



## Marine Sediment Contamination and Toxicity in Izmir Bay and Mytilene Harbor (Aegean Sea)

Rahime Oral<sup>1,\*</sup>, Maria Kostopoulou<sup>2</sup>, Marco Guida<sup>3</sup>, Anastasia Nikolaou<sup>2</sup>, Françoise Quiniou<sup>4</sup>, Marco Trifuoggi<sup>5</sup>, Ilaria Borriello<sup>3</sup>, Maria Vagi<sup>2</sup>, Antonello D'Ambra<sup>6</sup>, Giovanni Pagano<sup>3</sup>

<sup>1</sup> Ege University, Faculty of Fisheries, TR-35100 Bornova, Izmir, Turkey.

<sup>2</sup> Aegean University, Department of Marine Sciences, GR-81100 Mytilene, Greece.

<sup>3</sup> Federico II University, Department of Biological Sciences, Section of Hygiene, I-80134 Naples, Italy.

<sup>4</sup> BE/EX IFREMER, Centre de Brest, F-29280 Plouzané, France.

<sup>5</sup> Federico II University, Department of Chemistry, I-80126 Naples, Italy.

<sup>6</sup> 2<sup>nd</sup> Naples University, Department of Strategy and Quantitative Methods, I-81043 Capua (CE), Italy.

\* Corresponding Author: Tel.: +90.232 3113085; Fax: +90.232 3883685;  
E-mail: rahime.oral@ege.edu.tr

Received 06 January 2012  
Accepted 23 June 2012

### Abstract

The aim of the present study was to evaluate marine contamination and toxicity in sediment and pore water from the areas of Izmir Bay (Turkey) and Mytilene Harbor (Greece). The evaluation was performed in terms of selected toxic metals, polycyclic aromatic compounds (PAH) and toxicity to sea urchin (*Paracentrotus lividus*) early development and fertilization. Significantly higher levels of metals and total PAH were detected in Izmir vs. Mytilene sediment, and the highest contamination was detected in Izmir Bay innermost sites. Bioassays were carried out in *P. lividus* embryos and sperm, by evaluating developmental defects and changes in fertilization success by exposing sea urchin embryos or sperm to sediment/seawater suspensions. Whole sediment (WS) showed a higher toxicity of Izmir vs. Mytilene samples both in terms of developmental defects and of spermiotoxicity. Pore water (PW) and solid phase (SP) were separated by WS centrifugation and tested on embryos or sperm. The induction of developmental defects showed a significantly higher toxicity of WS compared to SP and, even more so, to PW. A significant decrease in fertilization success was observed following sperm suspension in SP from both Izmir and Mytilene sediment samples, whereas PW-exposed sperm failed to display any decrease of fertilization success vs. controls. The use of WS bioassays is suggested as a default procedure in sediment toxicity testing, especially in a topographic characterization of polluted sediments.

**Keywords:** sediment toxicity, whole sediment, pore water, sea urchins, Aegean Sea.

### İzmir Körfezi ve Midilli Limanı'nda (Ege Denizi) Denizel Sediment Bulaşması ve Toksikitesi

#### Özet

Bu çalışmanın amacı, İzmir Körfezi (Türkiye) ve Midilli Limanı (Yunanistan) bölgelerinden alınan sediment ve gözenek suyunda denizel bulaşmanın ve toksisitenin değerlendirilmesidir. Değerlendirme seçilmiş toksik metaller, polisiklik aromatik hidrokarbon (PAH) bileşikleri ve deniz kestanesi (*Paracentrotus lividus*) erken gelişimi ile fertilizasyonu üzerine toksisitesi açısından yapıldı. Midilli'ye göre İzmir sedimentlerinde daha yüksek metal ve toplam PAH değerleri tespit edildi. En fazla kirlenme İzmir Körfezi'nin iç bölgelerinde belirlendi. Biyodenyeler, sediment/deniz suyu karışımlarına maruz kalan deniz kestanesi embriyoları ve spermalarının gelişim bozuklukları ve fertilizasyon başarısındaki değişiklikler değerlendirilerek *P. lividus* embriyo ve spermaları ile gerçekleştirildi. Hem gelişim bozuklukları ve hem de spermiyotoksosite açısından tüm sediment (TS), Midilli'ye göre İzmir örneklerinde daha yüksek toksisite gösterdi. Gözenek suyu (GS) ve katı faz (KF), TS'in santrifüjü ile ayrılarak embriyolar ve spermalarla test edildi. Gelişim bozukluklarının induksiyonu KF ve hatta GS ile karşılaştırıldığında TS önemli derecede yüksek toksisite gösterdi. Hem İzmir ve hem de Midilli KF sediment örneklerine maruz kalan spermelerde dölleme başarısında belirgin bir azalma gözlenirken GS'na maruz kalan spermelerin dölleme başarısında ve kontrollerde herhangi bir azalma görülmedi. TS biyoteslerinin kullanımı özellikle kirli sedimentlerin topografik karakterizasyonu için sediment toksisitesinin test edilmesinde varsayılan bir yöntem olarak önerilmektedir.

**Anahtar Kelimeler:** sediment toksisitesi, tüm sediment, gözenek suyu, deniz kestanesi, Ege Denizi.

#### Introduction

Evaluating contamination and toxicity of marine sediment represents a major challenge in preventing

or ameliorating both hazards to marine biota and to human health, fisheries and leisure activities, as most anthropogenic chemicals eventually accumulate in sediments, especially in enclosed bays, fiords and

harbors. The concentrations of metals and polycyclic aromatic hydrocarbons (PAHs) are common indicators for marine sediment contamination (Ingersoll, 1995; Chapman *et al.*, 2002; Araújo *et al.*, 2009; Hübner *et al.*, 2009).

In studies of sediment contamination and toxicity, a major issue may be envisaged in the roles for whole sediment (WS) vs. its components, namely sediment solid phase (SP) and pore water (PW) (Pagano *et al.*, 2001; Ingersoll *et al.*, 2009). In fact, the microbial load of naturally occurring sediments prevents the implementation of WS toxicity testing in microbial bioassays or in other test systems requiring sterile conditions (Mucha *et al.*, 2003; Chen and White, 2004; Davoren *et al.*, 2005). Thus, a number of bioassays for sediment toxicity are run on PW, or on elutriates, or organic extracts, leaving the questions unsolved as to the realistic exposure conditions of benthic biota, or otherwise sediment-related biota. Moreover, testing PW or elutriate toxicity leads to disregarding the role(s) of SP in sediment toxicity (Pagano *et al.*, 2001).

Sediment toxicity tests by sea urchin (*P. lividus*) bioassays have been extensively utilized in evaluating a number of xenobiotics, model mixtures, and several complex mixtures (Pagano *et al.*, 1986; 1996; 2001; Guillou *et al.*, 2000; Sellem *et al.*, 2000; De Nicola *et al.*, 2004; 2007; Cesar *et al.*, 2004; Meriç *et al.*, 2005; Ignacio *et al.*, 2006; Manzo *et al.*, 2008; Salamanca *et al.*, 2009). In view of sediment toxicity testing, it should be noticed that sea urchin embryos, up to hatching (10 h post-fertilization) lay in direct contact with sediment samples, thus providing a well-fit model for sediment toxicity testing.

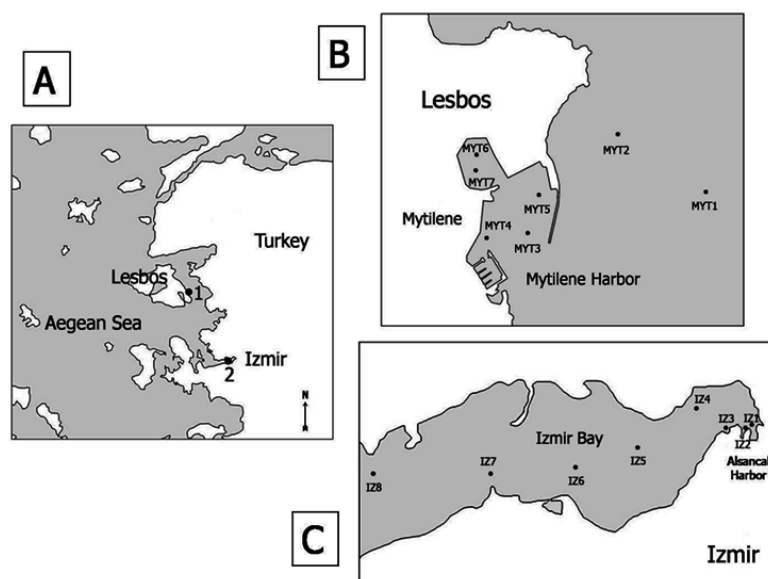
The aim of the present study is to evaluate the contamination of marine sediments and pore water, as well as the toxicity to sea urchins. The study areas were Izmir Bay (Turkey) and Mytilene Harbor (Greece). Sediment samples from Izmir Bay and

Mytilene Harbor (Aegean Sea, Eastern Mediterranean) (Figure 1 and Table 1) were evaluated for some selected pollution indicators, including metals and PAHs. Their toxicity to sea urchin early development and fertilization was also tested, by evaluating developmental defects and changes in fertilization success.

## Materials and Methods

### Sediment Sampling Location and Handling

Sediments were collected with a van Veen grab in Izmir Bay and Mytilene Harbor (Figure 1), whose coordinates are shown in Table 1. Samples were taken at ~10 cm depth as most of the macrofaunal species are located in this sediment strip (Mermillod-Blondin *et al.*, 2005). Sediment samples were carried to the laboratory in refrigerated boxes, then stored at +4°C and tested within maximum two weeks after collection. Prior to running bioassays, sediment samples were mixed thoroughly and subdivided in aliquots that were tested as wet WS or centrifuged (500×g for 10 min) to obtain SP and PW. Approximately 2-g aliquots of WS and SP samples were air dried prior to bioassays, by obtaining the dry weight equivalents utilized in running bioassays. Organic carbon was measured in the <2 mm fraction by the Walkley-Black method, adopted and modified by Jackson (1958). According to this method ~0.5 g of dried sediment was oxidized with 10 ml of 1 N K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and 20 ml of concentrated H<sub>2</sub>SO<sub>4</sub>. After 30 min, 200 ml of distilled water, 10 ml of 85% H<sub>3</sub>PO<sub>4</sub> and 0.2 g of NaF were added and the solution was back titrated with 0.5 N of Fe(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> in the presence of diphenylamine indicator to a one-drop end point. Pollutant analysis was carried out in the <0.63 μm fraction according to the methods described below.



**Figure 1.** The study area. A. Aegean Sea; B. Mytilene Harbor; C. Izmir Bay.

**Table 1.** GPS coordinates of Izmir and Mytilene sediment sampling sites

Sampling Stations	Depth (m)	Latitude	Longitude
Izmir	IZ1	13	38° 26' 8.07" N 27° 9' 9.53" E
	IZ2	9.6	38° 26' 7.09" N 27° 9' 5.79" E
	IZ3	9	38° 26' 7.09" N 27° 9' 2.55" E
	IZ4	9.7	38° 27' 2.24" N 27° 8' 0.82" E
	IZ5	17.5	38° 26' 2.02" N 27° 6' 0.37" E
	IZ6	15.7	38° 25' 6.15" N 27° 4' 0.89" E
	IZ7	11	38° 25' 4.87" N 27° 0' 9.77" E
	IZ8	7.8	38° 25' 6.39" N 26° 57' 1.13" E
Mytilene	MYT1	29.8	39° 6' 8.40" N 26° 34' 36.00" E
	MYT2	20.5	39° 6' 22.80" N 26° 34' 10.20" E
	MYT3	10.5	39° 6' 0.00" N 26° 33' 40.80" E
	MYT4	5.5	39° 5' 59.40" N 26° 33' 28.80" E
	MYT5	13.7	39° 6' 9.00" N 26° 33' 44.40" E
	MYT6	7.8	39° 6' 19.20" N 26° 33' 25.90" E
	MYT7	7.5	39° 6' 15.60" N 26° 33' 26.30" E

### Metal Analysis

Dry sediment samples were ground to fine powder with a hardened steel mortar, thereafter a digestion procedure was carried out. One-gram powder aliquots were treated in an open flask with boiling 10 ml 5% HNO<sub>3</sub> for 30 min, then filtered (US EPA, 1998). Solid residue was washed twice with 2-3 ml 5% HNO<sub>3</sub> and filtered; combined filtrates were treated with 1 ml 30% H<sub>2</sub>O<sub>2</sub> and boiled to a volume of 3 to 4 ml, then filtrates were treated with 20 ml H<sub>2</sub>O, 1 ml 30% H<sub>2</sub>O<sub>2</sub>, 1 ml HNO<sub>3</sub> and boiled to a volume of 3-4 ml twice. Finally it was treated with 20 ml H<sub>2</sub>O and 1 ml HNO<sub>3</sub> and boiled up to a volume of 3-4 ml; after cooling, 20 ml H<sub>2</sub>O and 1 ml HNO<sub>3</sub> were added, then diluted to 50 ml with water. The solutions were analyzed using Agilent Technologies 7500a ICP-MS spectrometer (Santa Clara, CA, USA), equipped with a quadrupole mass analyzer, nebulizer Babington type, glass spray chamber, quartz monobloc torch and auto-sampler. The gas flow (99.99% Argon) used for the plasma was 15 L/min; the auxiliary (cooling) gas flow was 1 L/min; the transport gas flow used to introduce the sample was 1.12 L/min. The radiofrequency generator worked at 1300 W; spray chamber was constantly kept at 2°C. The samples as well as the standards Romil (Cambridge, UK) and Fluka (Gillingham, Dorset, UK) ICP-MS multi-element standard solutions were analysed in a HNO<sub>3</sub> (1 to 10%) matrix to minimize the interferences and matrix effect. Before introducing the sample an auto-tuning sequence was implemented in order to optimize structural parameters, liquid and transport gas flows, to improve the sensitivity and mass resolution, and to limit oxide formation and double charges, by avoiding interferences (McCurdy and Potter, 2001). The sample was fluxed in the system for a conditioning period of 2 min before the acquisition of each metal was performed (mean duration 0.5 sec); five repeats were made and the final result was obtained as mean of readings. A good

reproducibility was observed, within the range 3-5% for measured elements. The instrumental detection limit for ICP-MS is usually calculated as equivalent concentration corresponding to 3 times the standard deviation on the results of these measurements (Ichihashi *et al.*, 2001). A precision of 0.5-1% for major element oxides, 2-2.5% for minor elements, 2-5% for trace elements of 50-150 ppm, 2-10% for trace elements of 10-50 ppm, and 5-25% for trace elements up to 5 ppm was found.

Replicated measures of international reference materials (PACS2), reagent blanks, and duplicated sediment samples (about 20% of the total number of samples randomly selected from the set) were used to assess accuracy (estimated as >95%) and precision (estimated on triplicate samples >93%).

### Polycyclic Aromatic Hydrocarbons (PAH) Analysis

Some selected PAHs were measured in marine sediment samples collected from Mytilene Harbor and Izmir Bay, namely the following seven PAHs, included in WFD 2000/60 (EC 2000) were determined by GC-MS: anthracene [A], fluoranthene [Fluo], benzo(ghi)perylene [BPer], benzo(b)fluoranthene [BbF], benzo(k)fluoranthene [BkF], benzo(a)pyrene [BaP] and indeno(1,2,3-cd)pyrene [IP] (Kostopoulou *et al.*, 2007). Two grams of homogenized, dried sediment were weighed directly in a centrifugation tube, where 2 ml CH<sub>3</sub>OH (Pestiscan grade), 1 g sodium sulphate (200°C for 24 h) and 20 ml CH<sub>2</sub>Cl<sub>2</sub> (Pestiscan grade, Lab-Scan, Dublin, Ireland) were added and ultrasonicated (frequency 50-60 Hz, Branson 2200) (Branson Ultrasonic Co., Danbury, CT, USA) at room temperature (20 to 22°C) for 15 min. The solution was left undisturbed for about 30 min and then centrifuged at ~600×g for 10 min. The supernatant was transferred to a round bottomed flask, where activated copper was added to desulphurize the

extract, reduced to approx. 1 ml using a rotary evaporator and then purified by using silica micro-columns chromatography. Elution was followed by using hexane and  $\text{CH}_2\text{Cl}_2$ , the purified extract was concentrated to a final volume of 0.5 ml and stored in a Teflon sealed vial at 4°C until analysis. The same sample preparation procedure was applied for sediments spiked with known concentrations of priority PAHs purchased from Supelco Co. (Park Bellefonte, PA, USA) and recoveries ranged from 65% to 100%.

The analyses were performed with a Hewlett Packard 6890GC–5973MSD (EI 70 eV) (GMI, Ramsey, MN, USA), equipped with a HP-5MS fused silica capillary column 30 m × 0.25 mm × 0.20 µm. Carrier gas was helium (purity 99.99%) with column flow of 0.6 ml/min. Injection was made in the splitless mode (purge delay 1 min, purge flow 30 ml/min). The oven temperature program was: 80°C for 1 min, rate 15°C/min to 220°C (for 1 min) and then up to 290°C with rate 5°C/min (for 15 min). MS source temperature was 180°C and transfer line 240°C. For higher sensitivity, the analysis was performed in Selected Ion Monitoring, (SIM) mode. For instrument calibration, a PAHs mixture solution (Supelco Co.) was used, from which secondary standard solutions were prepared, with concentrations ranging from 5 to 400 µg/L.  $R^2$  values for the 7 PAHs ranged from 0.995 to 1.000. The detection limits (LOD) for each determined compound, as well as the quantitation limits (LOQ) were calculated on the basis of signal-to-noise ratios 3 and 10 respectively.

### Sea Urchin Bioassays

Sea urchins from the species *P. lividus* were collected from East Mytilene coast; gametes were obtained and embryo cultures were run as described previously (Pagano *et al.*, 1986, 2001). Controls were conducted as untreated negative controls in natural filtered seawater (FSW) collected offshore from the isle of Mytilene, 3.5‰ salinity, and positive controls were exposed to  $2.5 \times 10^{-4}$  M  $\text{CdSO}_4$  (Pagano *et al.*, 1986). No “pristine” sediment control was utilized due to the expected – and verified – differences in sediment pollution and toxicity that allowed for distinctions within the sample sets according to their topographic distribution (e.g., inner vs. outer Izmir Bay sampling sites).

For embryo bioassays, sediment aliquots were laid in six-well (10 ml) culture plates for WS and SP (1% dry wt/vol) toxicity testing, or PW was diluted (1% vol/vol) in culture wells. The exposure of embryos (~20-30 embryos/ml) occurred throughout development from zygote (10 min after fertilization) up to the pluteus larval stage. Cultures were run at  $17.5 \pm 1^\circ\text{C}$  in the dark up to 72 h after fertilization.

Sperm bioassays were carried out by suspending *P. lividus* sperm for 1 h in stirred sediment (WS or SP) or in PW at the same 1% concentrations in 50-ml

polystyrene cups. Suspension medium was FSW collected offshore the isle of Mytilene. Sperm were suspended in FSW, with or without sediment aliquots, as a 0.2% suspension of concentrated sperm pellet for 2 h. After exposure, 0.5% supernatant sperm were used to inseminate untreated egg suspensions (~50 eggs/ml). Changes in the fertilization rate (FR = fertilized eggs%) of exposed sperm were determined by scoring the percent of fertilized eggs in live cleaving embryos (1 to 3 h post-fertilization).

Each bioassay was run in six replicates. The observations of larvae were performed on the first 100 pluteus larvae scored in each replicate culture, approximately 72 h after fertilization, and immobilized in  $10^{-4}$  M chromium sulfate 5 min prior to observation (Pagano *et al.*, 1986). The following outcomes in embryogenesis abnormalities were scored: i. pathologic (P1), malformed plutei; ii. pathologic embryos (P2), arrested at blastula /gastrula stages and unable to differentiate up to the pluteus stage, and iii. dead (D) embryos. The total percentages of P1+P2 were scored as % developmental defects (DD). Observations were carried out blind by trained readers, each one evaluating a complete set of cultures.

### Statistical Analysis

The data were analyzed using two inferential techniques, i.e.: a) test of hypothesis on means of two normal distributions with unknown variances, and b) test of hypothesis on the variance of two normal distributions, according to Hines and Montgomery (1980). The SPSS software was utilized.

## Results

### Organic Carbon

The concentrations of organic carbon and of some selected metals in sediment specimens from Izmir Bay and Mytilene Harbor are reported in Table 2. Organic carbon content in Izmir Bay showed to be higher at the innermost locations vs. the other locations in Izmir Bay. By comparing the data of the two areas, values of the organic carbon (% dry weight) were highest at locations subjected to anthropogenic activities, such as waste discharges and urban runoffs.

### Metal Analysis

The levels of the major metal pollutants (Cu, Ni, Cr, Pb, Zn) in Izmir Bay were higher in locations close to the inner part of the bay (IZ1 to IZ4) compared to the other locations (IZ5 to IZ8).

In Mytilene Harbor sediment, Zn had the highest values among metal pollutants, followed by Cr, Pb, Ni and Cu, without any clear-cut topographic differences in metal concentrations (Table 2).

**Table 2.** Concentrations ( $\mu\text{g/g}$  dry wt) of some selected inorganics in sediment specimens collected in Izmir (IZ) Bay and in Mytilene (MYT) harbor. Means of quintuplicate measurements. Measurements detected the following variation ranges: 0.5–1% for Al, Fe, Mg, Pb and Zn; 2–2.5% for Cr, Cu, Mn, Ni and Ti.

Elements	Al/27	Cr/53	Cu/63	Fe/57	Mg/24	Mn/55	Ni/60	Pb/208	Ti/47	Zn/66
Isotopes										
IZ1	8433	82.3	66.6	14030	14270	316.9	49.8	105.2	14.09	314.6
IZ2	7843	115.1	50.8	12940	14500	384.9	48.4	53.41	7.571	244.3
IZ3	7396	78.1	49.4	13370	13410	400.9	43.7	46.19	8.202	171.5
IZ4	8269	189.5	95.7	13770	15180	306.3	51.3	83.92	18.44	435.2
IZ5	5642	44.0	13.1	8210	17240	160.9	55.2	8937	3.622	47.43
IZ6	9626	65.6	38.5	16860	15670	331.7	59.8	32.73	39.53	159.1
IZ7	8068	40.9	21.3	13920	15260	414.5	58.0	22.36	55.81	84.73
IZ8	9066	41.3	22.4	13850	17710	367.1	56.9	17.15	88.15	75.44
MYT2	5901	36.7	19.0	7097	18420	150.3	46.7	43.96	4.432	59.09
MYT3	4186	29.5	48.9	7014	11900	136.8	30.4	68.96	10.21	172.6
MYT4	4581	32.5	29.7	7899	16130	145.0	38.3	39.43	2.321	93.09
MYT5	8607	125.3	52.0	15090	16700	305.4	56.6	55.43	35.51	285.4
MYT6	4889	33.2	19.5	6683	16400	138.3	42.5	42.39	2.64	67.16
MYT7	5313	37.3	53.4	8516	14860	141.4	37	56.12	4.058	160.7

### Polycyclic Aromatic Hydrocarbons

As shown in Table 3, the concentrations of PAHs examined ( $\Sigma 7\text{PAHs}$ ) varied for Izmir Bay sampling sites from 158 ng/g to 1,575 ng/g (dry wt) with a mean value of 599 ng/g. Mytilene Harbor values of ( $\Sigma 7\text{PAHs}$ ) varied from 110 ng/g to 615 ng/g (dry wt) with a mean value of 387 ng/g (dry wt).

Izmir sampling sites IZ1 to IZ4 showed peak values in PAH content (totaling  $885.9 \pm 244.5$  ng/g dw) that were significantly higher than either the outer Izmir Bay sites ( $311.5 \pm 61.1$  ng/g dw) ( $P=0.03$ ) or vs. a total of seven Mytilene sites ( $387.1 \pm 65.0$  ng/g dw) ( $P=0.012$ ), as shown in Table 3. However, the mean concentrations of PAHs from all Izmir sites were higher, yet did not differ significantly from all Mytilene sites ( $598.7 \pm 159.3$  vs.  $387.1 \pm 65.0$  ng/g dw, respectively).

### Sea Urchin Bioassays

A first bioassay showed that the highest rate of developmental defects (DD) was exerted by whole sediment (WS) from the innermost sampling site in Izmir Bay (#IZ1) ( $\text{DD} \cong 81\%$ ), compared to sediment from Mytilene Harbor ( $\text{DD} \cong 24\%$ ) and vs. controls ( $\text{DD} \cong 9\%$ ). When developmental toxicity was grouped along with a gradient from inner – and most

polluted – sites vs. outer sites in Izmir Bay, the most toxic WS samples coincided with the two innermost sites (#IZ1 and #IZ2), as shown in Figure 2, whereas the outer sites (#IZ6 to IZ8) failed to significantly differ from either Mytilene sites or from controls.

These early results prompted us to verify whether, and to what extent, sediment embryotoxicity was mainly associated with solid phase or pore water components of sediment (SP vs. PW). As shown in Table 4, SP displayed significantly higher developmental toxicity compared to PW from 4 out of 8 sediments samples from Izmir, while SP from the other 4 samples however showed higher, though not statistically significant developmental toxicity compared to the respective PW fractions. As for Mytilene, only site #MYT3 displayed a significantly higher developmental toxicity by SP vs. PW, while the other sediment samples failed to show any significant difference between SP and PW. In a subsequent bioassay developmental toxicity data from the overall sampling sites were plotted against the corresponding results obtained from WS vs. SP and vs. PW, as shown in Figure 3, and developmental defects were found to be ranking as:

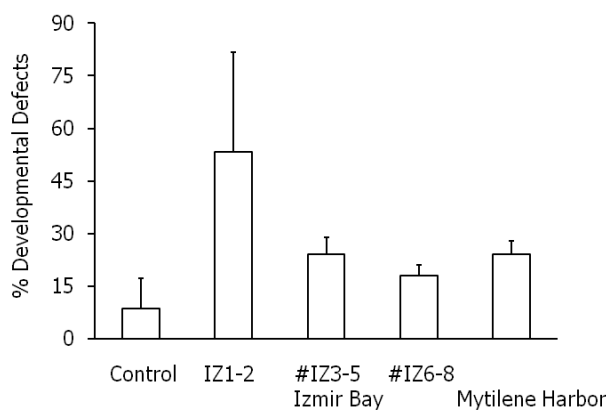
$$\text{WS} > \text{SP} > \text{PW} \cong \text{Ctr.}$$

This held true both for Izmir and for Mytilene sediment samples. Embryo exposure to 1% PW failed to show any difference in developmental defects vs. controls. On the other hand SP and, more severely, WS resulted in significant excess developmental toxicity vs. PW and vs. controls. Both WS and SP from Izmir were found more embryotoxic than the respective sediment components from Mytilene (Figure 3).

Sperm exposure to SP and PW sediment fractions resulted in a significant loss of fertilization success following SP, but not PW exposure, as shown in Figure 4, with IZ1 and IZ2 sites displaying the most severe spermotoxicity (Table 5). Sperm

**Table 3.** Mean values of individual PAHs and  $\Sigma\text{PAHs}$  concentrations (ng/g dw) in the sampled surficial sediments from Izmir Bay and Mytilene Harbor in March 2007, ( $n=2$ )

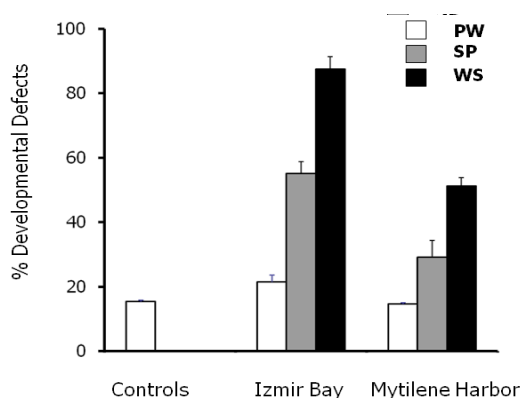
Sampling Sites	$\Sigma\text{PAHs}$ (ng/g dry wt.) – Means $\pm$ SEM
Izmir Bay	
All Sites	598.7 $\pm$ 159.3
Inner Bay (#IZ 1 to #IZ4)	885.9 $\pm$ 244.5
Outer Bay (#IZ5 to #IZ8)	311.5 $\pm$ 61.1
Mytilene Harbor	
All Sites	387.1 $\pm$ 65.0



**Figure 2.** Topographic distribution of whole sediment (WS) toxicity in Izmir Bay vs. Mytilene Harbor.

**Table 4.** Developmental toxicity of solid phase (SP) vs. pore water (PW) from sediment samples from Izmir Bay and Mytilene harbor. % Developmental defects in *P. lividus* larvae, means  $\pm$  SEM; six-replicate bioassay

Sampling sites/Schedules	% Developmental Defects		P (SP vs. PW)
	SP	PW	
Blank controls	13.0 $\pm$ 1.8		
Positive controls (CdSO <sub>4</sub> 2.5 $\times$ 10 <sup>-4</sup> M)	100.0 $\pm$ 0.0		
<b>Izmir Sites</b>			
IZ1	99.3 $\pm$ 0.5	32.2 $\pm$ 13.4	<0.01
IZ2	51.8 $\pm$ 14.0	19.6 $\pm$ 7.7	NS
IZ3	88.2 $\pm$ 7.9	10.0 $\pm$ 2.1	= 0.00
IZ4	28.2 $\pm$ 7.8	7.4 $\pm$ 2.3	<0.05
IZ5	61.0 $\pm$ 13.0	43.8 $\pm$ 18.9	NS
IZ6	30.8 $\pm$ 8.2	3.8 $\pm$ 0.6	<0.05
IZ7	42.3 $\pm$ 8.9	20.8 $\pm$ 7.2	NS
IZ8	49.7 $\pm$ 15.8	24.4 $\pm$ 4.9	NS
<b>Mytilene Sites</b>			
MYT3	57.8 $\pm$ 15.8	13.8 $\pm$ 6.7	<0.05
MYT4	8.2 $\pm$ 2.6	13.8 $\pm$ 8.8	NS
MYT6	7.5 $\pm$ 2.2	13.4 $\pm$ 5.1	NS
MYT7	42.8 $\pm$ 12.4	18.2 $\pm$ 7.3	NS



**Figure 3.** Effects of WS vs. SP and PW fractions to *P. lividus* developing embryos.

exposure to PW, both from Izmir and from Mytilene, provided overlapping results vs. controls. On the other hand, no significant differences in fertilization rate were detected by comparing the effects of sperm exposure to SP from Izmir vs. Mytilene sediment samples (Figure 4).

## Discussion

The results provided evidence for significant differences in sediment pollution and toxicity between Izmir Bay and Mytilene Harbor. The differences were particularly clear-cut by considering the measured

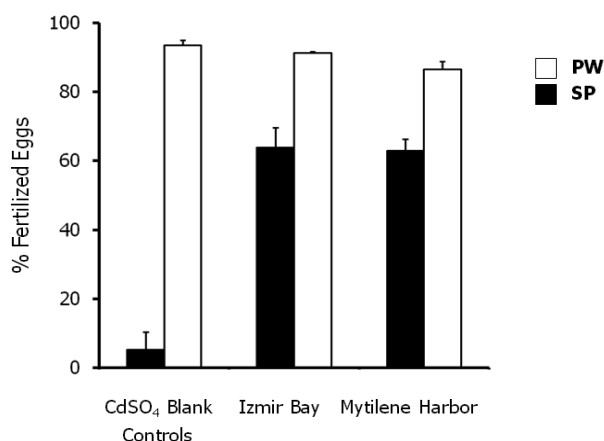
levels of some anthropogenic metals, in agreement with previous studies conducted in Izmir Bay and attributed to the heavily industrialized and urbanized coastal area as well as to the intense marine traffic in Izmir Alsancak Harbor (Küçüksezgin *et al.*, 2006). In the case of chromium, copper, lead and titanium, a further difference was detected when the innermost sampling sites in Izmir Bay (#IZ1 to #IZ4) were considered, which displayed higher concentrations of these contaminants vs. Mytilene Harbor, in agreement with the anoxic conditions occurring in inner Izmir Bay (Bizsel and Uslu, 2000; Özkan *et al.*, 2008). The same distinction applied to total PAHs, displaying significantly higher levels in samples from sites #IZ2 to #IZ4, both compared to Mytilene and to the remaining Izmir sites.

Unlike Izmir Bay, Mytilene Harbor failed to show any evidence for topographic differences in the levels of either metals or PAHs; thus we could not confirm the data by Aloupi and Angelidis (2001), who reported different lead levels in Mytilene Harbor sediment between inner and outer harbor sites.

The overall contamination levels detected in sediment from Izmir Bay overlapped with the data from several polluted coastal areas and lagoons (Accornero *et al.*, 2008; Rodríguez-Barroso *et al.*, 2008; Skoog and Arias-Esquivel, 2009), whereas anthropogenic pollution in Mytilene Harbor may be regarded as a relatively marginal phenomenon.

The bioassay data corroborate the feasibility of drawing a topographic pattern of sediment toxicity in enclosed coastal areas as, e.g., Mytilene Harbor and Izmir Bay, along with the parallel analytical determinations of inorganic contaminants and PAHs. Altogether, sediment samples from Izmir Bay displayed higher toxicity compared to Mytilene Harbor, and this finding was enhanced when the data from inner Izmir Bay were taken into account, consistent with a gradient of sediment pollution and contaminant bioaccumulation in Izmir Bay reported previously (Küçüksezgin *et al.*, 2006; 2008; Oral *et al.*, 2007) and consistent with our present analytical data.

The data also highlighted significant differences



**Figure 4.** Fertilization rate following exposures of *P. lividus* sperm to SP or PW from Izmir or from Mytilene.

**Table 5.** Spermotoxicity of solid phase (SP) vs. pore water (PW) from sediment samples collected in Izmir Bay and Mytilene Harbor. % Fertilized eggs following exposure of *P. lividus* sperm, means  $\pm$  SEM; six-replicate bioassay

	Sampling sites/Schedules		% Fertilized eggs
	SP	PW	
Blank Controls			93.5 $\pm$ 1.6
Positive Controls (CdSO <sub>4</sub> 2.5 $\times$ 10 <sup>-4</sup> M)			5.2 $\pm$ 5.2
IZ1	39.4 $\pm$ 10.7	91.5 $\pm$ 3.5	
IZ2	43.0 $\pm$ 13.9	90.8 $\pm$ 3.6	
IZ3	70.0 $\pm$ 5.5	91.0 $\pm$ 3.3	
IZ4	86.8 $\pm$ 3.5	89.0 $\pm$ 2.8	
IZ5	78.4 $\pm$ 7.2	91.7 $\pm$ 3.0	
IZ6	64.2 $\pm$ 7.5	91.6 $\pm$ 3.8	
IZ7	55.8 $\pm$ 11.4	93.3 $\pm$ 3.2	
IZ8	73.4 $\pm$ 5.8	92.0 $\pm$ 3.9	
MYT3	72.0 $\pm$ 4.7	92.0 $\pm$ 2.1	
MYT4	61.0 $\pm$ 3.8	81.7 $\pm$ 5.5	
MYT6	63.0 $\pm$ 8.1	88.7 $\pm$ 4.9	
MYT7	55.6 $\pm$ 5.1	83.6 $\pm$ 7.9	

in developmental toxicity to sea urchin embryos exerted by WS compared to its solid and pore water fractions (SP and PW). Whole sediment has been reported by a number of independent studies as a more realistic and effective matrix in evaluating sediment toxicity, compared to PW or to elutriates or other extracts (Geffard *et al.*, 2002; Hallare *et al.*, 2005; Dekker *et al.*, 2006; Phillips *et al.*, 2006; Ingersoll *et al.*, 2009). On the other hand, several authors have reported on PW or elutriate toxicity as a means of testing sediment toxicity (Mucha *et al.*, 2003; Davoren *et al.*, 2005; Losso *et al.*, 2009). Ammonia toxicity has been invoked as a confounding factor in sediment toxicity, and in justifying the use of PW or elutriates in sediment toxicity evaluation (Losso *et al.*, 2009); however, recent reports have demonstrated that ammonia is mainly confined to PW, and that a negligible role can be attributed to ammonia in WS toxicity (Carr *et al.*, 2006; Ingersoll *et al.*, 2009; Mehler *et al.*, 2010). Our previous findings had pointed to some dramatic differences in the toxicities of SP vs. PW, with some cases of “all-or-nothing”, namely 100% developmental defects induced by 1% SP compared to zero effect in embryos exposed to 1% PW in Kiel Fiord (Pagano *et al.*, 2001); similar outcomes were reported by Geffard *et al.* (2002), showing that SP displayed higher toxicity than elutriates both in sea urchin and in oyster embryos and sperm, by almost two orders of magnitude.

One might speculate about a possible discrepancy of “real” contaminant concentrations by comparing 1% dry weight/vol of WS and SP with 1% vol/vol of PW, by arguing that PW-associated toxicity would be detected at higher PW concentrations, e.g. ranging from 10% to 100%. However, such high PW concentrations might apply to sediment-dwelling biota that are, in fact, exposed to 100% PW; even so, the information arising from PW testing would in fact disregard the effects of the SP component. Apart from the specialized sediment-dwelling biota, one should consider the looser, yet effective interactions of sediment suspensions with a number of other benthic organisms that may be exposed to much lower sediment concentrations as, e.g., in the order of 1%, as adopted in the present investigation. Furthermore, one might concede that testing higher PW concentrations, such as 25%, might result in significant toxicity and this might be the case of developmental toxicity as shown in Table 4 (sites #IZ1 and #IZ5). Hence, one should anticipate ad hoc investigations focused on concentration-related trends for WS compared to SP and PW; as reported by independent studies. However, PW displayed lesser contaminant concentrations and toxicity compared to WS by several orders of magnitude (Geffard *et al.*, 2002; Martello *et al.*, 2007).

Thus, the present study provided evidence for the differences in developmental and reproductive toxicities of 1% SP vs. 1% PW. Moreover, we

explored the hypothesis that WS-associated toxicity might display any differences vs. SP- and/or PW-induced effects. One could figure out that WS induced overlapping effects vs. SP, or even lesser effects due to the presence, in WS, of PW content with expected lesser toxicity. Surprisingly, as shown in Figure 3, both Izmir and Mytilene sediment samples resulted in the highest rates of developmental in *P. lividus* embryos exposed to WS, significantly higher than in SP-exposed embryos, whereas PW-exposed embryos displayed overlapping rates of developmental defects vs. controls.

Following exposure of *P. lividus* sperm, no significant difference was observed between Izmir and Mytilene sediment samples in inducing loss of fertilization success. On the other hand, SP induced a significantly higher spermotoxicity vs. PW, which failed to induce any loss of fertilization success, again with overlapping results vs. controls both in Izmir and in Mytilene sediment samples.

The data altogether point to the role of solid component in evaluating sediment toxicity, while casting doubts about the relevance of PW (or elutriates) in sediment toxicity testing at realistic concentrations as 1%, as in the present study. Our data are consistent with the analytical data by Martello *et al.* (2007), who observed striking differences in the concentrations of total chromium and Cr(VI), i.e. approx. 50,000-fold, in WS compared to elutriate. Thus, even by considering that only minor fractions of SP-bound contaminants may be available to sediment-exposed biota, the data by Martello *et al.* (2007) provide support to our bioassay data and to the report by Geffard *et al.* (2002). Altogether, the available evidence suggests that evaluation of sediment toxicity on a topographic basis should rely on whole sediment as a prime choice matrix.

## Conclusions

The results showed different pollution and toxicity patterns in Izmir and Mytilene sites, with most severe damage in the innermost sampling sites in Izmir and an overall worse pattern in Izmir vs. Mytilene sediment samples. A noteworthy finding consisted of WS- and SP-associated toxicity to sea urchin embryos and sperm, whereas PW displayed lesser, if any, toxicity at the tested levels.

Evidence is provided for the evaluation of coastal sediment toxicity and contamination by comparing two enclosed coastal areas. For topographic assessment of sediment toxicity, whole sediment is found more reliable as a testing matrix compared to its components (SP or PW), which may underscore sediment toxicity or even result in false negative findings.

## Acknowledgements

This study was supported by NATO for Science



Programme (Harbor Sediments Pollution Assessment and Dredged Material Management, ESP.EAP.CLG 982446).

## References

- Accornero, A., Gnerre, R. and Manfra, L. 2008. Sediment concentrations of trace metals in the Berre Lagoon (France): an assessment of contamination. Archives of Environmental Contamination and Toxicology, 54: 372-385. doi: 10.1007/s00244-007-9049-6
- Aloupi, M. and Angelidis, M.O. 2001. Geochemistry of natural and anthropogenic metals in the coastal sediments of the island of Lesbos, Aegean Sea. Environmental Pollution, 113: 211-219. doi: 10.1016/S0269-7491(00)00173-1
- Araújo, C.V., Diz, F.R., Laiz, I., Lubián, L.M., Blasco, J. and Moreno-Garrido, I. 2009. Sediment integrative assessment of the Bay of Cádiz (Spain): an ecotoxicological and chemical approach. Environment International, 35: 831-841. doi: 10.1016/j.envint.2009.02.003
- Bizsel, N. and Uslu, O. 2000. Phosphate, nitrogen and iron enrichment in the polluted Izmir Bay, Aegean Sea. Marine Environmental Research, 49: 101-122. doi: 10.1016/S0141-1136(99)00051-3
- Bryan, G.W. and Langston, W.J. 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. Environ. Pollut., 76: 89-131.
- Buchanan, J.B. 1984. Sediment analysis. In: N.A. Holme and A.D. McIntyre (Eds.), Methods for the Study of Marine Benthos. Blackwell Sci. Publ., London: 41-65.
- Carr, R.S., Biedenbach, J.M. and Nipper, M. 2006. Influence of potentially confounding factors on sea urchin porewater toxicity tests. Archives of Environmental Contamination and Toxicology, 51: 573-579. doi: 10.1007/s00244-006-0009-3
- Cesar, A., Marin, A., Marin-Guirao, L. and Vita, R. 2004. Amphipod and sea urchin tests to assess the toxicity of Mediterranean sediments: the case of Portmán Bay. Sci. Mar., 68(1): 205-213.
- Chapman, P.M., Ho, K.T., Munns, W.R., Solomon, K. and Weinstein, M.P. 2002. Issues in sediment toxicity and ecological risk assessment. Marine Pollution Bulletin, 44: 271-278. doi: 10.1016/S0025-326X(01)00329-0
- Chen, G. and White, P.A. 2004. The mutagenic hazards of aquatic sediments: a review. Mutation Research, 567: 151-225. doi: 10.1016/j.mrrev.2004.08.005
- Davoren, M., Hartl, M.G., Sheehan, D., O'Brien, N.M., O'Halloran, J., van Pelt, F.N. and Mothersill, C. 2005. Assessing the potential of fish cell lines as tools for the cytotoxicity testing of estuarine sediment aqueous elutriates. Toxicology In Vitro, 19: 421-431. doi: 10.1016/j.tiv.2004.12.002
- Dekker, T., Greve, G.D., Ter Laak, T.L., Boivin, M.E., Veuger, B., Gortzak, G., Dumfries, S., Lücker, S.M., Kraak, M.H., Admiraal, W. and van der Geest, H.G. 2006. Development and application of a sediment toxicity test using the benthic cladoceran *Chydorus sphaericus*. Environmental Pollution, 140: 231-238. doi: 10.1016/j.envpol.2005.07.015
- De Nicola, E., Gallo, M., Iaccarino, M., Meriç, S., Oral, R., Russo, T., Sorrentino, T., Tünay, O., Vuttariello, E., Warnau, M. and Pagano, G. 2004. Hormetic vs. toxic effects of vegetable tannin in a multi-test study. Archives of Environmental Contamination and Toxicology, 46: 336-344. doi: 10.1007/s00244-003-2293-5
- De Nicola, E., Meriç, S., Cheggour, M., Della Rocca, C., Di Gennaro, A., Gallo, M., Iaccarino, M., Moukrim, A., Tünay, O. and Pagano, G. 2007. Wastewater toxicity of tannin- vs. chromium-based leather tanneries in Marrakesh, Morocco. Archives of Environmental Contamination and Toxicology, 53: 321-328. doi: 10.1007/s00244-006-0181-5
- Geffard, O., Budzinski, H., His, E., Seaman, M.N. and Garrigues, P. 2002. Relationships between contaminant levels in marine sediments and their biological effects on embryos of oysters, *Crassostrea gigas*. Environmental Toxicology and Chemistry, 21: 2310-2318. doi: 10.1002/etc.5620211108
- Guillou, M., Quiniou, F., Huart, B. and Pagano, G. 2000. Comparison of embryonic development and metal contamination in several populations of the sea urchin *Sphaerechinus granularis* (Lamarck) exposed to anthropogenic pollution. Archives of Environmental Contamination and Toxicology, 39: 337-344. doi: 10.1007/s002440010113
- Hallare, A.V., Kosmehl, T., Schulze, T., Hollert, H., Köhler, H.R. and Triebkorn, R. 2005. Assessing contamination levels of Laguna Lake sediments (Philippines) using a contact assay with zebrafish (*Danio rerio*) embryos. Sci. of the Total Environment, 347: 254-271. doi: 10.1016/j.scitotenv.2004.12.002
- Hines, W.W. and Montgomery, D.C. 1980. Probability and Statistics in Engineering and Management Science. John Wiley and Sons, New York, 634 pp.
- Hübner, R., Astin, K.B. and Herbert, R.J. 2009. Comparison of sediment quality guidelines (SQGs) for the assessment of metal contamination in marine and estuarine environments. Journal of Environmental Monitoring, 11: 713-722. doi: 10.1039/B818593J
- Ichihashi, H., Nakamura, Y., Kannan, K., Tsumura, A. and Yamasaki, S. 2001. Multi-elemental concentrations in tissues of Japanese common squid (*Todarodes pacificus*). Archives of Env. Cont. and Toxicology, 41: 483-490. doi: 10.1007/s002440010275
- Ignacio, L., Orenzo, J., Nieto, O. and Beiras, R. 2006. Anodic stripping voltammetry measures copper bioavailability for sea urchin larvae in the presence of fulvic acids. Environmental Toxicology and Chemistry, 25(1): 36-44.
- Ingersoll, C.G. 1995. Sediments test. In: G.M. Rand (Ed.), Fundamentals of aquatic toxicology: effects, environmental fate, and risk assessment. 2<sup>nd</sup> Edition. Taylor and Francis, Washington DC: 231-255.
- Ingersoll, C.G., Kemble, N.E., Kunz, J.L., Brumbaugh, W.G., MacDonald, D.D. and Smorong, D. 2009. Toxicity of sediment cores collected from the Ashtabula River in Northeastern Ohio, USA, to the amphipod *Hyalella azteca*. Archives of Environmental Contamination and Toxicology, 57: 315-329. doi: 10.1007/s00244-009-9379-7
- Jackson, M.L. 1958. Soil Chemical Analysis. Prentice Hall Inc, Englewood Cliffs, New Jersey, 498 pp.
- Kostopoulou, M., Mylona, A., Nikolaou, A., Lofrano, G., Meriç, S. and Belgiorno, V. 2007. Determination of the aromatic polycyclic hydrocarbons in the harbour sediments of Mytilene, Greece. Proceeding of 10<sup>th</sup> International Conference on Environmental Science and Technology, 5-7 September, Kos, Greece: 723-726. (<http://www.gnest.org/>).

- Küçüksezgin, F., Kondaş, A., Altay, O., Uluturhan, E. and Darılmaz, E. 2006. Assessment of marine pollution in Izmir Bay: Nutrient, heavy metal and total hydrocarbon concentrations. *Environment Int.*, 32: 41-51. doi: 10.1016/j.envint.2005.04.007
- Küçüksezgin, F., Kayatekin, B.M., Uluturhan, E., Uysal, N., Açıkgöz, O. and Gönenç, S. 2008. Preliminary investigation of sensitive biomarkers of trace metal pollution in mussel (*Mytilus galloprovincialis*) from Izmir Bay (Turkey). *Environ. Monit. and Assessment*, 141: 339-345. doi: 10.1007/s10661-007-9900-2
- Langston, W.J. and Spence, S.K. 1994. Metal analysis. In: P. Calow (Ed.), *Handbook of Ecotoxicology*. Oxford Blackwell Sci. Publ., London: 2(4): 45-78.
- Losso, C., Novelli, A.A., Picone, M., Ghetti, P.F. and Ghirardini, A.V. 2009. Porewater as a matrix in toxicity bioassays with sea urchins and bivalves: evaluation of applicability to the Venice lagoon (Italy). *Environment International*, 35: 118-126. doi:10.1016/j.envint.2008.09.010
- Manzo, S., Buono, S. and Cremisini, C. 2008. Predictability of copper, irgarol, and diuron combined effects on sea urchin *Paracentrotus lividus*. *Archives of Environmental Contamination and Toxicology*, 54: 57-68. doi: 10.1007/s00244-007-9009-1
- Martello, L., Fuchsmann, P., Sorensen, M., Magar, V. and Wenning, R.J. 2007. Chromium geochemistry and bioaccumulation in sediments from the lower Hackensack River, New Jersey. *Archives of Environmental Contamination and Toxicology*, 53: 337-350. doi: 10.1007/s00244-006-0164-6
- McCurdy, E. and Potter, D. 2001. Optimising ICP-MS for the determination of trace metals in high matrix samples. *Spectroscopy Europe*, 13: 14-20.
- Mehler, W.T., You, J., Maul, J.D. and Lydy, M.J. 2010. Comparative analysis of whole sediment and porewater toxicity identification evaluation techniques for ammonia and non-polar organic contaminants. *Chemosphere*, 78: 814-821. doi: 10.1016/j.chemosphere.2009.11.052
- Meriç, S., De Nicola, E., Iaccarino, M., Gallo, M., Di Gennaro, A., Morrone, G., Warnau, M., Belgiorno, V. and Pagano, G. 2005. Toxicity in a leather tanning wastewater treatment plant in sea urchins and in marine microalgae. *Chemosphere*, 61: 208-217. doi: 10.1016/j.chemosphere.2005.02.037
- Mermillod-Blondin, F., François-Carcaillet, F. and Rosenberg, R. 2005. Biodiversity of benthic invertebrates and organic matter processing in shallow marine sediments: an experimental study. *J. of Experimental Marine Biology and Ecology*, 315: 187-209
- Mucha, A.P., Leal, M.F., Bordalo, A.A. and Vasconcelos, M.T. 2003. Comparison of the response of three microalgae species exposed to elutriates of estuarine sediments based on growth and chemical speciation. *Environmental Toxicology and Chemistry*, 22: 576-585. doi: 10.1002/etc.5620220316
- Oral, R., Parlak, H., Boyacıoğlu, M., Çakal Arslan, Ö. and Küçüksezgin, F. 2007. An assessment of sediment quality at the streams flowing into Izmir Bay, Aegean Sea, Turkey. *Pakistan Journal of Biological Sciences*, 10: 1738-1742.
- Özkan, E.Y., Kocataş, A. and Büyükişik, B. 2008. Nutrient dynamics between sediment and overlying water in the inner part of Izmir Bay, Eastern Aegean. *Environmental Monitoring and Assessment*, 143: 313-325. doi: 10.1007/s10661-007-9984-8
- Pagano, G., Cipollaro, M., Esposito, A., Ragucci, E., Giordano, G.G. and Trieff, N.M. 1986. The sea urchin: Bioassay for the assessment of damage from environmental contaminants. In: Jr. J. Cairns (Ed.), *Community Toxicity Testing*. Association for Standard Testing and Materials, Philadelphia: 67-92.
- Pagano, G., His, E., Beiras, R., De Biase, A., Korkina, L.G., Iaccarino, M., Oral, R., Quiniou, F., Warnau, M. and Trieff, N.M. 1996. Cytogenetic, developmental and biochemical effects of aluminum, iron and their mixture in sea urchins and mussels. *Archives of Environmental Contamination and Toxicology*, 31: 466-474. doi: 10.1007/BF00212429
- Pagano, G., Korkina, L.G., Iaccarino, M., De Biase, A., Deeva, I.B., Doronin, Y.K., Guida, M., Melluso, G., Meriç, S., Oral, R., Trieff, N.M. and Warnau, M. 2001. Developmental, cytogenetic and biochemical effects of spiked or environmentally polluted sediments in sea urchin bioassays. In: P. Garrigues, C.H. Walker and H. Barth (Eds.), *Biomarkers in marine ecosystems: A practical approach*. Elsevier, Amsterdam, Netherlands: 85-129.
- Phillips, B.M., Anderson, B.S., Hunt, J.W., Huntley, S.A., Tjeerdema, R.S., Kapellas, N. and Worcester, K. 2006. Solid-phase sediment toxicity identification evaluation in an agricultural stream. *Environmental Toxicology and Chemistry*, 25: 1671-1676.
- Rodríguez-Barroso, M.R., Benhamou, Y., El Moumni, B., El Hatimi, I. and Garcia-Morales, J.L. 2008. Evaluation of metal contamination in sediments from north of Morocco: geochemical and statistical approaches. *Env. Monitoring and Assessment*, 159: 169-181. doi: 10.1007/s10661-008-0620-z
- Salamanca, M.J., Fernández, N., Cesar, A., Antón, R., Lopez, P. and Delvals, A. 2009. Improved sea-urchin embryo bioassay for in situ evaluation of dredged material. *Ecotoxicology*, 18: 1051-1057. doi: 10.1007/s10646-009-0378-2
- Sellem, F., Pesando, D., Bodennec, G., Abed, A. and Girard, J.P. 2000. Toxic effects of *Gymnodinium CF*. Mikimotoi unsaturated fatty acids to gametes and embryos of the sea urchin *Paracentrotus lividus*. *Wat. Res.*, 34(2): 550-556.
- Skoog, A.C. and Arias-Esquivel, V.A. 2009. The effect of induced anoxia and reoxygenation on benthic fluxes of organic carbon, phosphate, iron, and manganese. *Science of the Total Environment*, 407: 6085-6092. doi: 10.1016/j.scitotenv.2009.08.030
- U.S. Environmental Protection Agency 1998. Inductively coupled plasma-mass spectrometry, Method 6020. In *Solid Waste Methods, SW846, Update4*. U.S. Environmental Protection Agency, Environmental Monitoring System Lab., Cincinnati, Ohio.