Shellfish, especially oyster and mussel, are used as biomonitor organisms worldwide because of their ubiquity, sessile way of life, filtering mode of feeding, and relatively long biological half-life of metals in their body (Cooper et al., 1982; Presley et al., 1990; Schuhmacher and Domingo, 1996; Cantillo, 1997, 1998; Reinfelder et al., 1997; Beliaeff et al., 1998; O’Connor, 1998; Geffard et al., 2002; de Astudillo et al., 2005; Saha et al., 2006). Due to the well developed detoxification mechanisms these organisms tolerate much higher metal concentrations than the other living species, therefore in natural conditions a number of pathologial effects of metals in these organisms is relatively small (Avery et al., 1996; Rainbow, 1996; Geret et al., 2002).

In addition to the information on the level of environmental contamination, data on metal concentrations in the edible portion of oyster are of utmost importance for both the veterinary public health and the economic cost-effectiveness of farming this shellfish widely appreciated as a delicacy. The Mali Ston Bay has been known for centuries for European flat oyster (Ostrea edulis, Linnaeus 1758) farming. A planned production increase in the next decade from the present quantity of 100 tons to 5 000 tons demands detailed consideration of all potential risks.

Besides the fact that metal concentrations in water correlate positively with metal concentrations in the shellfish body, the metal tissue level and level of bioaccumulation are synergistically influenced by a number of abiotic and biotic factors. Among biotic factors, a major role is played by the shellfish age, sex, size, genetic type and physiologic condition, whereas major abiotic factors include the habitat, seawater circulation, chemical form of the metal present in water, between-metal competition, temperature, pH, dissolved oxygen, light, salinity,

**ABSTRACT**: The concentrations of cadmium, lead and zinc were determined in the soft tissue of oysters (Ostrea edulis L.,) from three locations in the Mali Ston Bay on the south-eastern coast of the Adriatic Sea. The sampling was performed during two periods of breeding cycle, in summer (age ~1 year) and winter (age ~20 months, consumption size). The concentrations of cadmium, lead and zinc measured in the oyster soft tissue showed considerable spatiotemporal variations that could be attributed to seasonal differences in the freshwater inflow which varied between the study locations. Median concentrations measured at different locations and in different time periods ranged from 0.303 to 0.969, 0.13 to 0.32 and 208.9 to 650.0 µg/g wet weight for cadmium, lead and zinc, respectively. Although metal concentrations do not exceed the maximal legally approved limits according to the legislation of the Republic of Croatia and EU, more knowledge of their spatiotemporal distribution – of cadmium in particular – would contribute to the sustainable future development of oyster farming in Croatian waters.

**Keywords**: cadmium; lead; zinc; Ostrea edulis L.
season, and degree of particular biotope contamination (Phillips, 1976; Martincic et al., 1980; Marcus and Thompson, 1986; Giordano et al., 1991; Goldberg-Bouchot et al., 1995; Hunter et al., 1995; Wang et al., 1996; O’Connor, 1998; Blackmore and Wang, 2004; Piano et al. 2004; Amiard et al., 2005; Lorenzo et al., 2005; Wang and Rainbow, 2005; Saha et al., 2006). In natural conditions, seasonal variations predominantly appear as a result of combined effects of the above-mentioned factors. They are often ascribed to changes in the freshwater inflow, changes in the metabolism rate of an organism and changes in soft tissue weight (Phillips, 1976; Cooper et al., 1982; Marcus and Thompson, 1986; Martincic, 1987; Mitra et al., 1994; Hunter et al., 1995; Morel and Koffi Koffi, 1995; O’Connor, 1998).

In this paper variations of cadmium, lead and zinc concentrations in the soft tissue of European flat oyster (Ostrea edulis L.) at three locations (Kuta, Hodilje, Brijesta) in the Mali Ston Bay during one breeding cycle are described.

**MATERIAL AND METHODS**

The Mali Ston Bay (Figure 1) is situated between the land and the Peljesac Peninsula in the southern part of the Adriatic Coast, some 60 km north-west of Dubrovnik, naturally continuing the Neretva Channel.

Oyster samples were collected from three oyster beds in the Mali Ston Bay: Kuta Cove, Hodilje and Brijesta Cove. The Kuta Cove (Figure 1) is situated in the inner end of the Bay. The nearest populated place is Mali Ston (159 citizens), a known tourist resort. The two most busy roads in this part of the Adriatic, i.e. Adriatic Highway (on the land) and the road connecting Peljesac Peninsula with the land, run along the two sides of the Bay. Hodilje (Figure 1) is a village in the inner, narrow part of the Mali Ston Bay. With 214 citizens and a small marina, Hodilje is the largest settlement in the study area. It is 4 km far from the main Peljesac road running through the central part of the peninsula. The Brijesta Cove (Figure 1) is located at the mouth of the Mali Ston Bay and is a part of the Small Sea. There are no settlements other than Brijesta (78 citizens) in the area, and the location is 8 km distant from the Peljesac road. A small oil refinery plant is located there.

One oyster bed was chosen at each of the three locations. Three clusters per bed were marked where oyster samples were collected in June 1999 and February 2000, when the oysters were aged ~12 and ~20 months (i.e. consumption size), respectively. Thirty pieces per location were sampled on each occasion. At all three locations, sampling was performed on the same day to avoid possible variations in metal concentrations due to weather conditions. The oysters collected at the three loca-

![Figure 1. Map of the investigated area (Mali Ston Bay)](image-url)
tions were packed separately and transported to the laboratory where they were frozen at ~21°C until analysis.

The metals were analyzed by atomic absorption spectrometry on an ATI Unicam 929 AAS at 228.8 nm for cadmium, 217.0 nm for lead, and 213.9 nm for zinc, with a deuterium lamp for background correction, after digestion with 3 ml 65% HNO₃ and 0.5 ml 30% H₂O₂ in a Milestone 1 200 M high performance microwave oven at 300 W for 5 min, then at 600 W for 10 min, which proved satisfactory. Working measurement standards were prepared from Titrisol solutions (Merck, Darmstadt). The Certified Standard Reference Material SRM 1566a (oyster tissue) from NIST, USA, was used to verify the procedure, yielding good agreement between the measured and verified concentrations (± 10%).

Statistics were processed by t-test and by calculating the median values which were presented graphically. Software package Statgraphic (ver. 4.0) was used.

RESULTS

Median cadmium concentrations in the edible portion of oysters were higher in older oysters (sampled in February 2000) as compared with younger ones (sampled in June 1999) at all the three locations (Figure 2). The difference was statistically significant ($P < 0.01$). In both sampling periods, the highest median cadmium concentrations were recorded in the oysters sampled from the Brijesta location, the difference from the other two study locations being statistically significant. The lowest median concentration of cadmium was found in the oysters sampled from the Hodilje location, and was statistically significantly lower as compared with the Kuta location for both sampling periods.

In Kuta, the median lead concentration was observed to decrease significantly ($P < 0.01$) between the first and second sampling period. The highest lead concentration in the oyster soft tissue was recorded in Kuta in the first sampling period and was statistically significantly higher ($P < 0.05$) than the concentrations measured at the other two locations. At the other two locations, however, the median lead concentration increased between the first and second sampling, the difference being statistically significant ($P < 0.05$) for Hodilje but not for Brijesta (Figure 3).

The highest zinc concentration was recorded in the oysters sampled at the Brijesta location in both sampling periods. The difference from the other

![Figure 2](image2.png)  
Figure 2. Median concentrations of cadmium with upper and lower quartiles in the edible part of oysters sampled at three researched locations (Kuta Cove, Hodilje and Brijesta Cove) in two different sampling seasons.

![Figure 3](image3.png)  
Figure 3. Median concentrations of lead with upper and lower quartiles in the edible part of oysters sampled at three researched locations (Kuta Cove, Hodilje and Brijesta Cove) in two different sampling seasons.
two locations was statistically significant (P< 0.01). In the first sampling period, the lowest zinc concentration was measured in the oysters collected from Hodilje, and was statistically significantly lower than the concentration measured in those from Kuta. On the second sampling, the lowest zinc concentration was recorded in the oysters from Kuta, however, the difference did not reach statistical significance (Figure 4).

DISCUSSION

In spite of the fact that the study area is relatively small, the results indicate significant spatiotemporal variations which are not equal for all three metals (Figures 2, 3 and 4). When considering these differences, it is necessary to take into account the characteristics of the Bay, properties of metals, and the fact that the metal concentration in shellfish, as sessile organisms, is a reflection of conditions in a very limited area.

The highest concentrations of cadmium and zinc were measured in the samples from Brijesta in both sampling periods (Figures 2 and 4), which could be ascribed to the strong submarine springs and vicinity of the Neretva River mouth (Caric et al., 2000). The influence of the Neretva River is manifested during the increase of its water level and wind from the west quadrant, and is strongest in the outer part of the Bay (Caric et al., 2000). It is important to mention that it rained heavily before the winter sampling and the water level of the river was extremely high.

In both sampling periods, the cadmium concentration in the oyster edible portion was higher in the samples from the Kuta Cove than in those from Hodilje (Figure 2), indicating that the submarine freshwater springs, which are quite strong in the Kuta Cove and do not exist in Hodilje, are considerably more contaminated by cadmium than by zinc. As these springs bring precipitation water (Sekulic and Branica, 1982), cadmium can well be presumed to mostly reach the area with precipitation, i.e. by wet deposition of the element from the atmosphere as well as by drainage of agricultural, urban and industrial surfaces of the area at large. Similarly, Korzeniewski and Neugebauer (1991) reported that the increased cadmium concentrations in shellfish from the coastal waters of the Gulf of Gdansk in Poland pointed to the increased deposition of the element from the atmosphere, whilst the zinc concentration pointed to the river influx of this element.

Many authors confirmed that an increased freshwater inflow from various sources, and a salinity decrease that followed it, significantly influenced the increase in cadmium and zinc concentrations in shellfish. So, Phillips (1976) reported seasonal variations in the concentrations of cadmium and zinc in mussels from the Port Phillip Bay in Australia. The fourfold summer concentrations measured in winter were ascribed to the increased freshwater influx during the winter. In laboratory conditions, Wang et al. (1996) found the influx of dissolved metal to increase in mussels 1.6 to 1.9 times for zinc, cadmium, cobalt, selenium and silver with salinity decline from 34‰ to 20‰. Investigating the level of metals in the tissue of American Crassostrea virginica oyster from three coastal lagoons in the southern part of the Bay of Mexico, Gold-Bouchot et al. (1995) found a significant negative correlation (r = –0.52) between salinity and cadmium concentration in the oyster tissue. The differences in the coefficients of correlation reported by these authors for cadmium and zinc (r = –0.32) clearly indicate that the increase in the effect of salinity decrease is more pronounced in the oyster tissue concentration of cadmium as compared to zinc. At the same time, a low positive correlation between lead and salinity was established (r = 0.03).
In addition to the role of the fresh water input which carries a certain amount of metals and influences the salinity decrease at the same time, some authors emphasize the negative correlation between the oyster body mass and the tissue level of metals, explaining it by the effect of tissue metal dilution which is expressed as a decreased metal concentration (Marcus and Thompson, 1986; Martincic, 1987; Hunter et al., 1995). In our study, there was no decline in the oyster tissue cadmium concentration at any of the study locations in the winter period (Figure 2), when the soft tissue mass and condition index of the species are highest (Hrs-Brenko et al., 1986). However, this factor probably influences the decrease in lead and zinc tissue concentrations in Kuta (Figures 3 and 4).

A low positive correlation between the condition index and soft tissue concentrations of cadmium, lead and zinc was reported by Avery et al. (1996) in Sydney Saccostrea commercialis oyster, and by Presley et al. (1990) in C. virginica oyster from the Bay of Mexico.

The spatiotemporal variations in the oyster soft tissue concentration of lead differed from those recorded for cadmium and zinc concentrations (Figures 2, 3 and 4), however, lead and zinc showed some similarity. As the oyster tissue concentration of lead depends mainly on its seawater concentration and creation of a complex with dissolved organic matter (Martincic et al., 1984; Martincic, 1987; Ikuta, 1988; Trefry et al., 1995), it is concluded that the freshwater contribution of lead was lower compared to the other two elements under study.

In Hodilje (Figure 1), where the freshwater influx is lowest, the lead concentration was highest in the winter period indicating that the seawater concentration of this element still exceeded the effect of dilution, i.e. there must have been a local source of contamination (Figure 3). The fact that there is a small marina near the sampling area is not sufficient for the explanation of such a variation, but it is presumable that the amount of lead that got into water through the combustion of fuels for motor boats, together with sewage waters of this mostly populated investigated location, contribute to the increase in the metal tissue concentration in the winter period.

As the highest oyster tissue lead concentration in the summer period was recorded in the Kuta Cove (Figure 1), the roads running along the coastline were presumed to be the main local source of this element. The roads are travelled most heavily in summer which, along with the tissue mass reduction, higher respiratory activity of all species and greater amount of liquid waste (Marasovic and Pucher-Petkovic, 1982) during the tourist season, leads to an increase in the oyster tissue lead concentration. Neither should the motor-boat traffic, although not too heavy, be neglected, since it also contributes to the elevated lead concentration in the seawater and shellfish from the area. Similar findings were reported by Marcus and Thompson (1986).

The measured metal concentrations do not exceed the highest legally approved limits according to the legislation of the Republic of Croatia and EU, and therefore from the public health aspect the Mali Ston Bay oysters are regarded as fit for the human consumption.

A comparison of oyster soft tissue lead concentrations in different geographic areas showed the lowest concentration in the coastal region of the USA (Hunter et al., 1995; Trefry et al., 1995; Beliaeff et al., 1998), which was ascribed to the restriction of the use of lead as a gasoline additive that started in this country a long ago (Beliaeff et al., 1998; O’Connor, 1998). Compared to the other world regions, a lower concentration of cadmium in oysters was reported in the coastal area of Spain (Schuhmacher and Domingo, 1996), France (Beliaeff et al., 1998) and in one part of the Gulf of Mexico (Hunter et al., 1995; Trefry et al., 1995).

Having in mind the concentrations of zinc, lead and cadmium reported in the Lim Channel (Martincic et al., 1980) and in the Gulf of Mexico (Presley et al., 1990), the Mali Ston Bay can be considered as an area with low anthropogenic impacts according to the cited authors. However, the increase in cadmium concentration in all three locations in the winter period in comparison with the summer period, as well as the concentration in Brijesta that is very close to the approved maximum, impose the commitment to pay a greater attention to this toxic metal (Figure 2). Monitoring should be carried out in a higher number of locations, in shorter (monthly) intervals, particularly in the winter period, when the consumption of this delicacy is the highest because of the best quality of meat. Regarding the economic significance of this shellfish, favourable natural farming conditions and planned production increase, more detailed knowledge of the spatiotemporal distribution of this element would allow the appropriate planning.
of production locations and sale of the final product in the market. In addition, increased metal concentrations can be expected owing to the global warming effect (Sokolova, 2004), and the planning of prevention measures, e.g. use of triploids for their faster growth (Amiard et al., 2005), demands more detailed knowledge and awareness of the current state.

REFERENCES


Martincic D., Nurnberg H.W., Stoeppler M., Branica M. (1980): Toxic metal levels in bivalves and their ambient
water from Lim Channel. Thalassia Jugoslavica, 16, 297–315.

Received: 2005–12–08
Received after revision: 2007–07–10
Accepted: 2007–07–11