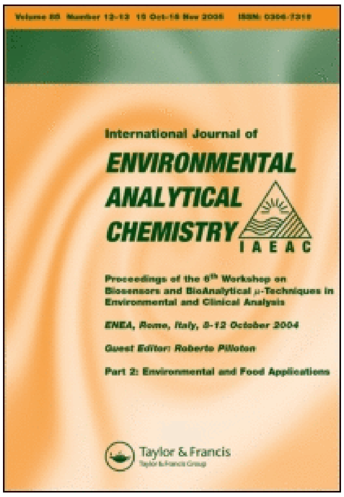

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DETERMINATION OF LEAD, CADMIUM AND MERCURY IN SURFACE MARINE SEDIMENTS AND MUSSELS

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Lead, cadmium and mercury were determined in sediments and mussels, and the ability of these indicators to record metal variations in coastal marine environment is described in this work. The results of an extended investigation of the status of three gulfs at Northern Greece are given, regarding the content of these metals in surface sediments and *Mytilus galloprovincialis*. The samples were collected during a four-year period. The total concentration of the above heavy metals was determined after digestion of the samples by suitable mixtures of acids, including nitric, perchloric and hydrofluoric acid. The digestion was carried out in a steel pressurised bomb with closed teflon vessels. Lead and cadmium were determined by means of electrothermal atomic absorption spectrometry (ETAAS), and mercury by cold vapour atomic absorption spectrometry (CVAAS). The results were statistically evaluated by analysis of variance, and emphasis was given to annual, seasonal and spatial sources of variation. The annual changes during the last four years and the spatial distribution of heavy metals load is also discussed.

Keywords: Marine sediment; mussel; atomic absorption spectrometry; lead; cadmium; mercury

INTRODUCTION

The coastal marine environment is the natural receiver of waters from rivers that drain large agricultural, urban and industrial areas. In addition, coastal cities usually dispose their communal sewages into the sea. Marine environment pollution is then demonstrated by the high concentration of heavy metals in water, sediments, and aquatic organisms[1].

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The heavy metal content of surface sediments is widely used as good indicator of the coastal zone seawater or estuarine water contamination for medium to long term studies, while mussel species are more used for short to medium term monitoring. Lead, cadmium, and mercury are common pollutants found in water systems, and a great part of these elements is coming from anthropogenic sources. The analytical determination of them is of high interest because of their toxicity to marine organisms and mammals. Introduction of these metals in the marine environment, to estuaries and lagoons[2,3,4] is due to contributors, like rivers and streams and ditches, which deliver large amounts of industrial wastewater and urban sewage.

Mytilus galloprovincialis is a very widespread and abundant mussel species, which is naturally found or cultured in coastal areas. This organism, as well as other similar species, like *Mytilus edulis and Mytilus californianus*, has been established as useful bio-indicator for the quality assessment of the aqueous environment[5,6] and for public health protection[7]. Their bio-accumulating ability is especially useful for monitoring heavy metals, which do not belong to the group of essential trace elements to this organism. Furthermore, it exhibits high accumulation factors to allow direct analysis without pre-concentration procedures.

In this work, lead, cadmium, and mercury were determined in coastal marine sediments and in *Mytilus galloprovincialis* by application of atomic absorption spectrometry. The method was applied to samples collected from three gulfs at the North Aegean Sea. The seasonal, annual and spatial variation of heavy metal concentrations in the surface part of the sediments were investigated, in order to estimate the pollution profile of these coastal ecosystems. The temporal sampling of mussels must be carefully designed, because the ability of a mussel to filtrate seawater and accumulate heavy metals is dependant on the age and some other factors like type of culture, physiological and nutritional state, etc. [1,5]. In addition, as Uthe and Clou noted[8], the sampling strategy must be designed for efficient statistical analysis. The use of two-way analysis of variance is a very well suited tool to test the significance of each source of variation, because the concentration of a metal is affected by many factors. Because of unavoidable large variation of environmental data, statistical analysis was applied to evaluate the significance of trends observed in either using annual means or using seasonal data.

MATERIALS AND METHODS

Studied area

The sediments and mussels were collected from three gulfs located at the North Aegean Sea, as illustrated in Figure 1. The coastline of Thermaikos gulf is approximately 90 km, and its surface area about 450 km^2. The second largest city of Greece, Thessaloniki (more than 1 million habitats), with the second commercial port of Greece, the most important one in the economy of the Balkan Peninsula, is located at this gulf. The gulf receives all domestic sewage from the city's sewage system, the industrial waste waters of the surrounding area and also water from three major rivers (Axios, Aliakmon, and Loudias). The gulf consists of the inner part (Thessaloniki Bay) at north, and the outer part at south. River Axios originates in former Yugoslavia, and after 220 km through its territory and 80 km through Greece, flows into the gulf. The pollutant sources, which flow into this river, are domestic and agricultural effluents, as well as industrial waste waters. River Strymon originates in Bulgaria and drains agricultural and industrial discharges into Strymonikos gulf. Kavala gulf borders the city of Kavala, a small industrial area, and several summer resorts.

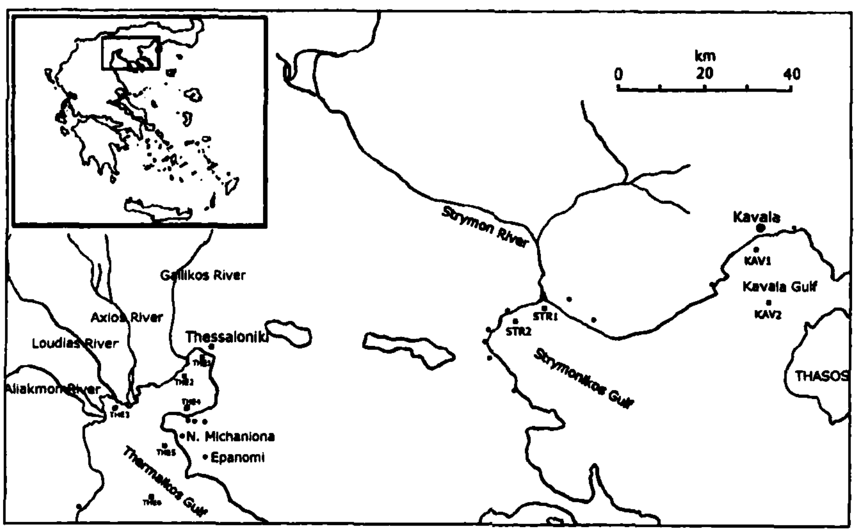

FIGURE 1 Map of Northern Greece, showing the studied areas and the locations of the sampling sites

Sampling and storage procedures

Surface sediments were collected during the period 1995–1998. Six coastal sampling sites were located in Thermaikos gulf (THE1, THE2, THE3, THE4, THE5, THE6), two sites in Strymonikos gulf (STR1, STR2) and two sites in Kavala gulf (KAV1, KAV2), as illustrated in Figure 1. The surface sediments were collected using a grab dredge sampler (0 to 10 cm from the bottom surface). Homogenized samples were sealed in polyethylene bags and then stored in a freezer, until analysed. Prior to analysis and after defrosting, a part of the sample was used for determination of heavy metals and another part was used for calculation of the dry mass of each sample. The final calculated concentrations are expressed in dry weight (DW).

Samples of *mytilus galloprovincialis* mussels were collected in three-months interval between 1996–1998, from six coastal sampling sites located at the three gulfs, (THE2, THE3, THE4, THE5, STR2, KAV1). The sampling sites for mussel collection were located preferably at mussel cultures near the coast. The corresponding sediment sample was also collected and analysed, under the same code number. Thermaikos and Strymonikos gulfs have great production of edible mussels. The first has more than 60 production units located at the north-western coast. Each sample consisted of more than 25 specimens. The mussels were rinsed with seawater, transferred in plastic bags and stored in frozen, until the analysis. After defrosting, a number of about 10 specimens from each sampling site was taken with uniform size each time, and chopped using an inox steel knife to prevent trace element contamination. The internal tissue of these specimens was blended and homogenised, to prepare a composite sample, which is more representative of each sampling site and date.

The collected specimens should be of same age, which practically means, they must have similar size. The length in the majority of specimens ranged between 5.0 – 6.5 cm, although in some cases the range was extended to 4.5–8.0 cm. The bigger specimens (7.0 –8.5 cm) were collected from cultures situated at sampling sites THE2, THE3, THE5 and STR2.

Digestion procedures

All chemicals were of analytical-reagent grade (Merck, Darmstadt, Germany), unless stated otherwise. All standard and sample solutions were made up with double de-ionized water. The samples of sediments and mussels were lyophilised in order to determine the wet to dry mass ratio (WW/DW).

The analysis of sediments was carried out by digesting an appropriate amount of the wet sample, corresponding to approximately 0.2 g dry weight as determined by lyophilization, in a steel pressurized bomb supplied with teflon vessels, at 130–140°C by using an acid mixture of HNO_3-$HClO_4$-HF[9–11]. This amount is the recommended capacity for the teflon vessels used in this work. The use of HF is necessary in order to recover the total amount of metals.

The analysis of mussel tissues was carried out by digesting an appropriate amount of the wet sample, corresponding to approximately 0.2 g dry mass (about 1.0 g of wet mass), in a steel pressurized bomb supplied with teflon vessels, at 130–140°C using concentrated HNO_3[11,12].

Atomic absorption spectrometry

Lead and cadmium were determined by means of electrothermal atomic absorption spectrometry (ETAAS) and mercury by cold vapor atomic absorption spectrometry (CVAAS) [10]. A Perkin Elmer model 5100 PC Atomic Absorption Spectrophotometer with Longitudinal Zeeman Background Corrector was used in electrothermal atomization mode. Peak area signal was evaluated in all cases. After an optimization of the time needed for the quantitative atomization of the metals it was proved that 6 s for lead and 5 s for cadmium were satisfactory.

Mercury was determined in the digests, by subsequent addition of $KMnO_4$, $NH_2OH{\cdot}HCl$ and $SnCl_2{\cdot}2H_2O$. The liberated mercury vapor was determined using a 15 cm long flow-through cell with quartz windows, attached to an atomic absorption spectrometer. The analytical wavelength used was 253.7 nm and peak height signal was evaluated.

The results for sediments are expressed in mg kg^{-1} dry weight (DW), and they are mean values from triplicate analysis of each sample. The recovery and accuracy of the analytical procedure was tested by analyzing reference materials SD-M-2 / TM (IAEA, marine sediment, certified values Pb: 22.8 mg kg^{-1} DW; Cd: 0.113 mg kg^{-1} DW; and Hg 0.054 mg kg^{-1}DW). The recoveries achieved were 94.6, 95.3 and 96.0 % for lead, cadmium and mercury respectively, and the relative standard deviation of the overall method (triplicate analysis) was less than 5 % for each element.

The results for mussels are expressed in mg kg^{-1} wet weight (WW), and they are also the mean values from triplicate analysis of each sample. The WW/DW ratio ranged between 5–6, because the percentage dry mass in tissues was usually 16–20%. The recovery was tested using the reference material CRM 278 (BCR, *Mytilus edulis*, certified values Pb: 1.91 mg kg^{-1} DW, Cd: 0.34 mg kg^{-1} DW, Hg: 0.188 mg kg^{-1} DW). The recovery of lead was 98.1 %, cadmium 96.7 %, and mercury 95.7 %. The relative standard deviation of the overall method (triplicate analysis) ranged between 5–8 % for the three elements.

RESULTS AND DISCUSSION

Marine sediments

The mean seasonal concentrations in sediments are summarized in Table I. The mean values, e.g. for winter, were calculated from all winter measurements during four years. Analysis of variance was selected to test the significance of the sources of variation for each metal separately, in order to gain in homogeneity of variance. The results for each metal at the same region are thought to follow a normal distribution, however, the ANOVA F-test is not too sensitive for departures from normality[13]. Application of two-way ANOVA on the data of Table I showed that the seasonal variation, taking into account the total load of each metal in the surface sediments, was not significant at 95% significance level (Table II). However, the spatial variation between sites was significant even at the higher significance level (99%).

TABLE I Mean seasonal concentrations of lead, cadmium and mercury in coastal marine sediments. (The standard deviation of each mean is given in parenthesis)

					Lead (mg kg^{-1} DW)					
Season	THE 1	THE 2	THE 3	THE 4	THE 5	THE 6	STR 1	STR 2	KAV 1	KAV 2
Winter	117.8	113.5	75.9	82.3	37.5	36.0	46.3	44.2	76.6	42.8
	(47.5)	(39.6)	(27.9)	(28.7)	(7.3)	(16.8)	(23.7)	(22.2)	(38.3)	(8.2)
Spring	132.8	76.3	94.8	42.8	47.3	32.8	32.8	34.0	63.8	69.0
	(50.4)	(16.9)	(51.1)	(16.7)	(13.0)	(12.5)	(20.1)	(14.0)	(10.6)	(38.4)
Summer	168.5	87.5	90.8	53.3	41.5	27.5	33.8	37.0	82.8	40.8
	(75.0)	(22.1)	(35.9)	(9.0)	(33.4)	(2.6)	(19.8)	(20.9)	(8.0)	(15.3)
Autumn	91.5	99.3	82.0	58.6	38.8	27.8	27.0	31.8	52.0	48.3
	(21.2)	(15.3)	(42.3)	(17.9)	(16.4)	(11.8)	(7.3)	(21.9)	(14.3)	(15.3)
					Cadmium (mg kg^{-1} DW)					
Season	THE 1	THE 2	THE 3	THE 4	THE 5	THE 6	STR 1	STR 2	KAV 1	KAV 2
Winter	1.41	1.73	1.93	0.83	0.52	0.52	1.15	0.87	1.15	0.68
	(0.61)	(0.31)	(1.20)	(0.46)	(0.17)	(0.18)	(0.62)	(0.86)	(0.59)	(0.46)
Spring	1.79	1.49	2.30	0.77	0.37	0.51	0.90	1.00	0.97	0.50
	(0.91)	(0.93)	(1.64)	(0.36)	(0.14)	(0.43)	(1.24)	(0.96)	(0.78)	(0.26)
Summer	1.50	1.74	1.65	0.60	0.54	0.31	0.91	1.06	0.82	0.38
	(0.59)	(1.24)	(0.42)	(0.21)	(0.23)	(0.14)	(0.98)	(0.60)	(0.76)	(0.21)
Autumn	0.96	1.20	1.28	0.63	0.58	0.41	0.52	0.81	0.57	0.74
	(0.45)	(0.99)	(0.49)	(0.16)	(0.44)	(0.12)	(0.42)	(0.93)	(0.53)	(1.12)

	Mercury (mg kg^{-1} DW)									
Season	THE 1	THE 2	THE 3	THE 4	THE 5	THE 6	STR 1	STR 2	KAV 1	KAV 2
Winter	1.44	0.90	1.35	0.62	0.52	0.56	0.44	0.52	0.62	0.59
	(0.84)	(0.34)	(0.94)	(0.35)	(0.37)	(0.29)	(0.18)	(0.24)	(0.52)	(0.25)
Spring	1.22	1.24	1.30	0.35	0.47	0.52	0.44	0.50	0.70	0.34
	(1.16)	(0.45)	(1.30)	(0.11)	(0.04)	(0.40)	(0.32)	(0.43)	(0.87)	(0.13)
Summer	0.90	1.08	1.28	0.41	0.34	0.33	0.42	0.47	0.50	0.19
	(0.81)	(0.42)	(0.87)	(0.11)	(0.17)	(0.19)	(0.33)	(0.29)	(0.57)	(0.07)
Autumn	1.12	0.94	1.14	0.44	0.31	0.48	0.29	0.26	0.33	0.45
	(0.97)	(0.45)	(0.62)	(0.19)	(0.14)	(0.40)	(0.15)	(0.13)	(0.26)	(0.38)

The analysis of variance for Thermaikos gulf showed the existence of higher sum of square values at sites THE1 to THE4. This is explained because these sites are located at the inner part of the gulf (Thessaloniki Bay) and are severely affected by the effluents coming from the industrial area and the city of Thessaloniki. In almost all cases during the studied period, sampling site THE6 was proved to be the less contaminated, because it was located at the outer gulf, near to open sea, where the mean depth is more than 60 m, thus, it can be used as a reference site for monitoring studies.

The mean annual concentrations are listed in Table III, and in this case, each annual mean was calculated from the serial data of the year, during the four seasons. The two-way ANOVA was performed taking into account the mean annual values of Table III and the results are given in Table IV. The conclusions for spatial variance even with this test showed acceptable agreement with the corresponding spatial results described in Table II.

TABLE II Results of two-way ANOVA of seasonal and spatial concentrations of metals in sediments

Region	*Source of variation*	*DF*	F_{exp} *Pb*	F_{exp} *Cd*	F_{exp} *Hg*	F_{crit} *95%*	F_{crit} *99%*
Thermaikos gulf	seasonal	3, 15	0.6207	3.2203	2.9785	3.2874	5.4170
	spatial	5, 15	17.5930	28.2436	43.6570	2.9013	4.5556
Strymonikos gulf	seasonal	3, 3	20.8142	1.7032	17.3100	9.2766	29.4567
	spatial	1, 3	1.4780	0.2924	2.9036	10.1280	34.1160
Kavala Gulf	seasonal	3, 3	0.3576	0.7613	1.0614	9.2766	29.4567
	spatial	1, 3	2.6411	3.7425	1.6075	10.1280	34.1160

The mean annual metal concentrations in sediments ranged between 23–180 mg kg^{-1} DW for lead, 0.3–2.8 mg kg^{-1} DW for cadmium and 0.2–2.6 mg

kg^{-1} DW for mercury. A comparison of these levels with other reported previously for nearly the same region[14] showed that there is not a significant increasing or decreasing trend during the studied period. The last 4 years (1995–1998), the lead level of sediments was not changed significantly, as it is concluded from the results of annual variation, listed in Table III. The significant variation, which was observed in case of cadmium and mercury, caused to the higher values obtained during 1995. In general, the pollution of the Thermaikos gulf is characterized as moderate only at the inner part of the gulf taking into account the surface sediments. The small mean depth at the north-western part of the gulf, in connection to the high half-life of water renewal of the gulf (6–8 months), are the main reason of rapid sedimentation of the suspended particles and the effective trapping of the major part of heavy metals.

TABLE III Mean annual concentrations of lead, cadmium and mercury in coastal marine sediments. (The standard deviation of each mean is given in parenthesis)

	Lead (mg kg^{-1} DW)									
Year	THE 1	THE 2	THE 3	THE 4	THE 5	THE 6	STR 1	STR 2	KAV 1	KAV 2
1995	84.0	86.0	51.7	59.2	39.1	23.0	27.0	20.7	66.3	49.0
	(4.9)	(20.1)	(20.2)	(2.4)	(2.4)	(1.4)	(7.3)	(3.4)	(16.6)	(13.9)
1996	180.3	84.8	94.5	43.3	26.3	25.8	22.0	22.0	70.3	63.5
	(52.4)	(24.4)	(14.4)	(23.6)	(4.3)	(9.3)	(9.7)	(8.5)	(16.8)	(31.9)
1997	104.5	94.5	76.5	62.5	42.5	34.0	40.0	45.8	58.8	51.3
	(43.5)	(12.9)	(37.5)	(10.3)	(19.7)	(10.5)	(16.4)	(12.8)	(16.9)	(30.2)
1998	141.8	11.3	120.8	72.0	57.3	41.3	50.8	58.5	79.8	37.0
	(56.0)	(42.8)	(36.6)	(37.2)	(24.9)	(13.5)	(24.2)	(9.9)	(38.0)	(9.4)
Mean	127.6	94.2	85.9	59.2	41.3	31.0	34.9	36.8	68.8	50.2
	Cadmium (mg kg^{-1} DW)									
Year	THE 1	THE 2	THE 3	THE 4	THE 5	THE 6	STR 1	STR 2	KAV 1	KAV 2
1995	1.25	1.95	2.18	0.71	0.57	0.69	2.05	2.14	1.78	1.29
	(0.20)	(0.61)	(0.39)	(0.08)	(0.06)	(0.30)	(0.70)	(0.22)	(0.34)	(0.80)
1996	0.99	0.66	1.29	0.54	0.31	0.26	0.25	0.30	0.59	0.28
	(0.18)	(0.58)	(0.11)	(0.40)	(0.08)	(0.14)	(0.28)	(0.14)	(0.55)	(0.18)
1997	1.39	1.07	0.89	0.65	0.35	0.31	0.53	0.50	0.48	0.33
	(0.91)	(0.30)	(0.44)	(0.35)	(0.20)	(0.03)	(0.33)	(0.19)	(0.30)	(0.22)
1998	2.03	2.48	2.80	0.92	0.79	0.49	0.65	0.81	0.67	0.39
	(0.75)	(0.47)	(1.40)	(0.25)	(0.29)	(0.16)	(0.21)	(0.25)	(0.27)	(0.21)
Mean	1.42	1.54	1.79	0.70	0.50	0.44	0.87	0.94	0.88	0.57

	Mercury (mg kg^{-1} DW)									
Year	THE 1	THE 2	THE 3	THE 4	THE 5	THE 6	STR 1	STR 2	KAV 1	KAV 2
1995	2.50	1.35	2.60	0.45	0.35	0.92	0.65	0.68	1.33	0.52
	(0.35)	(0.38)	(0.50)	(0.08)	(0.12)	(0.23)	(0.20)	(0.40)	(0.54)	(0.29)
1996	0.83	0.75	0.75	0.38	0.50	0.38	0.21	0.32	0.25	0.40
	(0.58)	(0.09)	(0.22)	(0.08)	(0.39)	(0.20)	(0.12)	(0.30)	(0.30)	(0.35)
1997	0.70	0.71	1.09	0.37	0.36	0.22	0.34	0.38	0.23	0.34
	(0.36)	(0.23)	(0.31)	(0.14)	(0.17)	(0.04)	(0.28)	(0.18)	(0.04)	(0.24)
1998	0.65	1.35	0.62	0.62	0.42	0.37	0.38	0.36	0.34	0.32
	(0.32)	(0.28)	(0.19)	(0.39)	(0.12)	(0.08)	(0.13)	(0.09)	(0.11)	(0.20)
Mean	1.17	1.04	1.27	0.45	0.41	0.47	0.40	0.44	0.54	0.40

In sediments of Strymonikos gulf, lead varied between 20 – 60 mg kg^{-1} DW, cadmium between 0.2 – 2.0 mg kg^{-1} DW, and mercury between 0.2 – 0.7 mg kg^{-1} DW (see Table III). The spatial variation between the two sampling sites was not significant, even at the lower significance level (95%) (Table IV), thus, confirming the normal distribution of the metals in surface sediments, in the vicinity affected by river Strymon. On the other hand, the seasonal variation was significant, especially for lead and mercury, with the higher values observed during winter every year (Table II). These rapid changes occurring in surface sediments are due to the strong effect of the river, so in order to screen the pollution profile in the gulf more reliably, more sampling sites could be established in longer locations. Furthermore, during the last four years, significant annual changes of surface sediment concentration were observed, as illustrated in Table IV, which were caused by the variation of river's flow rate. No explanation could be suggested for the elevated concentration of cadmium and mercury in 1995.

TABLE IV Results of two-way ANOVA of annual and spatial concentrations of metals in sediments

Region	*Source of variation*	*DF*	F_{exp} *Pb*	F_{exp} *Cd*	F_{exp} *Hg*	F_{crit} *95%*	F_{crit} *99%*
Thermaikos gulf	annual	3, 15	2.9372	7.9022	4.1631	3.2874	5.4170
	spatial	5, 15	13.1099	10.2296	3.1163	2.9013	4.5556
Strymonikos gulf	annual	3, 3	24.5215	421.492	44.0168	9.2766	29.4567
	spatial	1, 3	0.3199	2.7620	2.3098	10.1280	34.1160
Kavala gulf	annual	3, 3	0.3737	56.5347	1.8898	9.2766	29.4567
	spatial	1, 3	4.8842	17.9858	0.4119	10.1280	34.1160

In sediments of the Kavala gulf, lead varied between 37 – 80 mg kg^{-1} DW, cadmium between 0.3 – 1.8 mg kg^{-1} DW, and mercury between 0.2 – 1.3 mg kg^{-1} DW (See Table III). This gulf is an almost open gulf, with short water renewal half-life, high mean depth, and normal sedimentation processes, not affected by rivers. The analysis of variance (Table II) revealed that both the seasonal and spatial differences between the two sampling sites were not significant, although sampling site KAV1 is more affected by the port activities, while KAV2 is more than 6 km far and about 40 m deeper. It is then concluded that the absence of large industrial area and rivers are the main reason of the normal distribution of the pollutants in the sediments. The pollution profile was not significantly changed for lead and mercury during the 4-year period, although for cadmium, the high concentration of cadmium during 1995–96 caused the extraordinary F_{exp} values (see Table IV).

Mussels (*Mytilus galloprovincialis*)

The effect of seasonal changes on mean concentrations of lead, cadmium and mercury in *Mytilus galloprovincialis* is illustrated in Figure 2. The mean values, e.g. for winter, were calculated from all winter measurements during three years. The mean tri-annual concentrations and the ranges are listed in Table V. In this case, the annual means were calculated from the serial data of the year, during all seasons.

The mussels cultured at the north-western side of Thermaikos gulf (sites THE2, THE3) are more affected from heavy metals contamination in comparison to those of the south eastern side (THE4, THE5). Lead concentrations ranged between 0.231–0.680 mg kg^{-1} WW at THE2 (inner gulf), while at THE5 (outer gulf) ranged between 0.178–0.411 mg kg^{-1} WW. In comparison to previously reported values[15,16], referring to the same gulf, these levels are lower in average values, calculated and expressed as wet weight. Also, taking into account reports from other Mediterranean gulfs the concentration levels are lower for lead and similar for cadmium and mercury[17].

Metal concentrations in mussels from Strymonikos and Kavala gulfs were reaching only low levels. Lead ranged between 0.160–0.461 mg kg^{-1} WW, cadmium between 0.026–0.170 mg kg^{-1} WW and mercury between 0.018–0.081 mg kg^{-1} WW. Taking into account the mean annual values, *mytilus sp.* showed low metal contamination, similar to that observed in open non-polluted gulfs[17], and to those found at the outer part of Thermaikos.

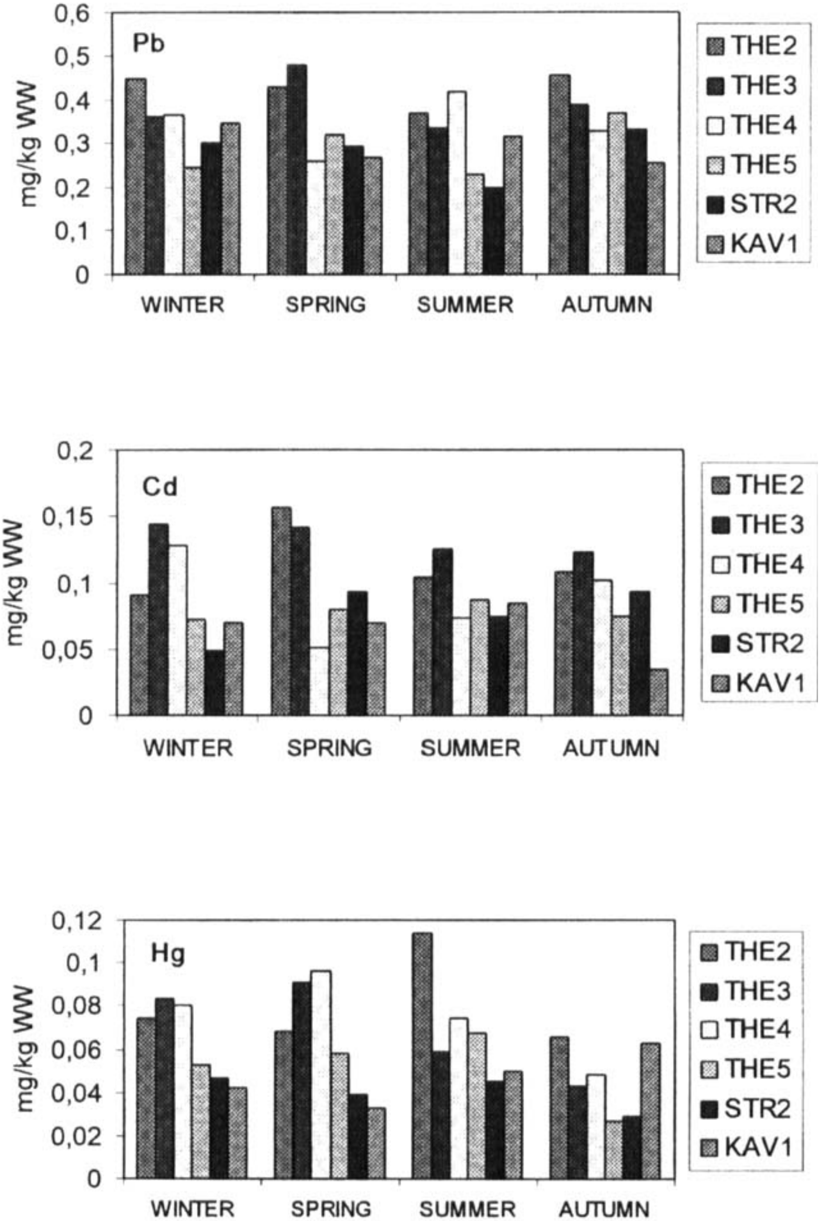

FIGURE 2 Mean seasonal concentration (mg kg^{-1} WW) of metals in *mytilus galloprovincialis* from different sampling sites

TABLE V Mean tri-annual concentration (mg kg^{-1} WW) and all-season ranges of lead, cadmium and mercury in *Mytilus galloprovincialis*

Region	*Sampling Site*	*Pb*			*Cd*			*Hg*		
		Mean	*s*	*Range*	*Mean*	*s*	*Range*	*Mean*	*s*	*Range*
Thermaikos Gulf	THE 2	0.510	0.123	(0.231–0.680)	0.115	0.039	(0.061–0.191)	0.080	0.031	(0.039–0.156)
	THE 3	0.391	0.109	(0.250–0.640)	0.134	0.049	(0.066–0.223)	0.069	0.032	(0.023–0.135)
	THE 4	0.344	0.130	(0.181–0.641)	0.089	0.064	(0.017–0.252)	0.074	0.031	0.030–0.142)
	THE 5	0.291	0.091	(0.178–0.411)	0.078	0.029	(0.035–0.135)	0.051	0.029	(0.014–0.111)
Strymonikos Gulf	STR 2	0.282	0.090	(0.160–0.452)	0.077	0.044	(0.026–0.170)	0.040	0.016	(0.018–0.070)
Kavala Gulf	KAV 1	0.297	0.090	(0.172–0.461)	0.065	0.031	(0.030–0.134)	0.047	0.017	(0.022–0.081)

Correlation analysis was performed between sediments and mussels collected from the same sampling site and date, for each metal separately. The values of correlation coefficient were low in all cases ($r_{Pb} = 0.62$, $r_{Cd} = 0.51$, $r_{Hg} = 0.63$), indicating the lack of significant dependence of metal load of mussels and corresponding sediments. The results of the application of two-way analysis of variance tests using the seasonal means for all gulfs are listed in Table VI. It can be seen that, although the spatial effect was significant (95%), the seasonal one was not significant.

TABLE VI Results of two-way ANOVA in seasonal and spatial results of metals in *Mytilus galloprovincialis*

Source of variation	*DF*	F_{exp} *Pb*	F_{exp} *Cd*	F_{exp} *Hg*	F_{crit} *95%*	F_{crit} *99%*
seasonal	3, 15	0.5982	0.1568	2.1651	3.2874	5.4170
spatial	5, 15	4.1695	4.7315	4.0569	2.9013	4.5556

CONCLUSIONS

This study investigated the possibility of screening either seasonal or annual changes of the pollution profile of coastal ecosystems, taking into account the metal content of surface sediments. The effect of seasonal variations was proved significant at vicinities nearby river Axios and Strymon outfalls or human activities' effluents like Thessaloniki Bay. The insignificant changes observed by analyzing annual variations in the sediments of the three gulfs indicated a stability of these coastal areas during the last four years. Separate two-way analysis of variance taking into account spatial, seasonal and annual mean values was proved a very convenient tool for rapid and reliable estimation of the significance of the sources of variation.

Mussels were proved to be good indicators of spatial distribution of metals in coastal areas, but their ability to indicate seasonal changes was not significant. Correlation between concentration of metals in mussels and the corresponding sediments from the same site and date is not reliable, generally. Higher concentrations of lead and cadmium were found in mussels from the inner and north western part of Thermaikos Gulf, and generally, at this part of the gulf the concentrations of metals in *Mytilus galloprovincialis* are medium to high in comparison to Strymonikos and Kavala gulfs. Finally, the range of concentration of the metals was found to be at levels similar to other reported for not heavily polluted coastal areas in Mediterranean Sea.

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