Global Nest: the Int. J. Vol 5, No 3, pp 119-126, 2003

DISTRIBUTION OF METALS & ORGANIC CONTAMINANTS IN MUSSELS FROM THERMAIKOS GULF

V.A. CATSIKI[1,*]
I. HATZIANESTIS[1]
F. RIGAS[2]

[1] *Hellenic Centre for Marine Research, Anavyssos Attikis, 19013, Greece*
[2] *National Technical University of Athens, School of Chemical Engineering, Athens 15700, Greece*

Selected from papers presented at the 7th Conference on Environmental Science and Technology, 3 - 6 September 2001, Ermoupolis, Syros island, Greece.

*to whom all correspondence should be addressed:
Tel: +(30) 22910-76371, fax: +(30) 22910-76347
e-mail: cats@ath.hcmr.gr

ABSTRACT

The levels and the distribution of metals, hydrocarbons and organochlorine compounds were studied in mussels (*Mytilus galloprovincialis*) and surface sediments collected from Thermaikos gulf in March 1999. Hydrocarbon values were indicative of moderate pollution, whereas in some cases clear evidence for the presence of petroleum residues was found. Polycyclic aromatic hydrocarbon, DDTs, PCBs and metal concentrations were low, but clearly higher than those measured in the same area during the previous years. Different spatial distribution patterns were observed between organic and inorganic contaminants bioaccumulated in mussels and between sediment and mussel values. In all cases the contaminant concentrations in mussels were well below the permissible limits for consuming seafood.

KEYWORDS: bioaccumulation, hydrocarbons, metals, organochlorines, mussels, Thermaikos gulf.

INTRODUCTION

Thermaikos gulf (North Aegean Sea) is influenced by many anthropogenic activities as it receives about 120,000 m^3 day^{-1} of sewage from the city of Thessaloniki and approximately 25,000 m^3 day^{-1} of industrial discharges from the industrial zone. In addition, three rivers, Axios, Loudias and Aliakmon, flow into Thermaikos gulf after the drainage of 93,500 ha of agricultural land. Previous pollution studies in the area have shown elevated values for heavy metals in sediments in the vicinity of the pollution sources, namely the industrial zone, the port of Thessaloniki and the sewage outfall (Voutsinou-Taliadouri *et al.*, 1998) and also the presence of some herbicide traces (Albanis *et al.*, 1994; Hatzianestis *et al.*, 2000).

The coasts of Thermaikos gulf and its adjacent area, host the most extended and productive mussel aquacultures of Greece (70% of the whole production). Mussels, as filter feeding organisms, accumulate pollutants in their tissues at elevated levels that are proportional to their biologically available concentration in the marine environment. Consequently they are worldwide used as pollution indicator organisms. In addition superficial sediments are considered as the "archives"

of the marine environment, imprinting information on contamination.

The present work aims to investigate the levels and the distribution of metals, hydrocarbons and organochlorine compounds in mussels from Thermaikos gulf. Superficial sediments were also studied in order to identify possible local pollution sources.

METHODOLOGY

Mussels *Mytilus galloprovincialis* of similar size (3-6 cm) and superficial sediments were collected during March 1999 by diving from seven coastal stations in Thermaikos gulf, representing both mussel farms and natural populations (Fig. 1). In the laboratory the shells of the mussels were removed and five pooled samples from the soft parts of 12 to 20 individuals were prepared for metals and one for organics. Sediments were silted and the fractions of <64 and <250μ were retained for metals and organics analysis respectively.

The analysis for metals included lyophilisation, homogenisation and digestion with HNO_3 under pressure for mussels (UNEP, 1984) and with a mixture of HNO_3, HF and HCl for sediments. The determination of metals was performed by atomic absorption spectrophotometry with flame for Cu, Cr, Ni, Zn & Mn and with graphite furnace for Cd & Pb. The accuracy and precision of the analytical methodology was tested with the reference material NRC-Dorm2 (Dogfish muscle).

For hydrocarbon analysis (UNEP, 1992) the freeze dried material (tissues or sediments), was spiked with known quantities of internal standards and Soxhlet extracted for 24 hrs with a mixture of dichloromethane-methanol 2:1. The extracts were saponified with methanolic KOH and the hydrocarbons were extracted with n-hexane and cleaned-up and fractionated on silica gel columns. The hydrocarbons were determined by gas chromatography-mass spectrometry. For the organochlorine analysis the freeze-dried material was Soxhlet extracted for 24 hrs with a mixture of dichloromethane-pentane 1:1, the extracts were cleaned-up and fractionated on deactivated alumina columns (Satsmadjis *et al.*, 1988) and the final determination was performed on a gas chromatograph equipped with an ECD detector.

The statistical treatment of the data, including analysis of variance and cluster analysis was performed with the software package STATGRAPHICS.

RESULTS-DISCUSSION

Metals

The results of metal analysis in mussels and sediments are reported in Table 1, expressed in ng g^{-1} for Cd and μg g^{-1} dry weight for the other metals. Generally, the measured metal concentrations were similar to those found in mussels from other non polluted Mediterranean areas (Coimbra *et al.*, 1991; Rodriguez *et al.*, 1995). In comparison with other Greek areas (Saronikos and Amvrakikos gulfs, Larymna Bay), the mussels from Thermaikos seem to be less contaminated

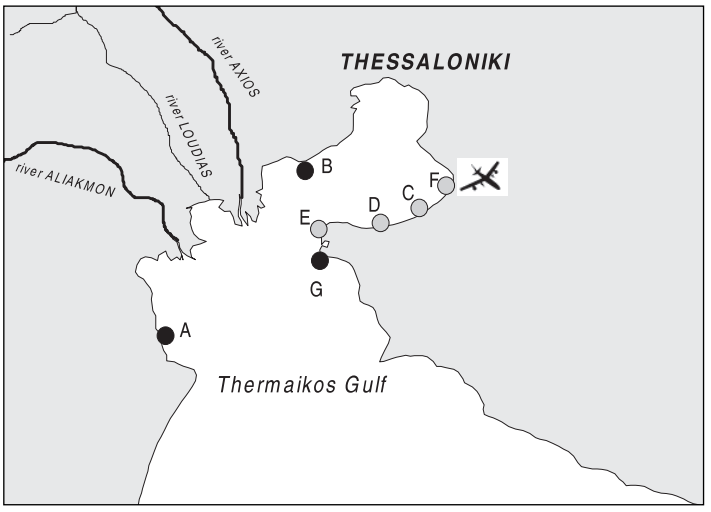

Figure 1. Sampling locations (in black: aquacultures, in grey natural populations)

by Cu, Cr, Ni and Zn (NCMR, 2000). However data were higher when compared with previous studies in the same area (NCMR, 2000). The latter could be attributed to the elevated natural seasonal variability of the bioaccumulation in mussels (NCMR, 2000).

The distribution of metals was not uniform along the coasts of Thermaikos gulf. Analysis of variance showed statistical differences among the sampling stations (Fig. 2). Mussels collected from station A presented the highest levels of metals probably due to a temporal impact of rivers during the winter season. Station F had also quite high concentrations obviously due to its vicinity to the Thessaloniki airport. On the other hand the remaining two aquaculture stations presented low metallic levels: station B hosted mussels with the lowest concentrations of Cd, Cr, Mn, Pb & Ni and station G with the lowest levels of Cu. We support the hypothesis that mussel farms act as pumping-plant, removing metals from the marine environment via mussels which are continuously taken away for consumption i.e. the amount of bioaccumulated Cr is as high as 300 Kg per year in Thermaikos gulf cultured mussels (NCMR 2000). The analysis of superficial sediments gave a different spatial distribution pattern: the stations located in the vicinity of Thessaloniki Bay (Table 1) were more contaminated than the others, fact attributed to the anthropogenic activities occurring in this area.

Hydrocarbons

Hydrocarbons presented their highest concentration (705,0 μg g^{-1} dry weight) in the natural population mussels collected from station E, whereas the lowest one (178,0 μg g^{-1}) was recorded in the mussel farms of station B (Table 2). In the rest stations total hydrocarbon content ranged between 256,1 and 451,9 μg g^{-1}. These values are generally similar with those found in other moderately polluted Mediterranean coastal areas (Granby and Spliid, 1995), but they are significantly higher than those recorded in previous studies in the area (Hatzianestis *et al.*, 2000). This differentiation is rather attributed to the high feeding rates of the mussels during winter (Baumard *et al.*, 1999), than to higher pollutant loads in the gulf. In sediments, a high value, indicative of petroleum pollution, was found only in station F close to the airport. In the rest stations total hydrocarbon concentrations were below 60 μg g^{-1}, values slightly higher than those reported for open sea sediments in Northern Aegean Sea (Hatzianestis *et al.*, 1998). The spatial distribution of hydrocarbons in sediments was generally different from that in mussels, suggesting that there are other important parameters, except the local pollution sources, controlling the accumulation of organic pollutants in mussels (lipid content, feeding conditions, physiology etc). In all samples the gas chromatographic traces of the aliphatic fraction were characterized by a unimodal hump corresponding to a mixture of unresolved compounds (UCM), which is generally well correlated with degraded or weathered petroleum residues. Values of the ratio unresolved/resolved compounds (U/R) greater than 4 are considered as clear evidence of petroleum related pollution (Mazurek & Simoneit, 1984). In the mussels the U/R ratio ranged between 1.2 at station A and 6.6 at station F (Table 2) indicating clear fossil fuel influence only at station F. Results from sediments indicated again station F as the most contaminated area.

N-alkane concentrations (C14-C34) presented good correlation with total aliphatic hydrocarbons, suggesting similar sources of n-alkanes and the petroleum originated UCM. Carbon preference index (CPI) values were low in mussels and higher in sediments indicating limited bioaccumulation of terrestrial n-alkanes, probably due to different particle association.

Polycyclic aromatic hydrocarbons (PAH) measured in this study included the parent compounds with 3-6 aromatic rings, retene, dibenzothiophene and the methylated derivatives of phenanthrene and dibenzothiophene. Their total concentrations, calculated as the sum of the 22 individual compounds accounted for less than 1% of the total hydrocarbons. These values can not be considered as elevated and they are generally lower than those expected for polluted coastal areas. The highest values were observed again in the natural populations of the eastern coast of the gulf (stations F, C, E). Benzo(a)pyrene, a known carcinogen, presented low values in all cases (Table 2). Mussels seems to preferentially bioaccumulate PAHs of petrogenic origin (compounds with lower MW, and higher water solubility), whereas in sediments the higher MB and more persistent pyrolytic PAH clearly predominate.

Table 1. Concentration of metals in mussels and superficial sediments in the coastal zone of Thermaikos gulf (in μg g^{-1} dry weight)

			A	B	C	D	E	F	G
Cd	sediment	AVG	0,60	0.66	1,51	-	-	2,40	0.51
	mussel	AVG±STD	0.95±0.12	0.77±0.07	0.93±0.07	1.0±0.11	1.2±0.06	1.11±0.18	0.92±0.06
		min-max	0.74-1.16	0.7-0.86	0.84-1.04	0.88-1.18	1.16-1.24	0.79-1.37	0.86-0.97
Cr	sediment	AVG	191	203	310	-	-	252	141
	mussel	AVG±STD	5.17±1.93	1.02±0.12	2.23±0.28	2.81±0.46	2.75±0.54	2.34±0.34	1.2±0.11
		min-max	3.52-9.31	0.9-1.16	1.66-2.53	2.3-3.35	2.37-3.13	1.72-2.68	1.91-2.16
Cu	sediment	AVG	38.35	55.35	42.31	-	-	56.95	42.31
	mussel	AVG±STD	5.75±0.5	5.26±0.21	6.11±0.58	5.2±0.41	5.65±0.21	5.53±0.77	4.46±0.22
		min-max	5.25-6.87	4.98-5.49	5.22-6.94	4.45-5.57	5.5-5.8	4.1-6.33	4.26-4.76
Mn	sediment	AVG	533	853	862	-	-	604	665
	mussel	AVG±STD	23.58±7.63	14.00±1.28	13.07±1.19	15.0±1.72	17.39±1.61	9.68±0.63	14.03±0.98
		min-max	14.82-38.98	12.53-14.89	12.16-15.08	12.63-17.11	16.26-18.53	9.13-10.37	12.78-14.85
Ni	sedim	AVG	166	124	85	-	-	165	91
	mussel	AVG±STD	9.78±2.73	1.47±0.11	3.57±0.32	3.8±0.44	3.94±0.06	3.21±0.55	2.75±0.11
		min-max	7.4-15.66	1.37-1.59	3.25-4.08	3.03-4.4	3.9-3.98	2.14-3.72	2.64-2.86
Pb	mussel	AVG±STD	1.73±0.54	0.97±0.35	1.5±0.88	2.16±0.44	2.05±0.22	1.77±0.36	1.96±0.22
		min-max	0.75-2.33	0.71-1.45	0.59-3.26	1.57-2.67	1.9-2.21	1.18-2.19	1.8-2,28
Zn	sedim	AVG	102	90	176	-	-	153	84
	mussel	AVG±STD	101.16±11.23	63.49±4.99	78.03±9.32	79.03±5.16	40.53	69.19±10.33	60.77±6.97
		min-max	79.89-114.89	57.42-67.83	62.16-87.12	72.24-85.33	-	49.71-78.39	51.2-67.09

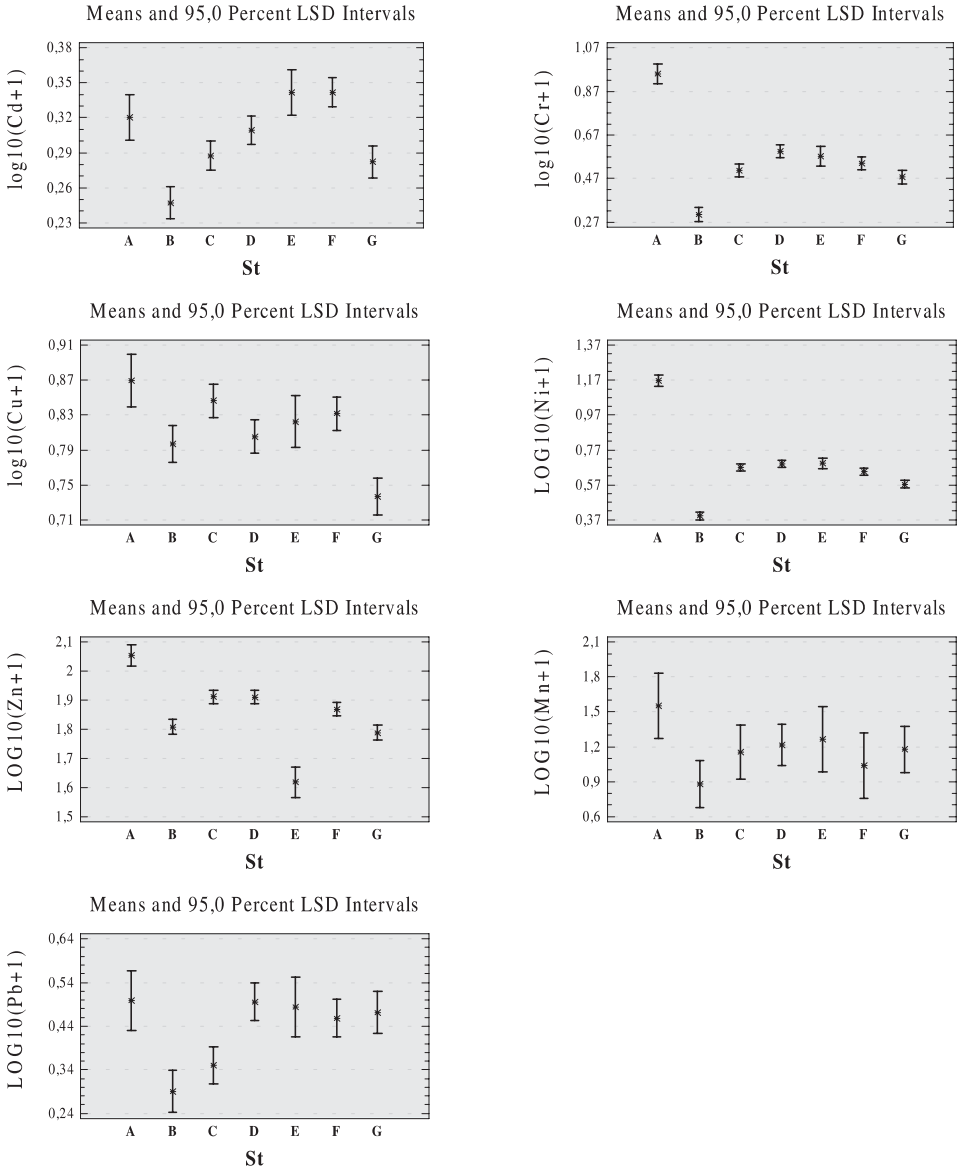

Figure 2. Plots of LSD significant differences among metal concentrations in mussels from the sampling stations.

Organochlorinated compounds

DDTs concentrations measured in mussel samples were generally low (18.2-84.0 ng g^{-1}, Table 2) and clearly below the limits considered dangerous for human consumption (Roach & Runcie, 1998). The maximum values were found again in the free populations of the eastern coasts (stations D, F, C). In sediments, DDTs values were also quite low (0.3-4.3 ng g^{-1}). p,p'-DDE, the main DDT metabolite was always the predominant component of the DDTs. However, in some cases (station G in sediments and D in mussels), unexpectedly high percentages of the parent compound DDT were recorded and this might indicate possible recent inputs of DDT in the gulf.

PCBs values correspond to the sum of the concentrations of seven congeners (IUPAC No 101, 118, 105, 153, 138, 156, 180) and varied between 5.9 and 19.1 ng g^{-1} in mussels and between 0.3 and 2.5 ng g^{-1} in sediments. These values are low and indicate that no significant pollution from PCBs occurred in the area. In the mussels the hexachlorobiphenyls 138 and 153 were always the dominant congeners followed by the pentachloro-118 and 101 (Figure 3). This pattern is commonly found in marine organisms as it is related to enzy-

Table 2. Selected parameters and analytical data of hydrocarbons and chlorinated compounds in mussels and sediments from Thermaikos gulf. THC: Total hydrocarbons, AHC: aliphatic hydrocarbons, UCM: Unresolved complex mixture, U/R: ratio of unresolved to resolved compounds, CPI: carbon preference index calculated in the 25-34 carbon range, ΣDDTs: sum of p,p'-DDT, p,p'-DDE, p,p'-DDD, PCBs: sum of seven congeners with No 101, 118, 105, 153, 138, 156, 180.

	A		**B**		**C**		**D**	**E**	**F**		**G**	
	Mussel	**Sedim**	**Mussel**	**Sedim**	**Mussel**	**Sedim**	**Mussel**	**Mussel**	**Mussel**	**Sedim**	**Mussel**	**Sedim**
Lipids (%)	10.4		12.2		12.2		12.3	9.7	14.0		7.4	
Organic carbon (%)		1.1		1.5		0.4				1.5		1.9
THC (μg g^{-1})	357.6	29.9	178.0	57.7	451.9	35.5	256.1	705.0	300.7	103.7	310.4	59.8
AHC (μg g^{-1})	357.2	29.7	177.3	57.0	450.8	33.9	255.4	704.1	298.9	103.1	310.0	59.3
n-alkanes (μg g^{-1})	10.2	2.9	5.2	3.3	8.9	3.0	5.8	32.5	8.1	3.4		3.5
UCM (μg g^{-1})	197.6	24.9	139.8	52.3	342.9	29.4	207.1	568.3	259.5	97.2	191.1	53.1
U/R	1.2	5.2	3.7	11.1	3.2	6.8	4.3	4.2	6.6	16.6	1.6	8.6
CPI (C25-C34)	2.7	4.9	1.6	4.1	2.0	2.9	1.9	1.6	1.6	4.8	2.9	4.2
ΣPAH (ng g^{-1})	190.2	165.2	489.5	305.8	681.1	586.6	409.6	686.0	750.4	483.1	248.4	392.4
Pyrolytic PAH	87.2	87.3	103.9	83.9	142.8	212.7	120.7	164.6	129.2	336.3	70.6	277.8
Petrogenic PAH	56.9	34.7	285.8	21.1	341.5	43.0	150.1	437.3	163.7	64.6	132.3	68.0
Biogenic PAH	5.9	23.1	2.5	91.6	0.9	10.1	1.8	7.0	1.2	17.1	0.5	9.6
Benzo(a)pyrene (ng g^{-1})	0.9	5.1	0.6	5.2	2.9	20.0	0.2	3.1	2.9	29.8	0.5	24.8
ΣDDTs (ng g^{-1})	21.4	1.9	27.3	1.0	42.0	0.3	82.0	24.4	44.3	4.3	18.2	2.3
p,p'-DDE	12.8	1.3	21.1	0.8	29.5	0.3	49.5	15.6	32.9	2.9	13.2	1.0
p,p'-DDD	4.3	0.3	2.9	0.1	7.9	n.d.	15.8	6.2	8.3	0.9	2.9	0.3
p,p'-DDT	4.3	0.3	3.3	0.1	4.6	n.d.	16.7	2.6	3.1	0.5	2.1	1.0
PCBs (ng g^{-1})	5.9	0.3	12.4	0.9	11.4	0.2	9.9	9.4	19.1	2.5	7.7	0.3

n.d.: not detected

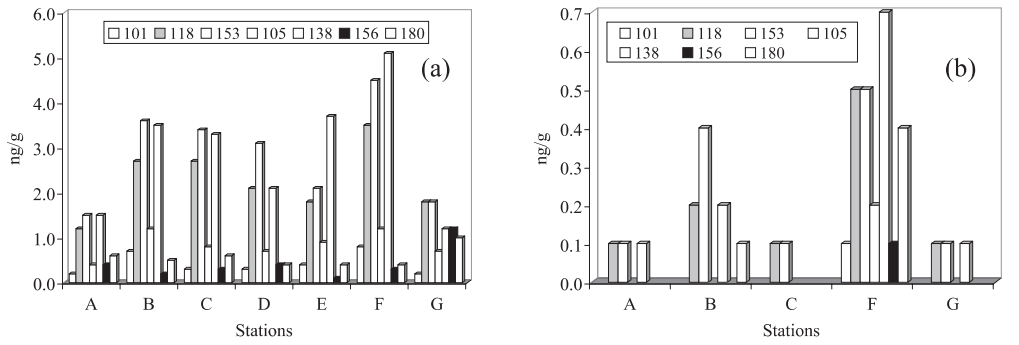

Figure 3. Distribution of the PCBs congeners. (a) mussels (b) sediments

matic metabolic processes in them (Boon & Eijgenraam, 1988). In the sediments the heptachloro- 180 was also detected in high percentages as in the commercial formulations of PCBs.

In order to classify the sampling stations and their state of pollution, we performed cluster analysis of the pollutant data in mussels (Fig. 4). Superficial sediment analyses were excluded from this treatment since not in all the sampling stations sediments were studied. At the 50% level the Cluster analysis revealed 4 groups of stations: a) the station A located at the west coasts of Thermaikos characterised by high concentrations of metals and low levels of organic pollutants, b) the stations E & G at the east coasts having low metallic levels and intermediate organics, c) the stations C & D at the north east coasts having similar environmental characteristics and d) the stations B & F located in Thessaloniki Bay. It seems that the cluster analysis grouped the sampling stations rather geographically.

In conclusion the concentration of contaminants in mussels was low, similar to other non polluted

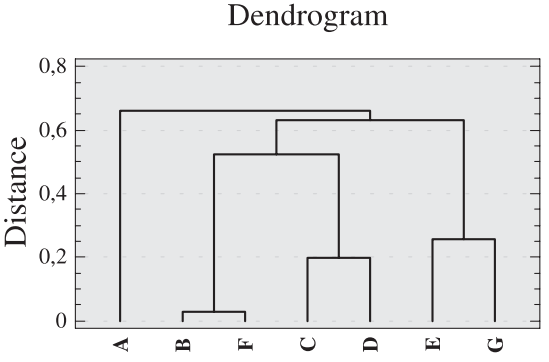

Figure 4. Cluster analysis of pollutant data in mussels from Thermaikos gulf

Mediterranean areas and well below the permissible limits for consumption. Their distribution did not follow a common pattern: Metals were higher in mussels collected from station A and in the sediments of the stations located in the vicinity of Thessaloniki Bay. Organic pollutants were enriched in the area of the airport (station F) and the neighbor station E. Finally the cluster analysis

REFERENCES

Albanis, T.A., Danis, T.G. and Kourgia, M.K. (1994), Transportation of pesticides in estuaries of the Axios, Loudias and Aliakmon rivers (Thermaikos Gulf), *Greece, Science of the Total Environment*, **156**, 11-22.

Baumard, P., Budzinski, H., Garrigues, P.,Narbonne, J.F., Burgeot, T. Michel, X., Bellocq, J. (1999), Polycyclic aromatic hydrocarbon (PAH) burden of mussels (Mytilus sp.) in different marine environments in relation with sediment PAH contamination, and bioavailability, *Marine Environmental Research,* **47**, 415-439.

Boon, J.P and Eijgenraam, F. (1988), The possible role of metabolism in determining patterns of PCB congeners in species from the Dutch Wadden Sea, *Marine Environmental Research,* **24**, 3-8.

Coimbra J., Carraca S. and Ferreira A. 1991. Metals in Mytilus edulis from the Noerthern coast of Portugal, *Marine Pollution Bulletin,* **22**, 249-253.

Granby, K., Spliid, N.H. (1995), Hydrocarbons and Organochlorines in Common Mussels from the Kattegat and the Belts and their Relation to Condition Indices, *Marine Pollution Bulletin,* **30**, 74-82

Hatzianestis, I., Sklivagou, E. and Georgakopoulou, E. (2000), Organic contaminants in sediments and mussels from Thermaikos gulf, Hellas, *Toxicological and Environmental Chemistry,* **74**, 203-216.

Hatzianestis, J., Sklivagou, E. and Friligos, N. (1998), Hydrocarbons in surface sediments from the Northern Aegean sea, *Rapp. Comm. int. Mer. Medit.,* **35**, 264-265.

Mazurek, M.A. and Simoneit, B.R.T (1984), Characterization of biogenic and petroleum-derived organic matter in aerosols over remote, rural and urban areas in Identification and Analysis of Organic Pollutants in Air, Keith L.H. ed.. Ann Arbor Science/Butterworth. Boston, 353-370.

NCMR (2000), Monitoring of the quality of the marine environment in Thessaloniki bay, Technical report (Pagou, K. ed), p.249.

Roach, A.C. and Runcie, J. (1998), Levels of Selected Chlorinated Hydrocarbons in Edible Fish Tissues from Polluted Areas in the Georges/Cooks Rivers and Sydney Harbour, New South Wales, Australia, *Marine Pollution Bulletin,* **36**, 323-344.

Rodriguez, C. Guerrero, J., Benedicto J.M. & Jornet A. 1995. Spatial distribution of heavy metals in mediterranean mussel mytilus galloprovincialis from spanish mediterranean coast. Rapp.Comm.int Mer Medit.,34:145.

Satsmadjis, J., Georgakopoulos-Gregoriades, E., Voutsinou-Taliadouri, F. (1988), Separation of organochlorines on alumina, *Journal of Chromatography*, **437**, 254-259.

UNEP/IOC/IAEA (1992), Determination of petroleum hydrocarbons in sediments, *Reference Methods for Marine Pollution Studies*. **No 20**, UNEP.

UNEP (1984), Determination of total Cd, Zn, Pd and Cu in selected marine organisms by atomic absorption spectrophotometry. Reference Methods for Marine Pollution Studies, No 11, Rev 1

Voutsinou-Taliadouri, F., Hatzianestis, I. and Georgakopoulou-Gregoriadou, E. (1998) Trace elements, pesticides and PCBs levels in sediments of a bay influenced by anthropogenic activities (Thermaikos bay, N.W. Aegean Sea). International Symposium on Marine Pollution, Extended synopses, pp. 680-68, Monaco.