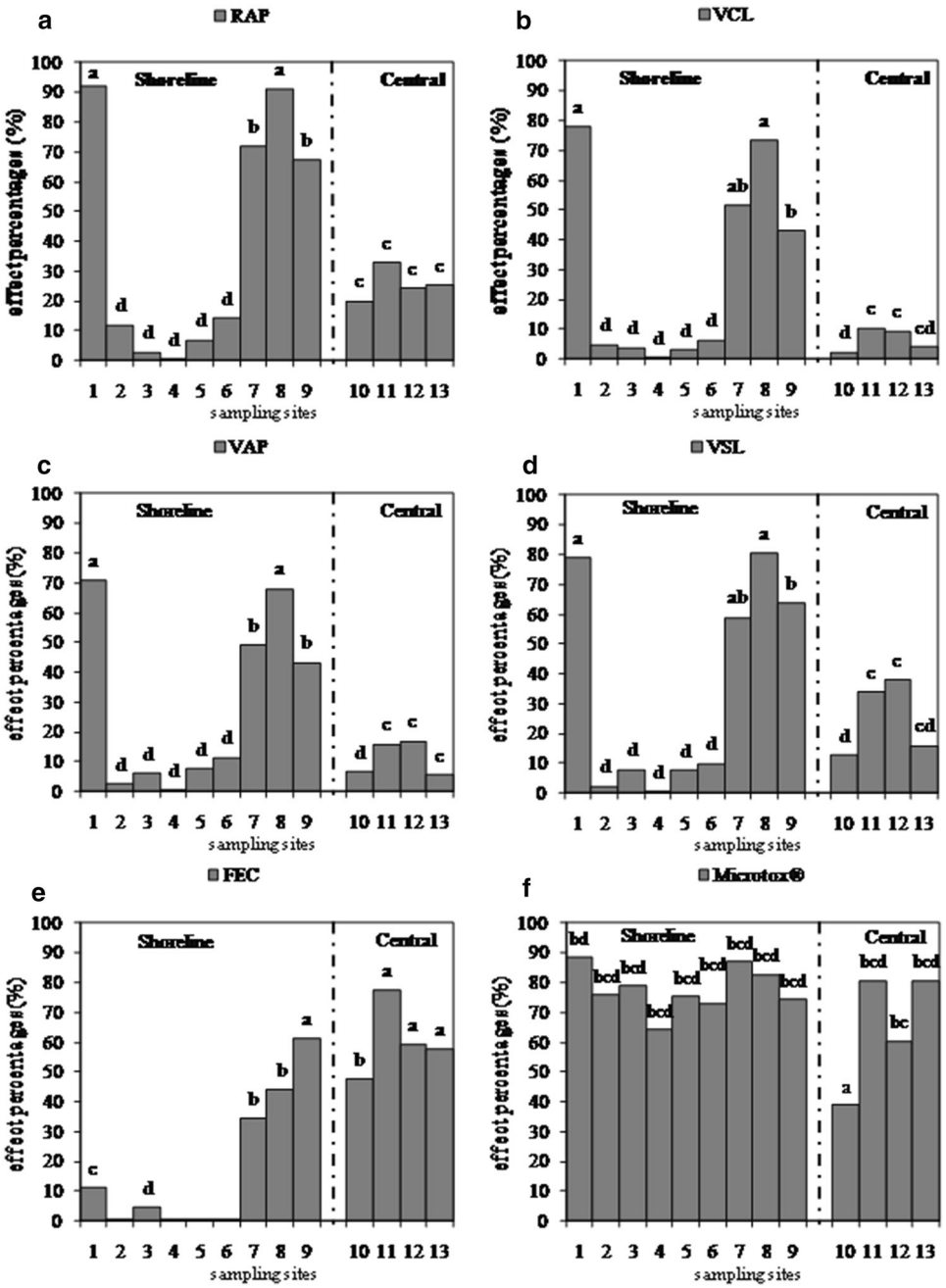

Fig. 3 Results (effect percentages) of bioassays performed on PW samples for 13 sampling sites. **a** Rapid spermatozoa. **b** VCL (μm s^{-1}). **c** VAP (μm s^{-1}). **d** VSL (μm s^{-1}). **e** FEC test (fertilization success). **f** Microtox test (decrease in bioluminescence). Values with different *lower-case superscript letters* are significantly different ($p < 0.01$)

ANOVA showed significant differences among the 13 sampling sites ($F_{1,12} = 4.86$). Duncan's post hoc test showed that the effect percentage at site 10 was significantly lower than all of the other sites. No differences were recorded among the other sampling sites except for site 12, where the toxicity was significantly lower than that at site 1. Moreover, no correlation was found with data from the FEC and MOT tests.

PCA

Applying PCA to all of the recorded data (Fig. 4), two factors accounting for 72 % of the total variance were obtained. Factor 1 was mainly related to metals (Cr, Pb, Ni, As, and Zn) and sediment grain size, whereas factor 2 was mainly related to PAHs and RAP, VCL, VSL, and VAP values. PCA clearly separated sampling sites into four distinct groups, which were moreover quite distant from each other.

The first two groups were each composed of a single site: site 1, characterized by sandy sediment, low metal concentrations, and a high response to ecotoxicological testing, and site 10, which was similar to site 1 in terms of particle size and metal concentration, but with higher OM content and a lower ecotoxicological response. The

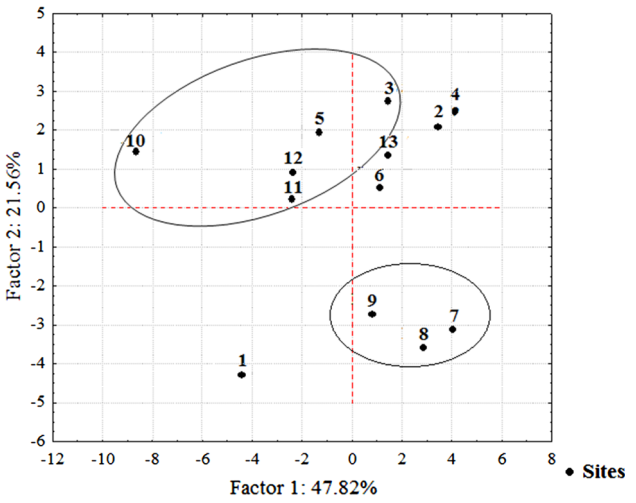

Fig. 4 PCA ordination diagram based on mean total PAHs, Hg, As, Cu, Cr, Cd. Ni, Pb, Zn, Mn, Fe, Al, grain size, organic matter, FEC test, Microtox test, MOT test. *Circles* group together, along the same factor loadings, sites with similar pollution profiles

segregation of these two groups is explained by variables associated with factor 2. The third cluster included sites 7–9, characterized by the lowest sand content (and, correspondingly, the highest mud content), the highest major metal concentrations, and the highest ecotoxicological response. In the fourth cluster were the remaining sites, which were separated from site 10 (along factor 1) by their differing metal content and from the third cluster (along factor 2) by their higher PAH concentrations and more significant ecotoxicological responses.

Discussion

In this work we assessed the impact of a particular climate event, such as a flood, on the sediment contamination level of a shallow microtidal lagoon, also evaluating the discriminatory ability with respect to the sediment contaminants of an innovative ecotoxicological test (MOT tests). Our results show that the occurrence of a flood significantly impacted the lagoon sediments because both the contaminant levels and the toxicity response recorded by the bioassay, increased with respect to previous observations (Fabbrocini et al. 2005; D'Adamo et al. 2008; Specchiulli et al. 2011); in particular, the innovative MOT test proved to give clearly different responses with sediments from different lagoon sites.

Regarding metals, with respect to values measured in the lagoon in 2004, increases in all metal concentrations, especially Cu and Zn, were observed (D'Adamo et al. 2008). Although the major metal [Al, Mn, and Fe (Table 2)] concentrations recorded in this study are similar to those found in previous studies performed in Lesina Lagoon (Andresini 2005), the data for the minor metals (As, Hg, Ni, Cd, Cr, Pb, and Zn) deserve some consideration.

Table 3 shows that Lesina Lagoon is comparable with other Mediterranean areas considered to have low human impact. Nevertheless, comparison with the Italian guidelines (DM 260/2010) and the NOAA international sediment-quality standards (Long et al. 1995) shows that some areas of the lagoon are moderately contaminated by Ni, Cu, and As. These areas, which are also characterized by EFs >3 (Bloundi et al. 2009), are confined to the eastern zone, along the southern border of the lagoon, near urban water and domestic waste discharges and agricultural inputs with waters rich in fertilizers. Moreover, the absence of correlation between Cu and other metals suggests a peculiar anthropogenic source, probably related to the use of Cu-based fungicides in vineyards to the southeast of the Lesina Lagoon (sites 7 and 8) as also reported in other areas (Pietrzak and McPhail 2004). Similarly to some metals, the concentrations of ΣPAHs and some specific congeners measured in this study are higher than the values recorded in a previous study in the same area (Specchiulli et al. 2011). Moreover, concentrations recorded in this study are higher than those measured in other Mediterranean areas (Table 4), and their distribution pattern is characterised by a wide range of concentrations (mean 2,230 ng g^{-1}, SD = 3,150 ng g^{-1}).

The highest concentrations were found in areas of high human impact, suggesting local sources. Moreover, applying the approach used for evaluating potential toxicity (ERL and ERM) described by Long et al. (1995), some congeners could have potentially adverse effects at sites 3, 5, and 10–12, which are grouped along positive factor 2 in the PCA. The highest pollution, found at sites 3 and 5, may be related to runoff from the extensive area south of the lagoon, which in the months before the sampling in 2010 was abundant. This part of the catchment area is intensively exploited for wheat crops, and open burning of biomass is a common technique for crop residue disposal and land preparation, thus representing a considerable source of pollutants. Deliberate agricultural burning for the purposes of vegetation removal is a frequent practice in the surrounding area and, as reported in other studies (Jenkins et al. 1996), wheat straw has high total PAH emission factors. Site 10 is located at the far eastern end where water residence time is low (50 days; Ferrarin et al. 2014), and anthropogenic discharges during rainy periods may be the main source of pollutants (Fabbrocini et al. 2005). Sites 11 and 12 are located in the central area, and their high organic contamination may be attributable to hydrodynamic conditions and the hydraulic gradient between the two seaward channels during intense wind events (Ferrarin et al. 2014). A comparison of the results of the ecotoxicological evaluation of the Lesina Lagoon sediments with

Table 3 Range of metal concentrations (μg g^{-1} dw) and mud levels (%) in sediments in the Lesina Lagoon and in other sites of the Mediterranean areas

Sites	Mud	Hg	Cr	Cu	Ni	Pb	As	Zn	References
Lesina Lagoon (I)	84.4	0.00–0.12	12–64	8–77	9–48	11–36	3-21	32–116	This study
Lesina Lagoon (I)			0.5–31	0.4–14		0.6–28		3–24	D'Adamo et al. (2008)
Berre Lagoon (F)	85.8	0.15–0.40	38–428	11–48	18–56	18–82	4-10	50–151	Accornero et al. (2008)
Venice Lagoon IZ (I)	74.5	0.0–98.5				103–1,890	15-244	356–16,880	Bellucci et al. (2002)
Venice Lagoon CZ (I)	71.8	0.0–1.25				44–146	6-55	200–1,428	Bellucci et al. (2002)
Northeastern lagoons (TN)			9–56	11–36	10–26	13–61		59–234	Hellal Mel et al. (2011)
Ölüdeniz Lagoon (TR)			21–56	5–9		5–10		10–24	Tuncel et al. (2007)
Albanian Adriatic coast		0.02–2.33	146–812	14–624	110–413	10–51		17–355	Çelo et al. (1999)
Gulf of Taranto, Ionian Sea (I)		0.04–0.41	75–103	42–52	48–61	45–75		87–129	Buccolieri et al. (2006)

IZ industrial zone, *CZ* central zone, *I* Italy, *F* France, *TN* Tunisia, *TR* Turkey

Table 4 Range of PAH levels (ng g^{-1} dw) and organic matter content (%) in Lesina Lagoon sediments and in other sites in the Mediterranean areas

Sites	OM	PAHs	References
Lesina Lagoon (I)	7.94	74–10,484	This study
Stagnone Lagoon (I)	2.1	65–17,700	Culotta et al. (2006)
Pialassa Baiona (I)		2,500–120,000	Fabbri et al. (2006)
Orbetello Lagoon (I)		1–279	Perra et al. (2009)
Venice Lagoon CZ (I)	10.68*	23–532	Secco et al. (2005)
Ghar El Melh Lagoon (TN)	2.9*	41–665	Ameur et al. (2010)
Lago di Faro e Ganzirri (I)		74–5,755	Giacalone et al. (2004)
Lesina Lagoon (I)		9–70	Specchiulli et al. (2011)
Varano Lagoon (I)		7–55	Specchiulli et al. (2011)
Santa Giusta Lagoon (I)		4–251	Specchiulli et al. (2011)
Mar Piccolo (I)		380–12,750	Cardellicchio et al. (2007)

CZ central zone, *I* Italy, *TN* Tunisia

those previously obtained in the same area (Fabbrocini et al. 2005) shows an increase in the toxic response for both the FEC and Microtox tests, thus reflecting the general increase in pollutant levels recorded with respect to previous samplings.

The MOT test, as previously observed for the sediment of the contiguous Varano Lagoon (Fabbrocini et al. 2010), yielded clearly different responses in the various sampling sites, thus showing a good discerning ability. It is worth noting that the toxicity levels recorded by the MOT test did not specifically correlate with any of the investigated pollutants; therefore, they can be considered as the reflection of the integrated effect on the sperm cells of all of the threatening factors present in the tested matrices.

To summarize and compare ecotoxicological and chemical data, the results of the toxicity bioassays were integrated into a unified toxicity score, which ranks samples in five classes (from class 1 = no toxicity response to class 5 = strong toxicity response in accordance with Ahlf and Heise 2005). As shown in Fig. 5, moderate to high toxicity (classes 4 and 5) was recorded in all case, with the highest toxicity scores at sites 1, 7, 8, 9, and 11–13. A mismatch between the toxicity levels predicted by the NOAA guidelines and those directly obtained by bioassays is seen in some cases: when following this approach, the bioavailability of toxicants and the susceptibility of test species thus must be taken into account (Hübner et al. 2009; Montero et al. 2013). A comparison of the individual toxicity tests with the results of the unified toxicity scores (Figs. 3, 5, respectively) shows that at the western end of the lagoon (sites 2–6), the toxicity score mainly depends on the Microtox results, whereas the MOT and FEC test end points indicated little or no toxicity. Similarly, the Microtox and FEC tests account for the toxicity scores of the central lagoon (sites 11–13), and Microtox and MOT tests for that of site 1. In contrast, at the eastern end of the lagoon (sites 7–9), positive toxic responses were returned by all bioassays.

The results of the present study highlight divergences in response among the performed bioassays. Toxicants may elicit different effects on different end points due to the physiological function they affect, thus leading to different sensitivity thresholds (Losso et al. 2007b; Fabbrocini et al. 2010). Heavy metals are known to impair the spermatozoa of aquatic species in a variety of ways, e.g., affecting

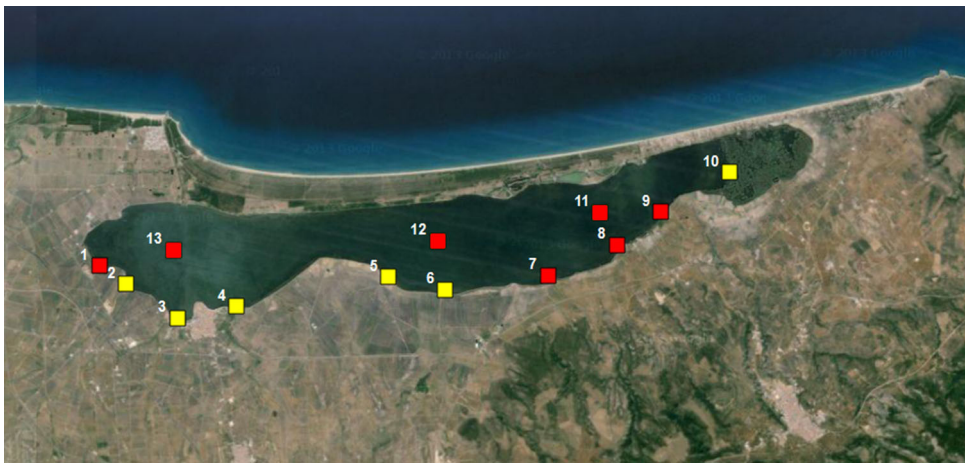

Fig. 5 Toxicity scores calculated for 13 sampling sites based on ecotoxicological tests (from class 1 = no toxicity response to class 5 = strong toxicity response following Ahlf and Heise 2005). *White squares* class 1, *blue squares* class 2, *green squares* class 3, *yellow squares* class 4, *red squares* class 5 (Color figure online)

mitochondrial *cristae* (Au et al. 2000) and plasma-membrane water channels (Abascal et al. 2007), displacing calcium ions (Dietrich et al. 2011), or inducing oxidative stress (Li et al. 2010), thereby altering both the motility and the fertilization ability of the sperm. In contrast, PAHs and their metabolites are highly liposoluble and therefore easily pass through cell membranes by passive diffusion (Evans and Nipper 2008), thus affecting both sperm cells and embryos mainly by oxidative damage (Pillai et al. 2003; Zhou et al. 2006). It is also necessary to take account of additive, synergic, or antagonistic interactions among toxicants as well as the presence of confounding factors, which may lead to unclear toxicological responses (Fernández and Beiras 2001). For example, simultaneous exposure of *P. lividus* sperm to a mixture of Cd and Zn appears to decrease Cd toxicity (Pagano et al. 1986), whereas an additive effect of Hg and Pb was recorded on *P. lividus* embryos (Fernández and Beiras 2001). Similarly, humic acids, which are a major component of dissolved OM (DOM) in aquatic environments, are known to decrease the bioavailability of some heavy metals such as Cu and Pb (Lorenzo et al. 2002), although contrasting effects on sea urchin larval development and microalgal growth, depending on the origins of the DOM, have been reported (Sánchez-Marín et al. 2010).

Moreover, the mud fraction, which in the collected sediments ranged from 45 to 99 % (see Table 1), could partly account for the diffuse high toxicity recorded by the Microtox test because it is strongly influenced by grain size (Stronkhorst et al. 2003). Finally, ammonia, which often acts as a confounding factor in ecotoxicological bioassays (Carr et al. 2006; Losso et al. 2007a), ranged in the PW samples from 1.2 to 12 mg l^{-1}, way below the EC_{50} levels for both the FEC test (Losso et al. 2007a) and the Microtox test (Qureshi et al. 1982). All of these considered, the MOT test proved an ability to discern among impacting sources, being more sensitive toward metals rather than PAHs. Because it seemed to be able to respond to the integrated effect of all threatening factors present in the tested matrices, but scarcely influenced by confounding factors, it is therefore a promising new bioassay to be used in ecotoxicological evaluations of aquatic environments.

Conclusion

Floods after storm events and the transport of materials lying along the edges of drainage streams may impact vulnerable systems such as the lagoon of Lesina. Indeed, both the chemical and the ecotoxicological analyses highlighted an increasing trend of pollution levels (critically high in some areas) compared with those recorded in previous studies.

Ecotoxicological tests are designed to provide a rapid and easy-to-perform evaluation of the synergistic and/or antagonistic effects of all factors that may adversely affect an aquatic ecosystem. The innovative MOT test proved to have good discriminatory ability, whereas the FEC test and Microtox test recorded diffuse nonspecific toxicity. Therefore, the MOT test can be considered a promising bioassay to be used not only as a part of routine biomonitoring programs but also as "quick response tool" in the case of exceptional events. Finally, in light of the European Water Framework Directive, this research shows importance for the preservation of the integrity of a lagoon ecosystem and for the correct management of its surrounding areas and of the anthropogenic activities that take place there.

Acknowledgments This work was supported by a Grant to Raffaele D'Adamo by Amministrazione Provinciale di Foggia, Settore Attività Produttive (Agreement number 2693 [4 August 2009]). The authors thank Tiziana D'Ambrosio (CNR-ISMAR, Lesina) for support in conducting field measurements and laboratory analyses and the CSA Laboratory in Rimini (Italy) for the grain-size analysis. George Metcalf revised the English text.

References

Abascal FJ, Cosson J, Fauvel C (2007) Characterization of sperm motility in sea bass: the effect of heavy metals and physico-chemical variables on sperm motility. J Fish Biol 70:509A–522A

Accornero A, Gnerre R, Manfra L (2008) Sediment concentrations of trace metals in the Berre Lagoon (France): an assessment of contamination. Arch Environ Contam Toxicol 54:372–385

Ahlf W, Heise S (2005) Sediment toxicity assessment. Rationale for effect classes. J Soils Sediments 5(1):16–20

American Society for Testing and Materials (2004) Standard guide for conducting static acute toxicity tests with echinoid embryos. In: Annual book of ASTM standards. ASTM, Philadelphia, p 18

Ameur WB, Trabelsi S, Driss MR (2010) Polycyclic aromatic hydrocarbons in superficial sediments from Ghar El Melh Lagoon, Tunisia. Bull Environ Contam Toxicol 85(2):184–189

Andresini A (2005) L'ambiente di sedimentazione recente e attuale del Lago di Lesina. Dissertation, University of Bari, Bari

Au DWT, Chiang MWL, Wu RSS (2000) Effects of cadmium and phenol on motility and ultrastructure of sea urchin and mussel spermatozoa. Arch Environ Contam Toxicol 38:455–463

Baumard P, Budzinski H, Garrigues P (1998) Polycyclic aromatic hydrocarbons (PAHs) in sediments and mussels of the western Mediterranean Sea. Environ Toxicol Chem 17:765–776

Bellucci LG, Frignani M, Paolucci D, Ravanelli M (2002) Distribution of heavy metals in sediments of the Venice Lagoon: the role of the industrial area. Sci Total Environ 295:35–49

Bloundi MK, Duplay J, Quaranta G (2009) Heavy metal contamination of coastal lagoon sediments by anthropogenic activities: the case of Nador (East Morocco). Environ Geol 56:833–843

Buccolieri A, Buccolieri G, Cardellicchio N, Dell Atti A, Di Leo A, Maci A (2006) Heavy metals in marine sediments of Taranto Gulf (Ionian Sea, Southern Italy). Mar Chem 99(227):235

Cardellicchio N, Buccolieri A, Giandomenico S, Lopez L, Pizzulli F, Spada L (2007) Organic pollutants (PAHs, PCBs) in sediments from the Mar Piccolo in Taranto (Ionian Sea, Southern Italy). Mar Pollut Bull 55(10–12):451–458

Carr RS, Biedenbach JM, Nipper M (2006) Influence of potentially confounding factors on sea urchin porewater toxicity tests. Arch Environ Contam Toxicol 51:573–579

Çelo V, Babi D, Baraj B, Çullaj A (1999) An assessment of heavy metal pollution in the sediments along the Albanian coast. Water Air Soil Pollut 111:235–250

Chapman PM (2002) Ecological risk assessment (ERA) and hormesis. Sci Total Environ 288:131–140

Culotta L, De Stefano C, Gianguzza A, Mannino MR, Orecchio S (2006) The PAH composition of surface sediments from Stagnone Coastal Lagoon, Marsala (Italy). Mar Chem 99:117–127

D'Adamo R, Di Stasio M, Fabbrocini A, Petitto F, Roselli L (2008) Migratory crustaceans as biomonitors of metal pollution in their nursery areas. The Lesina Lagoon (SE Italy) as a case study. Environ Monit Assess 143:15–24

Dietrich M, Dietrich GJ, Hliwa P, Ciereszko A (2011) Carp transferrin can protect spermatozoa against toxic effects of cadmium ions. Comp Biochem Physiol C 153:422–429

Evans AD, Nipper M (2008) The influence of biomass on the toxicity of hydrophobic organic contaminants. Arch Environ Contam Toxicol 54:219–225

Eyles N, Meriano M (2010) Road-impacted sediment and water in a Lake Ontario watershed and lagoon, City of Pickering, Ontario, Canada: an example of urban basin analysis. Sediment Geol 224:15–28

Fabbri D, Baravelli V, Giannotti K, Donnini F, Fabbri E (2006) Bioaccumulation of cyclopenta[*cd*]pyrene and benzo[*ghi*] fluoranthene by mussels transplanted in a coastal lagoon. Chemosphere 64(7):1083–1092

Fabbrocini A, D'Adamo R (2010) Gamete maturation and gonad growth in fed and starved sea urchin *Paracentrotus lividus* (Lamarck, 1816). J Shellfish Res 29(4):1051–1059

Fabbrocini A, D'Adamo R (2011) Gametes and embryos of sea urchins (*Paracentrotus lividus*, Lmk., 1816) reared in confined conditions: their use in toxicity bioassays. Chem Ecol 27(2): 105–115

Fabbrocini A, Guarino A, Scirocco T, Franchi M, D'Adamo R (2005) Integrated biomonitoring assessment of the Lesina Lagoon (Southern Adriatic Coast, Italy): preliminary results. Chem Ecol 21(6):479–489

Fabbrocini A, Di Stasio M, D'Adamo R (2010) Computerized sperm motility analysis in toxicity bioassays: a new approach to pore water quality assessment. Ecotoxicol Environ Saf 73(7):1588–1595

Fernández N, Beiras R (2001) Combined toxicity of dissolved mercury with copper, lead and cadmium on embryogenesis of early larval growth of the *Paracentrotus lividus* sea-urchin. Ecotoxicology 10:263–271

Ferrarin C, Zaggia L, Paschini E, Scirocco T, Lorenzetti G, Bajo M et al (2014) Hydrological regime and renewal capacity of the micro-tidal Lesina Lagoon, Italy. Estuar Coast 37(1):79–93

Fossato VU, Campesan G, Craboledda L, Dolci F, Stocco G (1996) Organic micropollutants and trace metals in water and suspended particulate matter. In: Lasserre P, Marzollo EA (eds) Venice Lagoon ecosystem, vol 1. UNESCO/MURST, Paris

Fossato VU, Campesan G, Dolci F, Stocco G (1998) Trends in chlorinated hydrocarbons and heavy metals in sediments of Venetian canals. Rapp Comm Int Mer Médit 35:258–259

Giacalone A, Gianguzza A, Mannino MR, Orecchio S, Piazzese D (2004) Polycyclic aromatic hydrocarbons in sediments of marine coastal lagoons in Messina, Italy: extraction and GC/MS analysis, distribution and sources. Polycycl Aromat Compd 24:135–149

Grasshoff K, Ehrhardt M, Kremling K (1983) Methods of seawater analysis. Verlag Chemie, Weinheim

Guerra R, Pasteris A, Ponti M (2009) Impacts of maintenance channel dredging in a northern Adriatic coastal lagoon. I: effects on sediment properties, contamination and toxicity. Estuar Coast Shelf Sci 85:134–142

Hellal Mel A, Hellal F, El Khemissi Z, Jebali R, Dachraoui M (2011) Trace metals in algae and sediments from the north-eastern Tunisian lagoons. Bull Environ Contam Toxicol 86(2):194–198

Hübner R, Astin KB, Herbert RJH (2009) Comparison of sediment guidelines (SQGs) for the assessment of metal contamination in marine and estuarine environments. J Environ Monit 11:713–722

International Atomic Energy Agency (2001) World-wide and regional intercomparison for the determination of organochlorine compounds, petroleum hydrocarbons and sterols in sediment sample. IAEA, Vienna

Jenkins BM, Jones AD, Turn SQ, Williams RB (1996) Emission factors for polycyclic aromatic hydrocarbons from biomass burning. Environ Sci Technol 30:2462–2469

Li ZH, Li P, Dzyuba B, Randak T (2010) Influence of environmental related concentrations of heavy metals on motility parameters and antioxidant responses in sturgeon sperm. Chem Biol Interact 188:473–477

Libralato G, Losso C, Arizzi Novelli A, Citron M, Della Sala S, Canotto E et al (2008) Ecotoxicological evaluation of industrial port of Venice (Italy) sediment samples after a decontamination treatment. Environ Pollut 156:644–650

Liß W, Ahlf W (1997) Evidence from whole-sediment, pore water and elutriate testing in toxicity assessment of contaminated sediments. Ecotoxicol Environ Saf 36:140–147

Long ER, MacDonald DD, Smith SL, Calder FD (1995) Incidence of adverse biological effects within ranges of chemical

concentrations in marine and estuarine sediments. Environ Manag 19:81–97

Lorenzo JI, Nieto O, Beiras R (2002) Effect of humic acids on speciation and toxicity of copper to *Paracentrotus lividus* larvae in seawater. Aquat Toxicol 58:27–41

Losso C, Arizzi Novelli A, Picone M, Marchetto D, Pantani C, Ghetti PF et al (2007a) Potential role of sulfide and ammonia as confounding factors in elutriate toxicity bioassays with early life stages of sea urchins and bivalves. Ecotoxicol Environ Saf 66:252–257

Losso C, Picone M, Arizzi Novelli A, Delaney E, Ghetti PF, Volpi Ghirardini A (2007b) Developing toxicity scores for embryotoxicity tests on elutriates with the sea urchin *Paracentrotus lividus*, the oyster *Crassostrea gigas*, and the mussel *Mytilus galloprovincialis*. Arch Environ Contam Toxicol 53:220–226

Manini E, Fiordelmondo C, Gambi C, Pusceddu A, Danovaro R (2003) Benthic microbial loop functioning in coastal lagoons: a comparative approach. Oceanol Acta 26:27–38

Montero N, Belzunce-Segarra MJ, Menchaca I, Garmendia JM, Franco J, Nieto O et al (2013) Integrative sediment assessment at Atlantic Spanish harbours by means of chemical and ecotoxicological tools. Environ Monit 185:1305–1318

National Oceanic and Atmospheric Administration (1999) Sediment quality guidelines developed for the National Status and Trends Program. http://archive.orr.noaa.gov/book_shelf/121_sedi_qual_guide.pdf. Accessed 21 May 2014

Pagano G, Cipollaro M, Corsale G, Esposito A, Ragucci E, Giordano GG et al (1986) The sea urchin: bioassay for the assessment of damage from environmental contaminants. In: Cairns J Jr (ed) Community toxicity testing. ASTM, Philadelphia, pp 67–92

Percival JB, Lindsay PJ (1997) Measurement of physical properties of sediments. In: Mudroch A, Azcue JM, Mudroch P (eds) Manual of physico-chemical analysis of aquatic sediments. CRC Press, Boca Raton, pp 7–45

Perra G, Renzi M, Guerranti C, Focardi SE (2009) Polycyclic aromatic hydrocarbons pollution in sediments: distribution and sources in a lagoon system (Orbetello, Central Italy). Transit Waters Bull 3:45–58

Pietrzak U, McPhail DC (2004) Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. Geoderma 122:151–166

Pillai MC, Vines CA, Wikramanayake AH, Cherr GN (2003) Polycyclic aromatic hydrocarbon disrupt axial development in sea urchin embryos through a β-catenin dependent pathway. Toxicology 186:93–108

Qureshi AA, Flood KW, Thompson SR, Janhurst SM, Inniss CS, Rokosh DA (1982) Comparison of a luminescent bacterial test with other bioassays for determining the toxicity of pure compounds and complex effluents. In: RNO (1998) Surveillance du milieu marin. Travaux du RNO. Ifremer et Ministere de l'Amenagement, du Territoire et de l'Environnement

Roselli L, Fabbrocini A, Manzo C, D'Adamo R (2009) Hydrological heterogeneity, nutrient dynamics and water quality of a non-tidal lentic ecosystem (Lesina Lagoon, Italy). Estuar Coast Shelf Sci 84:539–552

Sánchez-Marín P, Santos-Echeandía J, Nieto-Cid M, Álvarez-Salgado A (2010) Effect of dissolved organic matter (DOM) of contrasting origins on Cu and Pb speciation and toxicity to *Paracentrotus lividus* larvae. Aquat Toxicol 96:90–102

Secco T, Pellizzato F, Sfriso A, Pavoni B (2005) The changing state of contamination in the Lagoon of Venice. Part 1: organic pollutants. Chemosphere 58:279–290

Specchiulli A, Renzi M, Perra G, Cilenti L, Scirocco T, Florio M et al (2011) Distribution and sources of polycyclic aromatic hydrocarbons (PAHs) in surface sediments of some Italian lagoons exploited for aquaculture and fishing activities. Int J Environ Anal Chem 91(4):367–386

Storelli MM, Barone G, Garofalo R, Marcotrigiano GO (2007) Metals and organochlorine compounds in eel (*Anguilla anguilla*) from the Lesina Lagoon, Adriatic Sea (Italy). Food Chem 100: 1337–1341

Stronkhorst J, Schipper C, Brils J, Dubbeldam M, Postma J, van de Hoeven N (2003) Using marine bioassays to classify the toxicity of Dutch harbour sediments. Environ Toxicol Chem 22(7): 1535–1547

Tuncel SG, Tugrul S, Topal T (2007) A case study on trace metals in surface sediments and dissolved inorganic nutrients in surface water of Ölüdeniz Lagoon-Mediterranean, Turkey. Water Res 41(2):365–372

United States Environmental Protection Agency (1976) Method 245.1, mercury (manual cold vapor technique). USEPA, Washington, DC

United States Environmental Protection Agency (1994a) Method 3051A, microwave assisted acid digestion of sediments, sludges, soils and oils. USEPA, Washington, DC

United States Environmental Protection Agency (1994b) Method 200.7, determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry. USEPA, Washington, DC

United States Environmental Protection Agency (2007a) Method 6010C, inductively coupled plasma-atomic emission spectrometry. USEPA, Washington, DC

United States Environmental Protection Agency (2007b) Revision method 3546, microwave extraction. USEPA, Washington, DC

Zhou B, Liu W, Siu WHL, O'Toole D, Lam PKS, Wu RSS (2006) Exposure of spermatozoa to duroquinone may impair reproduction of the common carp (*Cyprinus carpio*) through oxidative stress. Aquat Toxicol 77:136–142

Zonta R, Collavini F, Zaggia L, Zuliani A (2005) The effect of floods on the transport of suspended sediments and contaminants: a case study from the estuary of the Dese River (Venice Lagoon, Italy). Environ Int 31:948–958