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RESEARCH ARTICLE

Sediment heavy metals of a Mediterranean coastal lagoon: Agiasma, Nestos Delta, Eastern Macedonia (Greece)

Christophoridis[1] A, Stamatis[2] N, Orfanidis[2]* S

[1]Technological Educational Institution, Department of Petroleum Technology & Natural Gas, 55 404 Kavala, Greece

[2]National Agricultural Research Foundation, Fisheries Research Institute, 64 007 Nea Peramos, Kavala, Greece

*corresponding author: e-mail: sorfanid@inale.gr;
tel.: +30 259 4029039, fax: +30 259 4022222

Abstract

1 - Sediment cores of Agiasma, Nestos Delta lagoon, characterized by the perennial angiosperm *Ruppia cirrhosa* and by the opportunistic macroalga *Ulva sp*. were analyzed for six heavy metals. The aim was to contribute (a) in quantifying origin and degree of heavy metal pollution, (b) in developing sediment pollutants base line data for eastern Mediterranean, and (c) in exploring the role of heavy metals in habitat type shifts.

2 - The maxima of total concentrations (in ppm dry weight) were the following: Cu (up to 37), Pb (up to 158.2), Ni (up to 59.2), Zn (up to 70.4), Cr (up to 112.3), and Hg (up to 0.1). These values are lower compared to the maxima measured in polluted lagoons and coastal ecosystems of the Mediterranean Sea. Mean total concentrations in *Ulva* habitat type were only for lead two times higher than in *Ruppia* habitat type. This result along with the Euclidean distance cluster analysis based on total metal concentrations suggested heavy metal pollution not to be a determinant factor of the habitat type shift in the Agiasma lagoon.

3 - Of two enrichment factors (r_{ef}, R), calculated only the "r_{ef}" indicated that habitat type of *Ulva* is slightly polluted by lead. The overall detected heavy metal surcharge could be attributed to local, e.g. fisherman, as well as to watershed, e.g. fresh waters, urban sewages, anthropogenic activities.

Keywords: transitional ecosystems, base line values, chemical status, *Ruppia*, *Ulva*, enrichment factor, WFD.

Introduction

Heavy metals enter marine environments from natural as well as from man-made sources through several pathways, e.g. weathering processes reaching aquifers mainly through subterranean waters and river run-offs, atmospheric depositions, point as well as non point pollution sources. Heavy metals released into water eventually are accumulated in the sediments, which act as recorders of heavy metal pollution events (Solomons, Foerstner, 1984). Therefore, sediment analyses play an important role in the assessment of the "Chemical Status" of transitional and coastal waters requested by WFD (EC, 2000) and generally in the assessment of environmental quality. Since marine sediments naturally contain different quantities of metals any measurement of total heavy metal concentration as a criterion to assess metal contamination in the sediment environment is not satisfactory to discriminate natural from anthropogenic sources. The evaluation of an enrichment factor (r_{ef}) and/or "total enrichment factor" (R) based on the usage of background values from relatively non polluted bottom sediments overcome this difficulty giving a simple quantitative criterion for characterizing the sediment according to the degree of metal pollution (Adami et al, 2000). Another possibility is the establishment of type-specific reference conditions (base line values) by sampling many minimally impaired sites of different ecosystems within the (sub) ecoregion also needed by WFD (EC, 2000).

Although heavy metals enter in the Mediterranean sea from natural sources, e.g. pedo-geochemical background (Bryan, 1976), industrialization and human activities has increased in the last decades the emission of heavy metals into the marine environment resulting in pollution hot spots close to urbanized coastal and transitional water areas (Sfriso et al, 1992, 1995; Barbieri et al, 1999; EEA, 1999; Voutsinou-Taliadouri et al, 2005). In the Greek coastal zone, including Kavala Gulf, urban, agricultural and industrial effluents are the main land-based pollution sources (Voutsinou-Taliadouri et al, 2005; Stamatis et al, 2002, 2006). Despite the numerous studies in the literature on the heavy metal contaminated sediments in lagoons or other coastal ecosystems (e.g. Brix and Lyngby, 1983; Dassenakis et al, 1995; Zabetoglou et al, 2002; Pekey et al, 2004; Okbah et al, 2005; Nasr et al, 2006; Karageorgis, 2007) no data exists on the heavy metal distribution in the Agiasma, Delta Nestos lagoon.

In coastal lagoons, including Agiasma lagoon, benthic vegetation form the basis of community structure and ecosystem functioning, which evolves through a succession driven mainly by nutrient loading and hydrological conditions (for an overview, see Viaroli et al, 2007). While pristine coastal lagoons are dominated by perennial seagrass species, since in oligotrophic waters rhizophytes take advantage of nutrient supply from sediment, eutrophic lagoons are dominated by macroalgae or phytoplankton species. Nutrient excess is considered to induce a shift between the two alternative states by favouring the rapid growth and/or the colonization ability of seaweeds to exclude angiosperms (Schramm and Nienhuis, 1996; Schramm, 1999). In such a vegetation switch hydrographic changes, grazing or interactions with other stressors e.g. heavy metals, cannot be excluded (Cloern, 2001; de Jonge et al, 2002).

The aim of the present work was to contribute (a) in quantifying origin and degree of heavy metal pollution, (b) in developing sediment pollutants base line data for eastern Mediterranean, and (c) in exploring the role of heavy metals in habitat type shifts. The concentrations of Cu, Pb, Ni, Zn, Cr, and Hg and the grain size distribution in different sediment fractions and the organic matter content in sediments of Agiasma lagoon dominated by the angiosperm *Ruppia cirrhosa* and by the opportunistic macroalga *Ulva sp.* were investigated.

Materials and Methods

Study area

Kavala Gulf, a modest populated area characterized by the presence of industrial (oil-platforms and refinery, oil repository, fertilizer and food production), port (shipbuilding, good stocking, commercial and touristic transaction), aquaculture (mussel cultivation), and agricultural activities (intensive agriculture in Chrysoupolis valley), is regarded as a hot spot of polluted Greek areas (Papathanasiou and Zenetos, 2005; Stamatis et al, 2006). In the eastern Kavala's Gulf shoreline Nestos Delta area of ca. 1700 ha exists. This Delta is characterized by an extensive coastal lagoon ecosystem protected by the Ramsar convention which it is also suggested to be included in the Natura 2000 network. The biggest Delta Nestos lagoon is Agiasma, having area of ca. 367ha and an average depth of 1m (Fig. 1). Extensive fish aquaculture, which prevents fish-immigrants to return to the sea by a system of mesh frames (metal grids) and a stationary entrapment system (fish barrier), is the main commercial use of Agiasma and other lagoons in the area. Previous studies on the level and symptoms of water nutrient contamination in the Nestos Delta lagoons showed Agiasma as one of less impacted lagoon at local as well as at national levels (Orfanidis et al, 2001, 2005, 2007; Nicolaidou et al, 2005). Two main habitat types, separated from each other by an inlet and metal grids, exist; one covering area B of the lagoon (ca. 86%), dominated by the angiosperm *Ruppia cirrhosa* (Petagna) Grande in summer and *R. cirrhosa* with *Gracilaria bursa-pastoris* (Gmelin) Silva in winter and indicative of less anthropogenic stress (Fig. 1); the other covering area A of the lagoon (ca. 10%), dominated by Cyanobacteria during winter and by the macroalga *Ulva* during summer and indicative of ecosystem degradation (Sfriso et al, 1992; Schramm and Nienhuis, 1996; Viaroli et al, 2001; Orfanidis et al, 2001). Using the biotic index EEI the two habitat type areas are classified in Good and Bad Ecological Status Class, respectively (Orfanidis et al, 2007). Within area B, there is a narrow recently deepened navigation channel (ca. 4%) with no or scarce vegetation consisting mainly of coarsely branched macroalgae, e.g. *Gracilaria*.

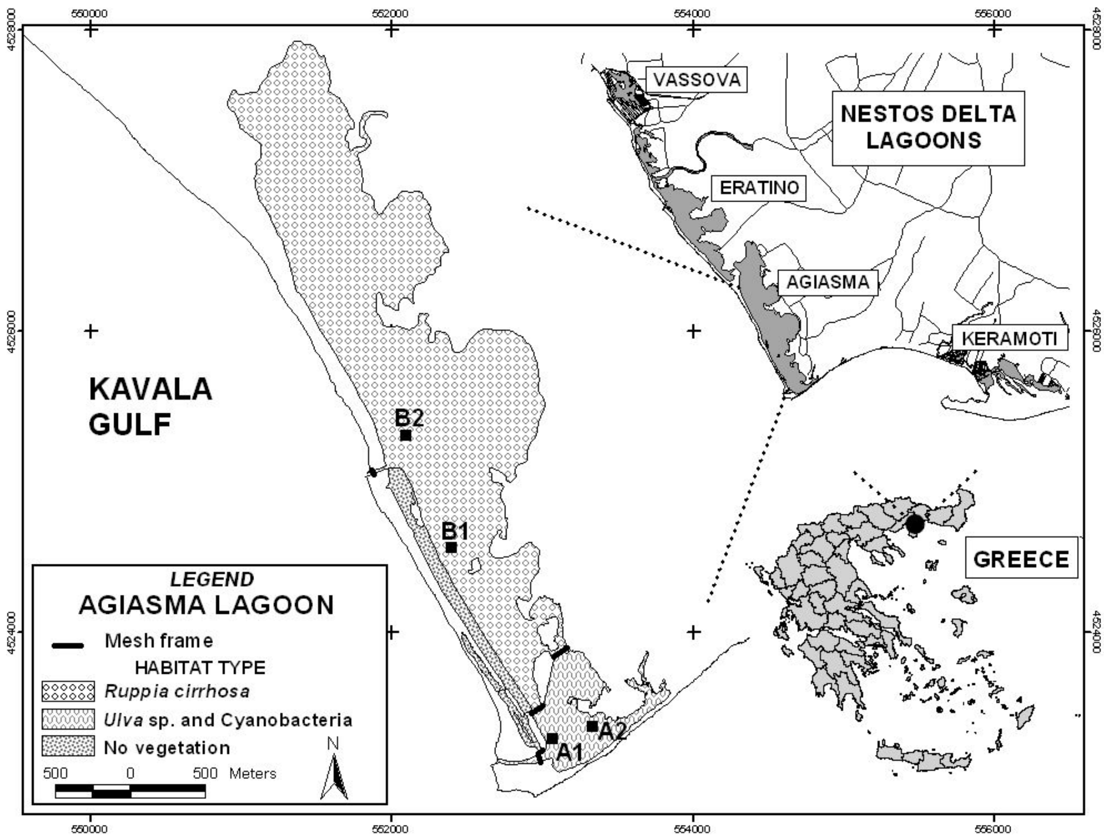

Figure 1. Map of the studied area with a general view of different habitat types.

Habitat type classification

Habitat type was defined by dominant vegetation: (1) macroalgae, when macroalgae or Cyanobacteria dominate, (2) angiosperms, when angiosperms alone or in coexistence with macroalgae or Cyanobacteria dominate or exist, (3) without vegetation.

Sampling

Figure 1 shows the four sampling sites in the Agiasma lagoon: two sites were randomly selected in the *Ulva* habitat type and two sites were randomly selected in the *Ruppia cirrhosa* (Petagna) Grande one. The sampling was carried out in July 2005. Sediment cores were collected using hand-held stainless steel box corer (17cm x 17cm x 15cm; length x wide x height), which was vertically pushed through the benthic vegetation and sediment. The selected sediment was divided in three slices down the length of the core, namely 0-2, 2-5 and 5-10cm. Afterwards the samples were immediately placed in labelled plastic bags, which have been cleaned with nitric acid (1N) for 1h and rinsed thoroughly with double distilled water, and kept on board in a cool box at $0^{o}C$. In the laboratory the samples were kept at -28°C until were further used for the digestions/extractions.

Digestion/Extraction

Sediment samples were defrosted at room temperature and wet sieved through polyester sieves in order to determine four fractions: (A) <64μm, (B) 64μm<x<250μm, (C) 250μm<x<1mm and (D) >1mm. Sediment metal concentrations were measured in each fraction as follows: sediment samples were dried at 50°C up to a constant weight and homogenized in a porcelain mortar with a pestle. A sediment sub-sample of 0.25g was digested in a mixture of 4ml of aqua regia and 1ml of concentrated HF for 30 minutes, using microwave heating, which incorporates reaching 1000W in approximately less than 25min and remaining at this stage (230°C, 20bar) for 5min for the completion of specific reactions. After cooling the clear, diluted (up to a volume of 25ml) solutions were used for the chemical analysis. Organic matter (OM) was measured in un-sieved sediment as follows: 1g of dry sample was heated at 450°C in porcelain crucibles for 12hrs. Organic matter was combusted to ash and carbon dioxide at this temperature. The weight loss was considered proportional to the amount of organic carbon contained in the sample (Dean, 1974).

Chemical Analyses

Concentrations of Cu, Pb, Ni, Zn, Cr, and Hg were determined in triplicate basis by atomic absorption spectroscopy (AAS) using a PC controlled Perkin-Elmer 5100ZL spectrometer, equipped with (1) a THGA graphite furnace, ZEEMAN background correction and AS-70 auto sampler (2) Mercury Hydride System MHS-15) and (3) a flame system. Quality assurance of analytical results was controlled using reference marine sediment material ("MESS 3"), certified by the Canadian NRC. In order to determine the precision of the analytical process, samples from the site A1 were analyzed four times. The standard deviation was calculated to 3.5 % and can be considered satisfactory for environmental analysis.

Enrichment factors

Enrichment factors (r_{ef}) for each metal (fraction <64μm), defined as the ratio $r_{ef}=(C_{sed}-C_{back})/C_{back}$ and a "total enrichment factor" (R), for each site defined as the ratio $R=(\Sigma^{ref})/n$ for each sampling site were calculated by adapting to Adami et al (2000) methodology. While C_{back} is the mean content of the same metal for all the background sediment samples (5-10cm depth), C_{sed} is the content of a metal in each surface (0-2cm depth) sediment sample. Using sedimentation rates in Amvrakikos delta (Poulos et al, 2005), a similar to Nestos Delta system, 5-10cm of sediments in Agiasma may corresponds to conditions 21-42 years before.

Statistics

For the interpretation of the data of the chemical analysis total metal contents were estimated using the sum of weighted concentrations of the each sediment sieved fractions. Spearman rank coefficient (r) was used to calculate inter-element correlations. Hierarchical cluster analysis of (log+1)-transformed not standardized data was applied to calculate correlations among sampling sites. All calculations were performed using the STATGRAFICS v. 7.1 software package.

Results and Discussion

Metal distribution

The metal concentrations of each sediment fraction are shown in Table 1. Chromium and Zn sediment concentrations composed of low natural metal concentration decreased with increasing grain size, suggesting their association with the fine fractions of the sediment. The concentrations of the rest metals (Cu, Pb, Ni, and Hg) showed not the same behaviour. Mercury was detected only at site A1 in the surface layer in three of the four fractions.

Mean total heavy metal concentrations (ppm) of the Agiasma lagoon sediments per slice of sampling site and per habitat type are shown in Table 2. The highest mean score values for each metal were found as follows: 37.04ppm Cu at site A1_2, 158.2ppm Pb at site A1_2, 59.02ppm

Ni at site B2_5, 70.44ppm Zn at site A1_2, 112.34ppm Cr at site B2_10, and 0.11ppm Hg at site A1_2. These values are at the same level or lower compared to the highest level for these metals measured in other lagoon and coastal ecosystems in the Eastern Mediterranean region (see Tab. 2; Fytianos and Vasilikiotis, 1982; Voutsinou-Taliadouri, 1984; Sakelariadou, 1987; Pavoni et al, 1987; Sharp and Nardi, 1987; Stamatis et al, 2002, 2006).

Table 1. Heavy metal distribution in various fractions and slices of Agiasma lagoon sediments: (A) <64μm, (B) 64μm<x<250μm, (C) 250μm<x<1mm, (D) >1mm; 2=0-2cm, 5=2-5cm, 10=5-10cm slices down the length of the sediment core. n.d.=not detected.

Station code	Fraction portion (%)	Fraction	Trace metal content, ppm dry weight					
			Cu	Pb	Ni	Zn	Cr	Hg
A1_2	12.0	A	30	170	70	110	210	0.62
	15.0	B	20	140	50	130	170	0.14
	52.8	C	50	160	50	60	80	0.02
	20.2	D	20	160	30	30	40	n.d.
A1_5	12.4	A	80	90	110	90	270	n.d.
	15.3	B	80	80	80	70	190	n.d.
	57.8	C	20	80	40	30	60	n.d.
	14.5	D	20	20	50	40	60	n.d.
A1_10	9.3	A	60	80	70	100	230	n.d.
	15.4	B	60	120	60	70	150	n.d.
	61.7	C	20	100	30	30	30	n.d.
	13.6	D	20	20	40	20	n.d.	n.d.
A2_2	9.0	A	20	110	40	80	230	n.d.
	5.8	B	n.d.	40	30	50	120	n.d.
	75.3	C	20	80	10	40	80	n.d.
	6.9	D	20	30	20	50	80	n.d.
A2_5	5.0	A	20	50	60	80	200	n.d.
	6.4	B	10	20	40	60	80	n.d.
	81.0	C	20	n.d.	50	50	100	n.d.
	7.6	D	10	110	60	60	110	n.d.
A2_10	4.6	A	20	30	80	80	240	n.d.
	5.9	B	20	30	50	50	110	n.d.
	82.0	C	10	50	40	40	50	n.d.
	7.5	D	60	30	70	60	50	n.d.
B1_2	14.9	A	30	20	70	80	200	n.d.
	11.6	B	20	n.d.	40	40	60	n.d.
	68.2	C	10	40	40	30	20	n.d.
	5.3	D	10	180	50	30	30	n.d.
B1_5	12.6	A	20	140	70	70	190	n.d.

	11.1	B	10	80	40	30	60	n.d.
	71.8	C	10	10	30	20	10	n.d.
	4.5	D	n.d.	20	50	20	30	n.d.
B1_10	12.0	A	20	40	80	70	220	n.d.
	13.5	B	20	n.d.	50	40	130	n.d.
	69.7	C	10	n.d.	30	30	40	n.d.
	4.8	D	20	30	50	40	50	n.d.
B2_2	23.9	A	30	20	30	70	190	n.d.
	10.5	B	20	20	20	50	130	n.d.
	57.2	C	20	40	40	40	70	n.d.
	8.4	D	20	40	50	40	60	n.d.
B2_5	18.4	A	30	80	90	80	220	n.d.
	9.2	B	20	20	70	60	150	n.d.
	64.2	C	20	40	50	40	70	n.d.
	8.2	D	20	n.d.	50	40	60	n.d.
B2_10	20.4	A	30	40	80	80	220	n.d.
	10.5	B	20	40	70	50	140	n.d.
	60.7	C	20	80	50	40	80	n.d.
	8.4	D	20	40	60	40	50	n.d.

Furthermore, the values are comparable to those for metals detected at sites in the open Aegean Sea or at sites in non-polluted coastal ecosystems (Voutsinou-Taliadouri, 1995, 1998; Voutsinou-Taliadouri et al, 2005). The highest mean score values for all metals were found in the *Ulva* habitat type but with only lead to be more than double higher than in *Ruppia* habitat type. While the highest mean score values of Agiasma *Ruppia* habitat type were slightly higher than similar habitat types dominated by the angiosperm *Zostera marina* L. of Venice and Thau lagoons (Rigollet et al, 2004), the highest mean score values of Agiasma *Ulva* habitat type were by far lower than similar values in polluted sites of Venice lagoon, dominated by *Ulva* (Pavoni et al, 1987). Therefore, *Ruppia* habitat type of Agiasma lagoon can be treated as a sediment heavy metal pollutants base line ecosystem in the Eastern Mediterranean area.

Inter-element correlation coefficients

Copper concentration values are significant correlated ($p<0.05$) with those of Pb ($r=0.63$), Zn ($r=0.69$), and Cr ($r=0.62$) (Tab. 3). Nickel values are significant correlated with those of Zn ($r=0.59$), and Zn values are significant correlated with those of Cr ($r=0.77$). High concentrations of these metals at site A1 sediments, which is close located to fisherman infrastructures in the main lagoon outlet, indicate the sources of these metals as effluents from fisherman activities. High Cr values were also measured at site B2, which is close located to the main freshwater sources, indicating a transport in the lagoon as weathering products of drainage basin ophiolites due to Nestos river runoffs. A high (0.57) but not significant correlation between Ni and Cr may further support this hypothesis. High Cr values were measured in Amvrakikos Gulf lagoons were also attributed to natural weathering process (Karageorgis, 2007). Mercury detected at site A1 sediments showed no significant correlations with other metals come probably from limited local non point sources of pollution.

Table 2. Organic matter and mean total heavy metal concentrations (ppm) of the Agiasma lagoon sediments per slice of sampling site and per habitat in comparison to relevant literature. Literature: 1=Pavoni et al (1987); 2=Rigollet et al (2004); 3=Kornilios et al (1997); 4=Karageorgis (2007); 5=Voutsinou-Taliadouri (1995); 6=Voutsinou-Taliadouri (1998). For more information see Table 1.

Coastal zone	Station code	Habitat type	Organic matter (%)	Metal content (ppm dry weight)					
				Cu	Pb	Ni	Zn	Cr	Hg
Agiasma lagoon	A1_2	Macroalgae	2.64	37.04	158.20	48.36	70.44	101.02	0.11
	A1_5		2.65	36.62	72.54	56.25	45.01	105.93	n.d.
	A1_10		1.93	29.88	90.34	39.70	41.31	63.00	n.d.
	A2_2		1.49	18.24	74.53	14.25	43.67	93.42	n.d.
	A2_5		1.38	18.60	12.14	50.62	52.90	104.48	n.d.
	A2_10		1.29	14.80	46.40	44.68	43.93	62.28	n.d.
	Mean		1.9 (± 0.25)	25.86 (± 4.04)	75.69 (± 19.94)	42.31 (± 6.05)	49.54 (± 4.48)	88.36 (± 20.38)	0.02 (±0.02)
	B1_2	Angiosperms	1.39	14.14	39.80	45.00	38.61	51.99	n.d.
	B1_5		1.24	10.81	34.60	37.05	27.41	39.13	n.d.
	B1_10		1.15	13.03	6.24	39.66	36.63	74.23	n.d.
	B2_2		3.31	22.39	33.12	36.35	48.22	104.14	n.d.
	B2_5		2.87	21.84	42.24	59.20	49.20	104.14	n.d.
	B2_10		2.42	22.04	64.28	59.06	49.21	112.34	n.d.
	Mean		2.06 (± 0.38)	17.38 (± 2.15)	36.71 (± 7.63)	46.05 (± 4.32)	41.55 (± 3.63)	81.00 (± 30.64)	n.d.
Venice lagoon	Mean	Macroalgae[1]		463	278		5930		27.4
		Angiosperms[2]		8.4	6.1	12.8	61.6	47.3	
Thau lagoon	Mean	Angiosperms[2]		18.7	13.8	8.9	36.1	21.8	
Gialova Lagoon	Range	Angiosperms[3]		0.24-14.1	0.04-1.13		3.6-19.5	5.5-38.2	
Rodia Lagoon	Mean	No vegetation[4]		37	36	124	72	231	
Tsoukalio Lagoon	Mean	Angiosperms[4]		31	26	131	76	274	
Logarou Lagoon	Mean	No vegetation[4]		44	25	221	105	302	
Tsopeli Lagoon	Mean	Macroalgae-Angiosperms[4]		48	26	168	100	295	
Messolonghi Lagoon	Mean	No vegetation[5]		23	12	80	60	73	
Kavala Bay	Mean	No vegetation[6]		16-154	30-670	22-33	67-345	105-168	

Cluster analysis

Cluster analysis for total heavy metal concentrations classified sampling sites in three groups (Fig. 2). The first group is comprised the sites A2 and B2, the second the site B1 and the third the site A1. This result indicates the influence of natural sources through weathering processes reaching the lagoon through fresh water sources and the fisherman activities in the distribution of heavy metals at sites A2, B2 and A1, respectively. The site B1 differs from the other sites due to distance from fresh water sources and fisherman activities.

Table 3. Spearman Rank Order Correlations between heavy metals and organic matter (OM). Marked (bold) correlations are significant at $p<0.05$.

	Cu	Pb	Ni	Zn	Cr	Hg	OM
Cu	1.00						
Pb	**0.63**	1.00					
Ni	0.38	0.15	1.00				
Zn	**0.69**	0.26	**0.59**	1.00			
Cr	**0.62**	0.09	0.57	**0.77**	1.00		
Hg	0.48	0.48	0.13	0.48	0.04	1.00	
OM	**0.80**	0.40	0.34	**0.59**	**0.61**	0.22	1.00

Enrichment factors

The enrichment factor r_{ef} values (Tab. 4) were except Pb below of unity. The enrichment factor r_{ef} values for Pb were 1.13 for the related to fisherman activities site A1 and 2.67 for the related to freshwater and limited urban sewages site A2. Lead can be considered as indicator of urban metal pollution and used for evaluating the degree of pollution of surface lagoon sediments. As Hg was only detected at surface sediments can not be calculated. The total enrichment factor R values shown in Table 4 were below unity indicating low degree of metal pollution.

Table 4. Values for metal enrichment factors (r_{ef}) and for total enrichment factors (R) for the sampled sites. n.d.=not determined

Site	$r_{ef\ Cu}$	$r_{ef\ Pb}$	$r_{ef\ Ni}$	$r_{ef\ Zn}$	$r_{ef\ Cr}$	$r_{ef\ Hg}$	R
A1	-0.50	1.13	0.00	0.10	-0.09	n.d.	0.25
A2	0.00	2.67	-0.50	0.00	-0.04	n.d.	0.53
B1	0.50	-0.50	-0.13	0.14	-0.09	n.d.	0.13
B2	0.00	-0.50	-0.63	-0.13	-0.14	n.d.	0

Organic matter and grain size distribution

Organic matter contents and grain size distribution are summarized in Table 1. Organic matter shows in general low values around the mean content of 1.98%, with the highest score (3.31%) measured at site B2 in the middle layer of the sediment. Much higher organic matter contents (6-9%) were measured at the deeper sites of the adjoining ecosystem of the Gulf of Kavala (Stamatis et al, 2006).

The increased organic matter content (3.31%) at one site probably reflects the long-standing

deposition of effluents, coming from aggravating fresh water forwarded into the lagoonal area or decay of plant material produced there. First hypothesis is supported by significant correlation of organic matter with Cu (r=0.80), Zn (r=0.59) and Cr (r=0.61).
Fraction A of this study was mostly abundant at site B2 in all the slices, and especially in the upper layer (23.9%). Lower mud percents were observed at site A2 in all the slices, and especially in the dipper layer (4.6%).

Heavy metals and habitat types
Correlations among sampling sites based on heavy metal concentrations (Fig. 2) along with mean total concentrations (Tab. 2) showed that heavy metals may not be the determinant factor for the habitat shift from *Ruppia* to *Ulva*. However, higher metal concentrations were detected at site A1 (Tab. 1, 2) and the enrichments factor (r_{ef}) was higher than unity at site A2 (Tab. 4), where the *Ulva* dominated. Hydrographic changes should be also taken under consideration. The system of mesh frames used for aquaculture proposes can operate as barriers for water exchanges between the two habitat types as well as between the *Ulva* habitat type and the sea causing water stratification and high nutrient concentrations in water and in sediment (Orfanidis et al, 2007), that finally may contribute to the formation of *Ulva* macroalgal blooms (Sfriso et al, 1992; Schramm, 1999).

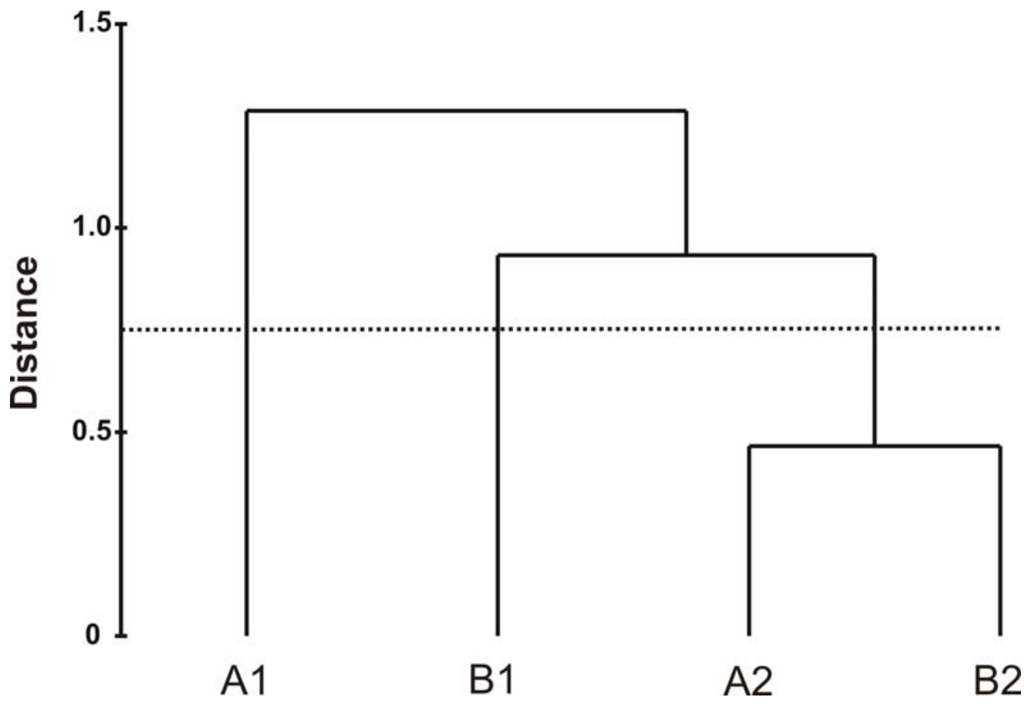

Figure 2. Euclidean distance cluster analysis based on mean heavy metal concentrations of the studied sites.

Conclusions

Sediment of *Ruppia* habitat type of Agiasma lagoon can be considered as a base line for brackish to saline lagoons of eastern Mediterranean Sea. The detected heavy metal surcharge in sites dominated by *Ulva* stands could be attributed to local, e.g. fisherman, as well as to watershed, e.g. fresh waters, urban sewages, activities.

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