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Biomonitoring of trace metals (Cu, Cd, Cr, Hg, Pb, Zn) in Mali Ston Bay (eastern Adriatic) using the Mediterranean blue mussel (1998-2005)

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In Mali Ston Bay, located on the eastern Adriatic coast, mussels and oysters have traditionally been cultivated. However, previous studies showed that water and sediments from Mali Ston Bay contain elevated levels of some trace metals, which was attributed to natural processes of sedimentation and drainage. In order to assess the bioavailability of trace metals in the bay, and to assess the health risk of consumption of shellfish species from Mali Ston Bay, a passive monitoring program was carried out in the period from December 1998 to August 2005. Samples of the whole soft tissue were collected seasonally at 4 shellfish breeding farms and analyzed for essential (Cu, Zn) and non-essential (Cd, Cr, THg, Pb) metals. Mean concentrations of analyzed metals in the edible tissue of mussels (Cd: 1.15 mg kg^{-1} d.wt.; Cr: 1.65 mg kg^{-1} d.wt.; Cu: 5.6 mg kg^{-1} d.wt.; THg: 0.15 mg kg^{-1} d.wt.; Pb: 1.09 mg kg^{-1} d.wt.; Zn: 139 mg kg^{-1} d.wt.) fell in the range of values usually found in low to moderately contaminated marine coastal areas. Examination of the spatial pattern of contaminants showed that there were no statistically significant differences among stations in Mali Ston Bay regarding trace metal content. Concentrations of Cd, Cu, Cr and THg were significantly higher during the autumn-winter period, while concentrations of Pb and Zn did not depend on the sampling season. Among six analyzed metals, only concentrations of Cd and THg were significantly negatively correlated with the condition index of the mussels. Analysis of temporal trends during the 7 years of monitoring showed that metal concentrations had not changed with time. According to EU and WHO legislation, consumption of the edible tissue of the mussels was not harmful for humans since all the values were well below the permissible limits for fresh seafood. In addition, results of the evaluation of the risks to human health associated with consumption of the mussels containing trace metals suggest that there is no health risk for moderate shellfish consumers.

Key words: Adriatic Sea, biomonitoring, heavy metals, mussels, trends

INTRODUCTION

Trace metals are serious pollutants of the marine ecosystem (FÖRSTNER & WITTMANN, 1979). Non-essential metals (e.g. Pb, Cd, Hg) are held to be the most dangerous, since continuous exposure of marine organisms to low concentrations may result in bioaccumulation, and subsequent transfer to man through the food web. Estuaries and coastal zones are the most endangered as they are exposed to the largest concentrations of these contaminants (FÖRSTNER & WITTMANN, 1979).

Although Mali Ston Bay is an important bivalve mariculture area and one of the most studied areas in the Adriatic Sea, very little is known about the present situation in this area regarding trace metal contamination. The last comprehensive research of the ecological properties in Mali Ston Bay was performed 20 years ago (ROGLIĆ & MEŠTROV, 1981). Most of the studies in this and the following research were focused on hydrographical (e.g. VUČAK *et al.*, 1981; VUKADIN, 1981) and hydro-geological characteristics of the area (e.g. BAHUN, 1981), or phytoplankton and zooplankton communities (e.g. MARASOVIĆ & PUCHER-PETKOVIĆ, 1981; VILIČIĆ *et al.*, 1994). Rare studies of trace metal distribution in different environmental compartments in this area (VUKADIN, 1989; VUKADIN *et al.*, 1994; VUKADIN *et al.*, 1984) indicated that water and sediments from Mali Ston Bay contained elevated levels of some trace metals in comparison to the open sea. Unfortunately, even though the meat of oysters and blue mussels cultivated in the bay is an important component of the diet in the coastal region as well as an important export item, after pioneering works of VUKADIN *et al.* (1984) and VUKADIN (1989), who analyzed trace metal content in three fish species, there have been no follow-up systematic studies on trace metals content in mussels.

In this respect, the goal of the present study was to analyze spatial and temporal variations of selected trace metals (Cd, Cr, Cu, THg, Pb, Zn) in the edible tissue of mussels from Mali Ston bay used for human consumption, in order to estimate the health risk for seafood consumers. In addition, the collected data were analyzed to detect possible temporal trends in trace metal concentrations during a 7 year period.

MATERIAL AND METHODS

Study area

Mali Ston Bay is a scarcely inhabited, closed and well-indented area (Fig. 1) which is under a strong influence of the surrounding land. The continental area close to the coast is composed of a high permeable limestone mass, which has specific hydro-geological characteristics (BAHUN, 1981). The groundwater drainage towards the sea, and the underground connections between swallow holes and submarine springs are intensive, especially during the rainy periods. Due to open drainage, the relative underground flow speed is up to 5 cm s^{-1}, leaving almost no possibility for natural filtration of water (BAHUN, 1981). Consequently, any kind of underground pollution would inevitably cause contamination of springs and surrounding sea water (BAHUN, 1981). Mali Ston Bay extends towards the northwest where there is considerable freshwater influx from the Neretva River. Sediments in the study area are mainly muddy. This type of sediment has an extraordinary absorption capacity of trace elements from the water column (VUKADIN, 1989; VUKADIN *et al.*, 1984). During the winter, in homogenous conditions, the estuarine type of circulation prevails in the bay. The only exceptions are meteorological situations with northerly winds forcing the surface water to enter the bay (VUČAK *et al.*, 1981). During the summer both ingoing and outgoing surface currents are possible, depending mainly on the wind direction (VUČAK *et al.*, 1981). According to hydrographic conditions and phytoplankton photosynthetic rate, Mali Ston Bay belongs to highly productive Adriatic coastal ecosystems (MARASOVIĆ & PUCHER-PETKOVIĆ, 1981). Based on the phytoplankton biomass, Mali Ston Bay belongs to moderately eutrophicated and ecologically stable ecosystems (VILIČIĆ *et al.*, 1994).

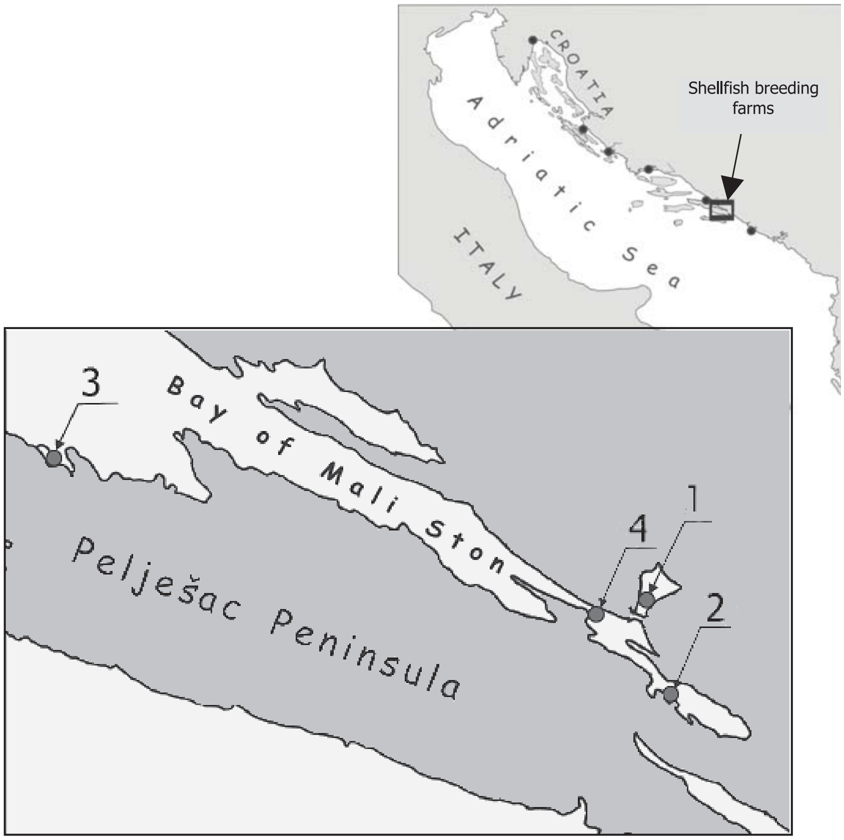

Fig. 1. Sampling stations in Mali Ston Bay

Sampling of mussels

Samples of mussels for heavy metal analysis were collected from natural populations at 4 shellfish breeding farms in Mali Ston Bay (Fig. 1). Samples were collected four times a year (in March, June, August and December) in the period from December 1998 to August 2005. At every site, water temperature and salinity were recorded. Mussels were collected from a plastic/fibreglass boat by hand. Twenty individuals of similar shell length were collected at each sampling site, placed in plastic bags, and transported to the laboratory. According to workers on shellfish farms, the average age of all analyzed mussels is 2-2.5 years (personal communication). Mussels were scrubbed with a brush to remove adhering detritus, washed with seawater and dissected in the laboratory (BERNHARD, 1976). The gut content of mussels was not depurated prior to analysis, although this could influence the results (COSSA, 1989; ROBINSON *et al*., 1993). Dissected tissues were weighed, and the shell length and weight of each specimen were measured. After dissection, composite samples of mussel tissue were freeze-dried and homogenized. Water content in soft tissues varied between 84.96 and 90.23%. The condition index (CI) of mussels was calculated as the ratio between dry weight of the whole soft tissue (g) and the shell length (cm) of mussels.

Temperature, salinity, pH and dissolved oxygen were measured at all stations during the experiment. Dissolved oxygen was determined using the Winkler method (GRASSHOFF, 1976), while temperature, pH and salinity were determined by CTD probe.

Determination of Cu, Zn, Cd, Cr, Mn and Pb

Mussel tissues for trace metal analysis (approximately 0.12 g of dry homogenized tissue) were digested with a mixture of concentrated HNO_3 (65% Merck, Suprapur) and H_2O_2

(30% Merck, Suprapur) in a High Microwave Digestion System (ODŽAK *et al.*, 2001). Prior to decomposition, samples with added reagents were allowed to digest at room temperature in loosely capped Teflon beakers for at least 1h, in order to remove the excess of nitrous-oxide vapors. Digested samples were diluted to 25 ml with 1.0% HNO_3.

The analysis of Cd, Cr and Pb were performed using Graphite Furnace Atomic Absorption Spectrometry (Perkin-Elmer, Analyst 800, with Zeeman background correction). Zinc and copper concentrations were determined using Flame Atomic Absorption Spectrometry on the same instrument. All results are reported on a dry weight basis. The accuracy of the applied analytical procedure for the determination of trace elements in mussels was tested using SRM 2976 (Mussel homogenate; NIST) certified reference material. The obtained results showed very good compliance with the referred values (Table 1).

Table 1. Trace metal concentrations in standard reference material SRM 2976 (values are expressed as mg kg^{-1} dry wt. ± 1 SD)

	Mussel homogenate SRM 2976 (mg kg^{-1} dry wt.)	
	certified	obtained
Cd	0.82 ± 0.16	1.00 ± 0.11
Cu	4.02 ± 0.33	4.25 ± 0.37
Cr	0.50 ± 0.16	0.45 ± 0.11
HgT	0.0610 ± 0.0036	0.0604 ± 0.0026
Zn	137 ± 13	145 ± 7
Pb	1.19 ± 0.18	1.16 ± 0.05

Determination of total mercury

Approximately 0.1-0.2 g of lyophilized and homogenized tissue was weighed directly in a Teflon digestion vessel and, after the addition of 4 cm^3 of conc. HNO_3 (low Hg), the vessel was closed and the mixture was left to react at room temperature for 1 hour. Digestion was finished by heating in an Al-block at 90 °C overnight on a hot plate. After the digestion, samples were left to cool to room temperature and diluted to the mark (V=25.80 cm^3) with Mili-Q water (HORVAT *et al.*, 1991).

Total mercury concentrations were measured using cold vapor atomic absorption spectrometry (CV-AAS) on a Perkin Elmer Flow Injection Mercury System (FIMS-100), after reduction with $SnCl_2$. A detailed description of the method has been given elsewhere (HORVAT *et al.*, 1991). The accuracy of the applied analytical procedure for the determination of total mercury was tested using the certified reference material SRM 2976 (Mussel homogenate; NIST). The obtained results showed good compliance with the referred values (Table 1).

Statistical analysis

Regression analysis was used to examine metal vs. metal and metal vs. condition index correlations in tissues. In all cases, the level of significance was set at $P<0.05$. Spatial patterns of trace metals were investigated using both the Kruskall-Wallis non-parametric test for independent samples (AMBROSE & PECHAM AMBROSE, 1995) and principal component analysis (PCA).

RESULTS AND DISCUSSION

Field data

Metal levels in tissues represent a time-integrated response to bio-available metal in food and water (PHILLIPS & RAINBOW, 1993). The resulting bioaccumulation is influenced by numerous environmental (salinity, temperature, dissolved oxygen, pH, dissolved organic carbon, food availability, etc.) and biological factors (seasonal growth cycles, size, sex, sexual maturation, reproduction stages, etc.) (COSSA, 1989; PHILLIPS & RAINBOW, 1993; KRAMER, 1994).

a) Hydrographic parameters

Results of measurement of the main physical and chemical parameters (Table 2) in the period from December 1998 to August 2005 showed that waters at all 4 stations were well mixed and oxygenated (4.00-9.06 ml O_2 dm^{-3} in surface layer; 4.46-8.64 ml O_2 dm^{-3} in the 7 m deep layer). Temperatures varied seasonally between 7.60 and 27.40 ºC in the surface layer, and between 10.10 and 27.20 ºC in the bottom layer. Thermal stratification was not recorded probably due to shallowness of this area. In the surface layer, salinity varied from 19.80 to 38.10, with the highest variations recorded at station 4. In the bottom layer variations of salinity were less pronounced (31.90-39.00).

Temporal changes of salinity in the surface layer are the consequence of the prevailing circulation pattern of waters in the bay (VUČAK *et al*., 1981). Namely, during the winter an estuarine type of circulation prevails in the bay - less saline surface waters which are leaving the bay in the surface layer are replaced with more saline sea water entering the bay in the bottom layer (VUČAK *et al*., 1981). During the summer both incoming and outgoing surface currents are possible, depending mainly on the wind direction (VUČAK *et al*., 1981).

b) Mussels biometry

The length of mussels varied from 5.9 to 7.9 cm during the monitoring period, with an average coefficient of variation of 6.05% (Table 3). The condition index of mussels, which provides information on somatic growth and induction of reproductive cycle, varied significantly during the experimental period depending on the sampling location (Table 3). The highest variations were recorded at station 2 (CV=30.3%), and the lowest at station 4 (CV=16.8%). Observed fluctuations in condition index can be attributed to seasonal changes and associated nutritional and reproductive states (PHILLIPS & RAINBOW, 1993; KRAMER, 1994). Differences between stations indicate that the environmental conditions at those stations were somewhat different. Although mussels at all stations were in a similar nutritional state (similar CI) owing to the high availability of organically rich suspended particulate matter in the bay, the spawning at these stations (indicated by the decline of the condition index) occurred in different periods.

Table 2. Important hydrographic parameters of surface and bottom waters at 4 stations in Mali Ston Bay for the period December 1998 to August 2005

Station		S1		S2		S3		S4	
		0 m	5 m	0 m	5 m	0 m	5 m	0 m	5 m
Temperature (°C)	Min	11.30	12.10	9.00	10.10	10.20	11.30	7.60	11.50
	Max	27.20	27.20	26.50	26.50	27.40	26.10	26.90	26.80
	Mean	17.37	17.26	16.93	17.40	17.04	17.10	16.76	17.24
	St. dev.	5.37	4.36	5.81	5.17	5.23	4.12	5.43	4.45
Salinity	Min	26.70	32.10	29.40	34.60	22.90	31.90	19.80	32.20
	Max	37.60	39.00	37.40	38.00	38.10	38.80	37.80	38.60
	Mean	34.70	37.28	34.90	36.36	32.95	37.05	34.75	36.90
	St. dev.	2.51	1.37	2.14	0.84	3.98	1.51	3.46	1.47
Oxygen (ml l^{-1})	Min	4.57	4.51	4.51	4.46	4.00	4.79	4.57	4.59
	Max	9.06	8.64	7.00	8.19	8.30	7.75	7.53	6.39
	Mean	6.04	5.89	5.79	5.78	5.91	5.83	5.79	5.45
	St. dev.	1.05	1.11	0.65	0.76	0.85	0.68	0.72	0.51

Table 3. Mean length (cm), soft tissue body weight (g d.wt.) and condition index (g d.wt./cm) of mussels collected at 4 shellfish breeding farms in Mali Ston Bay, in the period from December 1998 to August 2005

	Length (cm)				Soft tissue weight (g d.wt.)				Condition index (g d.wt./cm)			
Station	1	2	3	4	1	2	3	4	1	2	3	4
Dec-98	6.84	6.84	7.06	6.97	4.11	1.88	4.16	5.07	0.097	0.038	0.080	0.117
Mar-99	6.96	7.20	6.85	7.33	5.20	5.05	4.00	7.04	0.110	0.112	0.083	0.147
May-99	7.15	7.31	6.75	7.88	5.31	5.85	3.45	6.88	0.121	0.136	0.075	0.137
Aug-99	6.94	6.96	7.33	6.04	6.25	5.77	5.85	4.11	0.133	0.166	0.137	0.093
Dec-99	7.02	6.90	6.25	7.18	6.11	4.56	4.55	5.81	0.143	0.093	0.099	0.126
Mar-00	7.22	7.03	6.68	6.96	7.00	5.25	5.51	5.97	0.132	0.102	0.112	0.117
May-00	6.39	6.17	6.12	6.00	5.00	5.04	3.64	4.54	0.107	0.111	0.081	0.103
Aug-00		7.56	7.32	5.94		7.72	5.82	4.19		0.139	0.108	0.096
Dec-00	6.19	6.83	6.18	6.85	2.89	5.40	4.14	6.44		0.108	0.091	0.128
Mar-01	6.47	7.22	6.42	6.66	4.99	5.71	3.86	5.73	0.104	0.074	0.067	0.103
May-01	6.57	7.45	7.12	6.66	5.46	6.64	4.97	5.45	0.141	0.117	0.103	0.109
Aug-01	7.26	7.43	7.04	7.12	5.04	5.22	4.37	5.39	0.100	0.045	0.092	0.127
Dec-01	6.49	7.66	6.72	6.67	4.44	6.38	3.63	6.51	0.072	0.092	0.058	0.118
Mar-02		6.22	4.11	6.88	2.75	2.99		4.06	0.081	0.099	0.093	0.126
May-02	6.19	6.99	4.44	7.09	2.89	5.78		5.43	0.063	0.123	0.102	0.110
Aug-02												
Dec-02												
Mar-03	6.56	7.48	7.03	6.47	4.06	5.05	5.32	4.79	0.066	0.090	0.078	0.132
May-03												
Aug-03												
Dec-03	6.91	7.28	6.94	7.04	3.81	4.81	4.05	4.40	0.062	0.085	0.056	0.076
Mar-04	6.37	6.97	6.91	6.50	4.03	3.97	4.73	4.93	0.085	0.053	0.081	0.110
May-04	6.07	6.92	6.39	6.66	3.89	4.63	4.18	4.34	0.132	0.110	0.126	0.111
Aug-04	6.93	7.13	6.57	6.42	5.11	5.13	4.26	4.13	0.135	0.119	0.103	0.105
Dec-04	6.59	6.59	6.80	6.62	4.28	4.07	4.31	3.57	0.112	0.092	0.109	0.083
Mar-05	7.30		6.82	6.17	6.05		4.64	4.59	0.135		0.096	0.122
May-05	6.79	6.64	7.19	6.71	4.46	3.53	4.26	4.00	0.092	0.087	0.090	0.084
Aug-05	7.08	6.78	6.36	6.25	5.10	3.85	4.03	3.32	0.112	0.077	0.103	0.082
Min	6.07	6.17	4.11	5.94	2.75	1.88	3.45	3.32	0.06	0.04	0.06	0.08
Max	7.30	7.66	7.33	7.88	7.00	7.72	5.85	7.04	0.14	0.17	0.14	0.15
Median	6.82	6.99	6.78	6.67	4.99	5.05	4.26	4.86	0.11	0.10	0.09	0.11
Mean	6.74	7.02	6.56	6.71	4.71	4.97	4.44	5.03	0.11	0.10	0.09	0.11
SD	0.37	0.39	0.79	0.46	1.10	1.25	0.68	1.04	0.03	0.03	0.02	0.02
RSD (%)	5.48	5.54	11.98	6.81	23.37	25.10	15.27	20.69	24.73	30.28	21.00	16.82

Metal concentrations

Mean concentrations of trace metals in whole soft tissue of natural mussels from 4 shellfish breeding farms in Mali Ston Bay, collected in the period from December 1998 to August 2005, are given in Table 4. All data were found to be log normal distributed.

Examination of the spatial patterns of trace metals using both the Kruskall-Wallis non-parametric test for independent samples (AMBROSE & PECHAM AMBROSE, 1995) and principal component analysis (POPHAM & DAURIA, 1983) showed that there were no statistically significant differences among stations in Mali Ston Bay regarding trace metal content. The highest variations of values during 7 years of monitoring were recorded for lead (56%) and the lowest for copper (27%). Values obtained for Cd, Cr and Zn in the mussels from Mali Ston Bay (Table 4) are similar to or even higher than the average values usually found in more urbanized areas on the Croatian coast (KLJAKOVIĆ-GAŠPIĆ *et al.*, 2006). Our results provide evidence that even the mussels from areas with no known point sources of contamination may have measurable body burdens of some metals, especially cadmium. This is probably due to the processes of natural weathering in the hinterland, and supply by rivers and submarine springs (VUKADIN *et al.*, 1984). However, this topic could not be explored in detail since the potential source area is located on the territory of the neighboring state. Unlike Cd, Cr and Zn, concentrations of copper, total mercury and lead in mussels from all shellfish-breeding farms (Table 4) were substantially lower than the average values usually found at 'hot spots' located on the Croatian coast (KLJAKOVIĆ-GAŠPIĆ *et al.*, 2006). More detailed comparison of data obtained in this work (Table 4) with previously published values for the Mediterranean and Europe (Table 5) showed that the obtained concentration ranges of analyzed metals in mussels from Mali Ston Bay are not seriously elevated. In fact, they fall in the range of values usually found in lowly contaminated marine coastal areas.

O'CONNOR & BELIAEFF (1995) summarized National Status & Trends (NS&T) data on trace metal concentrations in mussels using geometric means and geometric means plus one standard deviation to define 'high' concentrations. That assumption was based on the log normal nature of the data distributions and the fact that the concentration range beyond one standard deviation from the mean contained about 17% of the data. According to CANTILLO (1998), a simpler way to summarize data is to use medians and 85th percentiles, which does not require the assumption that the data should be log normal. Comparison of the data of three long term Mussel Watch programs (CANTILLO, 1998) showed that even in the absence of human activity there would be measurable concentrations of trace elements in mussels. CANTILLO (1998) suggested that the lowest of the 85th percentiles from the

Table 4. Mean trace metal content (mg kg^{-1} d.wt.) and other important statistical parameters for mussels collected at 4 shellfish breeding farms in Mali Ston By, in the period from December 1998 to August 2005

	Valid N	Mean	Std. Dev.	Median	Minimum	Maximum	Lower Quartile (25%)	Upper Quartile (75%)	85% Percentile	CV(%)
Cd	85	1.15	0.453	1.03	0.39	2.4	0.82	1.45	1.56	39.4
Cr	82	1.65	0.748	1.52	0.41	4.61	1.16	2.05	2.33	45.4
Cu	85	5.61	1.537	5.35	1.98	10.98	4.63	6.33	7.08	27.4
HgT	39	0.15	0.054	0.13	0.08	0.28	0.12	0.18	0.22	35.7
Pb	85	1.09	0.608	0.91	0.24	3.69	0.71	1.22	1.58	55.8
Zn	85	138.76	53.76	127.11	49.36	418.3	106.4	166.56	181.83	38.7

Table 5. Overview of trace metal concentrations in the whole soft tissue of Mytilus species collected in European coastal waters; REF-reference, SP-species. All the values are expressed in mg kg^{-1} d.wt. If necessary, values originally expressed on wet weight were recalculated to dry weight by multiplying the original values with average conversion factor=6

AREA	REF	SP	Cd	Cu	Cr	THg	Pb	Zn
Gdansk Bay, Poland	RAINBOW *et al.*, 2000	MT	2.9-8.3	8.2-14.6			7.6-36	77-276
The Bay of Piran, N Adriatic	KOSTA *et al.*, 1978	MG	0.6-0.9	6.5-7.6		0.08-0.1		102-108
Portugal. southern coast	BEBIANNO & MACHADO, 1997	MG	1.2-3.8	4.3-7.2				165-545
NW Mediterranean	FOWLER & OREGIONI, 1976	MG	0.4-5.9	2.4-154	0.5-28.8	0.18-0.96	2.7-117	97-644
Mediterranean	JEFTIĆ *et al.*, 1990	MG		0.4-36 (6-9.1)				19-586 (84-240)
Kaštela Bay, Adriatic	VUKADIN *et al.*, 1995	MG	0.4-0.5	2.6-4.1		1.08-2.52		93-105
Lim channel, Croatia	MARTINČIĆ *et al.*, 1984	MG	1	11.5			1.37	149
Eastern Adriatic coast	MARTINČIĆ *et al.*, 1987	MG	0.7-1.7	4.2-17.7		0.08-0.20	1.2-8.3	109-189
Krka river estuary, Adriatic	MARTINČIĆ *et al.*, 1992	MG	0.8-2.3	4.6-7.7			0.5-4.1	124-269
Eastern Adriatic coast	KLJAKOVIĆ-GAŠPIĆ *et al.*, 2006	MG	0.2-2.0	3.1-60	0.3-5.4	0.04-8.58	0.9-15.1	44-387
French coast	CLAISSE, 1989	MG				0.04-0.49		
Kaštela Bay, Adriatic	MIKAC *et al.*, 1985	MG				0.3-79.7		
Krka river estuary, Adriatic	MIKAC *et al.*, 1989	MG				0.07-0.35		
Thermaikos gulf, Greece	CATSIKI *et al.*, 2001	MG	0.74-1.37	4.1-6.9	0.9-9.3		0.6-2.7	50-115
Mali Ston Bay	This work	MG	0.4-2.4	1.98-11	0.41-4.6	0.08-0.28	0.24-3.7	49-418

MT–*Mytilus trossulus* MG– *Mytilus galloprovincialis*

Table 6. Comparison of trace metal concentrations in mussels from Mali Ston Bay with concentrations of trace metals in mussels that are indicative of contamination (in mg kg^{-1} d.wt.), as suggested by CANTILLO (1998)

Metal	85-th percentile concentration from NS&T (mg kg^{-1} d.wt.)	Trace metal concentrations in mussels from Mali Ston Bay (mg kg^{-1} d.wt.)		Percentage of our data >85-th percentile from NS&T (%)
		Range	85th percentile	
Cd	3.7	0.39-2.4	1.56	-
Cr	2.5	0.41-4.61	2.33	8.5
Cu	10	1.98-10.98	7.08	2.4
Hg	0.23	0.08-0.28	0.22	12.8
Pb	3.2	0.24-3.69	1.58	1.2
Zn	200	49-418	182	8.2

NS&T or RNO Mussel Watch programs could be used as indicative of contamination (Table 6).

Although CANTILLO (1998) was aware that there are mussels with lower concentrations that are contaminated and conversely, that there are sites where local mineralogy causes mussels to have higher concentrations of some elements and are not contaminated, he suggested that this value, as a first approximation, can be used for classifying concentrations reported by monitoring programs from around the world. Results of the comparison of our data with the NS&T data (Table 6) showed that all the data for cadmium, almost all data for Pb and Cu, and more than 85% of the data for the other three metals were lower than the NS&T 'threshold' values. Chromium and zinc values higher than the 'threshold' values were recorded in the winter period (December) (Fig. 2), probably owing to local hydrology and intensive leaching of the rocks in surrounding area during the winter (rain, storms).

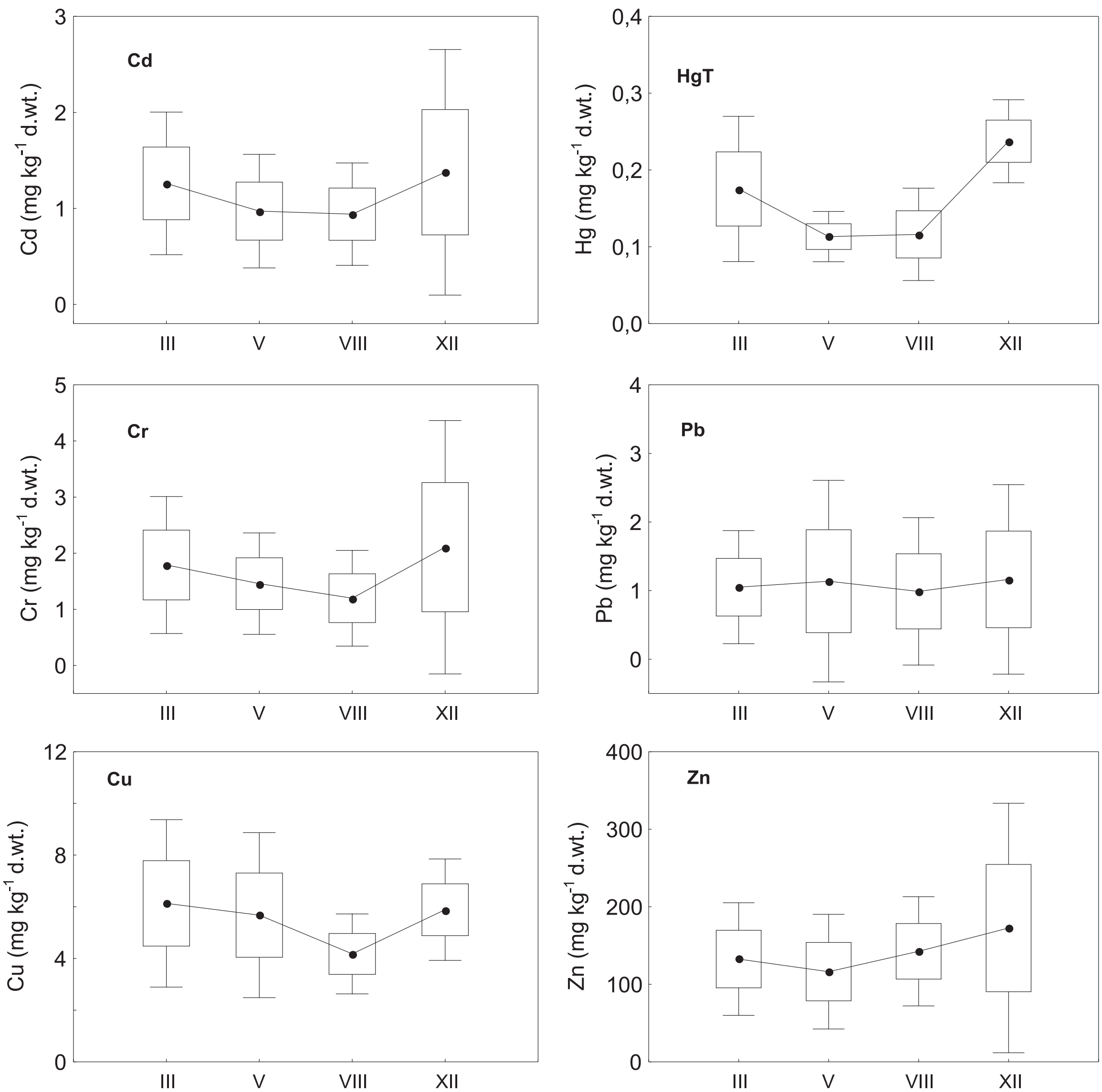

Fig. 2. Seasonal variations of the average trace metal content in the whole soft tissue of mussels collected at 4 shellfish breeding farms in Mali Ston Bay

Seasonal differences

Concentrations of trace metals in the whole soft tissue of mussels varied from 22% (Cu, station 4) to 64% (Pb, station 3) during the monitoring period. According to seasonal patterns (Fig. 2) and element-element correlations (Table 7), the analyzed metals could be divided into two groups. The first group includes elements such as Pb and Zn whose concentrations did not depend on the sampling season (Fig. 2). Unlike Pb and Zn, concentrations of elements forming the second group (Cd, Cu, Cr and Hg) were significantly higher during the autumn-winter period in comparison to the spring-summer period (Fig. 2).

It is well documented that trace metal concentrations in mussels depend on numerous environmental and biological factors, which were summarized in detail by KRAMER (1994) and COSSA (1989). Comparison of the trace metals content in mussels with their condition index has shown that only concentrations of Cd and Hg are significantly negatively correlated with the condition index of mussels (Table 7). This is probably due to the well known effect of 'dilution' of trace metal concentration due to enlargement of the body weight (COSSA, 1989; PHILLIPS & RAINBOW, 1993). Also, we found a significant positive correlation between cadmium and mercury ($r=0.64$; $p>0.05$) (Table 7), which indicates that the spatial distributions of this pair of metals is regulated by similar input and removal processes. Concentrations of other metals in the whole soft tissue were generally negatively correlated with changes in the condition index, although no clear seasonal trend could be established.

When speaking about environmental factors, numerous processes (e.g. freshwater runoff, particulate matter re-suspension, primary production) can affect the bioavailability of trace metals in Mali Ston Bay. Many of these processes are highly variable on monthly and even daily scales. Among different environmental factors, over-land drainage is probably outstanding since the main input of contaminants into the Croatian coastal area is known to take place via fluvial or ground water discharges (KUŠPILIĆ, 2005). Although average river and ground water flows are low during the entire year, in autumn and winter significant increase of maximum flows occurs. Consequently, groundwater drainage towards the sea in the area of Mali Ston Bay is especially intense during the rainy periods. Due to open drainage, the relative underground flow speed is high, leaving almost no possibility for natural filtration of water (BAHUN, 1981). That is why these periods of high flows are also periods of intensive pollution transport to the sea. Given variations in natural sources of metals and natural factors affecting their accumulation in mussels, the high variations of results in Mali Ston Bay were to be expected.

Table 7. Coefficients of linear correlations (r) between trace metals concentration and condition index (N=85 except for mercury where N=40)

	Cd	Cr	Cu	HgT	Pb	Zn	C.I.
Cd		0.23*	0.22*	0.64*	0.09	0.26*	-0.40*
Cr			0.21	0.41*	0.28*	0.27*	-0.09
Cu				0.25	0.22*	0.01	-0.11
HgT					0.05	0.46*	-0.60*
Pb						0.30*	-0.02
Zn							-0.16
C.I.							

*statistically important correlations

Risk assessment

When considering the trace metal content in organisms suitable for human consumption, the most important aspect is their toxicity to humans. Similar to many other Mediterranean and European countries, Croatian law defines legal limits on the permissible concentrations for only a few metals and metalloids in fresh seafood, mainly the most toxic ones (Table 8). For those elements whose concentrations are regulated by law (Cd, Cr, THg, Pb), we found out that all the values, when expressed on a wet weight basis, were significantly lower than the maximum permissible levels (MPL) for trace metal content in fresh shellfish (ANONYMOUS, 1994, ANONYMOUS, 2001).

Similar to FUNG *et al.* (2004), environmental health risk in this study was assessed by a comparison between environmental status (represented by the concentrations of trace metals in mussels) and 'threshold' values which may cause adverse effects in human consumers. Risk quotient (RQ) was calculated as the ratio between concentration of trace metal in the mussels and the level of concern (LOC) for that metal (FUNG *et al.*, 2004). A level of concern (LOC), which is a 'threshold concentration' of a chemical above which a hazard to human health may exist, was calculated as the ratio of Tolerable Daily Intake (TDI) and the Rate of Shellfish Consumption (RSC) (FUNG *et al.*, 2004). For the purpose of this calculation, it was assumed that total trace metal exposure was derived solely from shellfish.

Table 8. Preview of the Maximum Permissible Levels of metals and non-metals (mg kg^{-1} w.wt.) in fresh shellfish in Croatia and the European Union

Metal	Pb	Cd	Hg	MeHg	As
Croatia[a]	1	1	1	0.8	8
EC[b]	1.5	1	0.5		

[a] *ANONYMOUS, 1994*

[b] *ANONYMOUS, 2001*

Data on average national rate of shellfish consumption (RSC) were provided by the Central Bureau of Statistics of the Republic of Croatia (http://www.dsz.hr). The rates of the consumption of shellfish for the Croatian population vary between 2.4-3.4 g/person/day (statistical data for 2003-2005 period). In this study, the highest available consumption rate of 3.4 g/person/day was used to calculate the level of concern for the average shellfish consumption group. However, it should be pointed out that exposure estimates for trace metal intake from shellfish consumption based on national average shellfish consumption data may not be suitable for estimating exposures of particular subpopulations or individuals residing in specific regions of the country, such as islands, where more than 77% of the families consume 3-7 meals of seafood per week (BUZINA *et al.*, 1989).

In the absence of health criteria in Croatia, we used Tolerable Levels of Intake (TDI) and Estimated Safe and Adequate range of Daily Dietary Intake Levels (ESAADI) for heavy metals provided either by the US Food and Drug Administration (http://vm.cfsan.fda.gov), FAO/WHO or the National Research Council (NRC) of the US National Academy of Sciences (NAS) to calculate the relevant level of concern for each metal.

Results of the evaluation of the risks to human health associated with consumption of the mussels containing trace metals are summarized in Table 9. The calculation of Risk Quotient for worst-case scenario (RQwcs) provides a simple way of screening chemicals that may require a more refined analysis. For cases where RQ<1 the chemicals involved are unlikely to cause harm to human consumers (FUNG *et al.*, 2004). It is noteworthy that all RQ's, were 2 (Pb) to 81 times lower than "1", suggesting that probably no health associated problems might be encountered, at least not in moderate shellfish consumers. Overall, our assessment indicates that metals may not pose a health risk to seafood consumers. It should, however, be noted that consumption data used in the assessment are based on national average consumption rates. As

Table 9. Risk analysis for the minimum and maximum concentrations of metals present in the mussel samples (in μg/g d.wt.) from Mali Ston Bay

Metal	Min. conc. (ug/g)	Max. conc. (ug/g)	TDI or ESADDI (ug/p/d)	RSC (g/p/d)	LOC (ug/g)	CLOC (g/p/d)	RQwcs	
							1 (for min. conc.)	2 (for max. conc.)
Cd	0.39	2.4	60[a]	3.34	16.5	22.9		0.146
Cr	0.41	4.61	50-200[b]	3.34	59.8	43.4	0.308	0.077
Cu	1.98	10.98	2000-3000[b]	3.34	897.7	273.2	0.018	0.012
HgT	0.08	0.28	33-43[c]	3.34	12.9	153.6	0.028	0.022
Pb	0.24	3.69	25-75[d]	3.34	22.4	20.3	0.493	0.164
Zn	49.4	418.3	5600-15000[e]	3.34	4488.3	35.9		0.093

Legend:
TDI-Tolerable Daily Intake (in μg/person/day)
ESAADI-Estimated Safe and Adequate range of Daily Dietary Intake levels (in μg/person/day) for all foods set by the National Research Council of the National Academy of Sciences of the USA
RSC-Rate of Shellfish Consumption in Croatia (g/person/day) for the year 2005 provided by the Central Bureau of Statistics of the Republic of Croatia (http://www.dsz.hr)
LOC-Level of Consumption (in μg/g)
CLOC-Consumption Level of Concern (in g/person/day)
RQwcs- Risk Quotient for worst-case scenario: 1-For lowest value of TDI or ESADDI range
2-For highest value of TDI or ESADDI range
[a]-Provisional Tolerable Daily Intake of cadmium (FAO/WHO, 2003); calculated from the PTWI for 60 kg human ($PTWI_{Cd}$=7 μg/kg body weight/week).
[b]-Estimated Safe and Adequate range of Daily Dietary Intake levels (in μg/person/day) for Cr and Cu set by the National Research Council of the National Academy of Sciences of the USA.
[c]-Provisional Tolerable Daily Intake of total mercury; set by FAO/WHO.
[d]-Provisional Tolerable Daily Intake of lead; FDA, 1993 (http://vm.cfsan.fda.gov/~frf/guid-pb.html)
[e]-Dietary reference value for zinc; WHO, 2001

seafood, particularly shellfish consumption rates can vary greatly between specific sections of a community, the numbers obtained should only be used for screening purposes. Clearly, further assessment is necessary before more a definitive conclusion can be drawn.

CONCLUSION

A comparison of data obtained in this work with previously published values for the Adriatic and the Mediterranean showed that values obtained herein fall in the range of values usually found in low to moderately polluted areas of the Mediterranean. This means that the ecosystem of the bay is not seriously polluted with trace metals. However, values obtained for Cd, Cr and Zn in the mussels from Mali Ston Bay are similar to or even higher than the average values usually found in more urbanized areas on the Croatian coast. Our results provide evidence that even the mussels from the areas with no known point sources of contamination may have measurable body burdens of some metals.

Although concentrations of some trace metals vary seasonally, analysis of temporal trends revealed that metal concentrations had not changed with time during the monitoring period of 7 years.

All the values obtained in the edible tissue of mussels, when expressed on a wet weight basis, were significantly lower than the maximum permissible levels (MPL) for fresh shellfish in Croatia and the EU, implying that consumption of their meat is not harmful for humans. In addition, the results of the evaluation of the risks to human health associated with consumption of the mussels containing trace metals suggest that there is no health risk for moderate shellfish consumers.

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Biološki monitoring metala (Cu, Cd, Cr, Hg, Pb, Zn) u Malostonskom zaljevu (1998-2005) korištenjem dagnje kao biološkog indikatora

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SAŽETAK

Malostonski zaljev je područje tradicionalnog uzgoja dagnji i kamenica. Rijetke prethodne studije su pokazale da morska voda i sedimenti iz Malostonskog zaljeva sadržavaju povišene masene udjele nekih metala, što se smatralo posljedicom prirodnih procesa ispiranja tla u okolnom području i sedimentacije. Stoga smo u razdoblju od prosinca 1998. do kolovoza 2005. proveli projekt pasivnog biološkog monitoringa dagnji u Malostonskom zaljevu s ciljem da utvrdimo biološku dostupnost tragova metala u zaljevu i sazanamo kolika je opasnost po zdravlje ljudi koji konzumiraju školjkaše iz zaljeva. U uzorcima dagnji, koji su sakupljani sezonski (4 puta godišnje) na 4 uzgajališta, određen je sadržaj esencijalnih (Cu, Zn) i neesencijalnih (Cd, Cr, THg, Pb) metala. Utvrđeno je da su prosječni maseni udjeli analiziranih metala u jestivom tkivu dagnji (Cd: 1.15 mg kg^{-1} s.m.; Cr: 1.65 mg kg^{-1} s.m.; Cu: 5.6 mg kg^{-1} s.m.; Hg: 0.15 mg kg^{-1} s.m.; Pb: 1.09 mg kg^{-1} s.m.; Zn: 139 mg kg^{-1} s.m.) u rasponu vrijednosti koje su uobičajene za nezagađena do umjereno onečišćena obalna područja Jadrana i Mediterana. Sadržaj metala u dagnjama se nije značajno razlikovao između pojedinih postaja. Maseni udjeli Cd, Cu, Cr i Hg su bili značajno viši u jesensko-zimskom razdoblju, dok udjeli Pb i Zn u školjkašima nisu značajno ovisili o sezoni uzorkovanja. Od 6 analiziranih metala jedino su maseni udjeli Cd i Hg značajno negativno ovisili o indeksu kondicije dagnji. Analiza vremenskih trendova je pokazala da se maseni udjeli metala u dagnjama nisu značajno promijenili tijekom 7 godina monitoringa. Maseni udjeli metala u dagnjama značajno su niži od maksimalno dozvoljenih koncentracija (MDK) propisanih hrvatskim i EU zakonima, pa se ovi organizmi, s obzirom na sadržaj metala, mogu bez zapreke koristiti u ishrani stanovništva. Slične rezultate dala je i studija procjene opasnosti po ljudsko zdravlje, prema kojoj nema opasnosti za zdravlje ljudi koji konzumiraju umjerene količine dagnji iz Malostonskog zaljeva.

Ključne riječi: Jadransko more, biomonitoring, metali, školjkaši, trendovi