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Heavy metal concentrations in sediment and fish species from Boka Kotorska Bay

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ABSTRACT

The concentrations of lead, mercury, copper and zinc were determined in sediments and muscle tissues of red mullet (*Mullus barbatus*) sampled from Boka Kotorska Bay, the Montenegrin coast. The average concentrations of the heavy metals found in sediment samples were: 31.8 ± 7.04 mg/kg for Pb, 0.388 ± 0.056 mg/kg for Hg, 57.7 ± 27.4 mg/kg for Cu and 111 ± 31.1 mg/kg for Zn, dry weight, while the average concentrations of heavy metals in red mullet samples were: 0.045 ± 0.011 mg/kg for Pb, 0.65 ± 0.14 mg/kg for Hg, 0.26 ± 0.04 mg/kg for Cu and 2.39 ± 0.21 mg/kg for Zn, wet-weight. According to sediment quality guidelines, included in the Canadian Environmental Quality Guidelines, only the concentrations of Cu and Hg exceeded the threshold effect levels (TEL). Also, considering USEPA Sediment Quality Guidelines, the sediments were classified as non-polluted (Pb) and moderately polluted (Cu and Zn). Metal concentrations in the red mullet samples in both seasons were lower than limits on permissible levels, defined by the National and European legislation.

Keywords: heavy metals, sediment, *Mullus barbatus*, Boka Kotorska Bay

INTRODUCTION

Over the last few decades, heavy metal pollution in marine environments has become an issue of a great concern, primarily because of the potential human health risks. Heavy metals from natural and/or anthropogenic sources are continually released into seas and they represent serious threats because of their toxicity, long persistence, bioaccumulation, and biomagnification in the food chain (Cardellicchio *et al.*, 2008). Heavy metal contamination of water and their uptake by different fish species are a direct consequence of an urban and industrial pollution (Türkmen *et al.*, 2005).

Fish absorb heavy metals from the surrounding environment depending on a variety of factors, including characteristics of the examined species, the exposure period, the concentration of the element, as well as abiotic factors such as temperature, salinity, pH and seasonal changes (Ginsberg & Toal 2009; Has-Schon *et al.*, 2006). In the marine environment, harmful substances like heavy metals are potentially accumulated in marine organisms and sediments, and consequently transferred to humans via the food chain (Tüzen, 2003). Therefore, the consumption of fish contaminated by toxic elements can pose risks to human health.

The objective of this study was to determine the accumulation of Pb, Hg, Cu and Zn in sediments and red mullet, which is a very important component of the human diet. Also, the comparison of the obtained results with national and international limits on permissible concentrations, as well as with studies of the surrounding countries, was performed.

MATERIALS AND METHODS

The sediment samples were collected from three sites along the Boka Kotorska Bay: Sveta Nedelja (S1), Shipyard of Bijela (S2), Herceg Novi (S3) (Fig. 1). The sediment samples were sampled in October 2019. The upper 5 cm of the surface sediment samples, collected by a Petite Ponar Grab (Wildco - dimensions 15x15 cm), were placed in polypropylene boxes and stored in a cold place (4 °C) until transferring to the laboratory. To prepare those sediment samples for analysis, after the homogenization which was done by conning and quartering, the samples were frozen and then freeze-dried at -40 °C for 48 h in a freeze-dryer (CHRIST, Alpha 2-4 LD plus, Germany). After the freeze-drying procedure, the samples were sieved and the fraction less than 63 µm was used for trace metal analysis. Before the analysis, the prepared sediment samples (0.2 g) were digested in a closed vessel microwave digestion system using 5 ml of HNO_3, 2 ml of HF and 2 ml of H_2O_2. The microwave digestion was performed in two steps, first with these reagents and the second after adding H_3BO_3. The digested samples were diluted using Milli-Q water and for each batch of analysis two blank digests were prepared in the same way.

The mineralized samples were analyzed for Pb, Hg, Cu and Zn content by using flame and hydride vapor generation atomic absorption spectrometer (F-AAS, HVG-AAS, Shimadzu A7000). The obtained results of the investigated elements in sediments are expressed in mg/kg of sample dry weight (dw). The accuracy of the analytical procedure was checked using the certified reference material, IAEA 158 (Marine sediment), which was also digested and analyzed along with the samples. The recovery rates for heavy metals in the standard reference material were between 86 and 106 %. In order to determine the precision of the analytical methods, the samples were prepared and analyzed in triplicate.

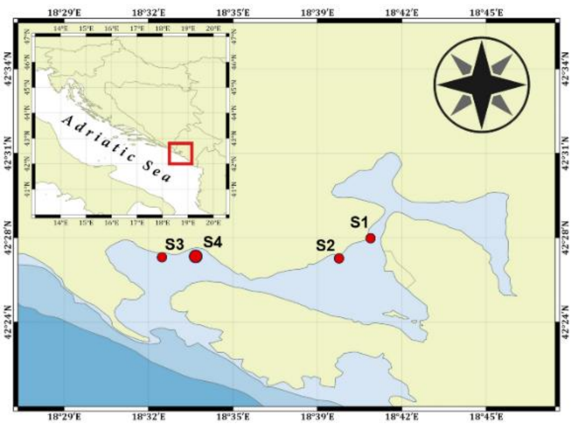

Figure 1. Map of investigated area

Samples of *Mullus barbatus* (Linnaeus, 1758) were collected in 15–20-m depths from the area in the coastal waters of Boka Kotorska (S4) in spring and fall of 2019

(Fig. 1), by using fishing methods such as trammel nets (mesh size 56 mm and length 160 m). The fish samples were dissected in order to separate the muscle tissues, washed with distilled water, weighed, and then the tissues were packed in polyethylene bags and stored at −18°C until the chemical analysis. Homogenized samples (1 g) were weighed and digested in a microwave oven (Speedwave Xpert, Berghof.) with 5 ml of HNO_3 and 2 ml of H_2O_2. The digested samples were then diluted to a final volume of 10 ml with ultrapure water, and these solutions were analyzed by an inductively coupled plasma–optical emission spectrophotometry, ICP-OES, Thermo iCAP7400 Duo. The analyses of Pb were performed by a graphite furnace atomic absorption spectrophotometry (GF-AAS), using a Shimadzu AA 6800 instrument, while Hg levels were determined by an Advanced Mercury Analyzer AMA 254, Altec. Performance of the method was evaluated in terms of linearity, recovery and precision. The certified reference materials, IAEA 407 (Fish homogenate) and IAEA 436 (Tuna Fish Tissue Homogenate), were also digested and analyzed along with the samples. The recovery rates for investigated heavy metals in these reference materials were between 90 and 105 %. In order to determine the precision of the analytical methods, the samples were prepared and analyzed in duplicate. All metal concentrations were determined on a wet-weight basis and expressed in mg/kg.

RESULTS AND DISCUSSION

The concentrations of trace metals (Pb, Hg, Cu and Zn) investigated in sediment samples, collected during fall of 2019 at the three sites along the Boka Kotorska Bay, are summarized in Table 1. Considering all the investigated metals and sampling sites of this study, the obtained mean values decreased in the following order: Zn>Cu>Pb>Hg. Based on the obtained values, the location with the highest contents of the examined elements was ex Shipyard of Bijela (S-2). Comparison of trace metal concentrations in surface sediments from Boka Kotorska Bay with values used to evaluate natural levels of these elements (Dolenec *et al.*, 1998; Žvab Rožič *et al.*, 2012) showed that concentrations of certain trace metals in sediments of Boka Kotorska Bay were significantly higher than those background levels.

Table 1. Concentrations of heavy metals in sediments (mg/kg (dw))

Location	**Pb**	**Hg**	**Cu**	**Zn**
S - 1	27.1	0.364	39.9	86.3
S - 2	39.9	0.452	89.2	146
S - 3	28.4	0.347	43.9	102

The highest concentrations of Pb, Hg, Cu and Zn were found in sediments from the site S2, with the values 39.9, 0.452, 89.2 and 146 mg/kg, respectively. The concentrations of these elements at site S2 were higher, but the concentrations found for other sites were lower than the natural levels. High concentrations of Zn and Cu (in comparison with the natural levels) are often the consequence of anthropogenic pollution. Different factors, such as municipal runoff, domestic and industrial effluents, but also the atmospheric deposition contribute to the high Cu levels (Khan *et al.*, 2017).

The highest Hg content was recorded for sediments at the site S-2, while at the other two sites the concentrations were similar. Due to the human activities, Hg contents are enriched and Hg can easily reach the offshore regions through wet and dry atmospheric deposition. The average concentrations of Pb, Hg, Cu and Zn in surface sediments from Boka Kotorska Bay were higher than those in surface sediments from the Southern Adriatic Sea (Dolenec *et al.*, 1998; Ilijanic *et al.*, 2014; Komar *et al.*, 2015). However, except for the maximum concentrations of these elements at the site S-2, at other two sites the results were in the same range as the results obtained for the different parts of the Adriatic and the Mediterranean (Sprovieri *et al.*, 2007; Acquavita *et al.*, 2010; Joksimovic *et al.*, 2011; Tanaskovski *et al.*, 2014; Stamatis *et al.*, 2019). Similar results are obtained from the comparison with Sediment Quality Guidelines of the USEPA (Pekey *et al.*, 2004), Table 2. Considering these guidelines, the investigated sediments were classified as non-polluted (Pb) and moderately polluted (Cu and Zn), except for Cu at the site S2, where its contents indicated heavy pollution. Also, compared with Sediment Quality Guidelines (MacDonald, 1994; WDOE, 1995), included in the Canadian Environmental Quality Guidelines (CCME, 2019), levels of Cu and Hg were higher than the TEL values, while Pb and Zn contents were below the TEL values, except for the sediments at the site S2, as shown in Table 2.

Table 2. Sediment quality guidelines for elements (mg/kg) in marine sediments

		US EPA Guidelines			**Sediment Quality Guidelines (SQG)**	
Elements	This work	Non-polluted	Moderately polluted	Heavily polluted	TEL	PEL
Cu	39.9-89.2	<25	25-50	>50	18.7	108
Pb	27.1-39.9	<40	40-60	>60	30.2	112
Zn	86.3-146	<90	90-200	>200	124	271
Hg	0.35-0.45	-	-	-	0.13	0.70

TEL threshold effect level, PEL probable effect level

The Montenegrin coast, especially Boka Kotorska Bay, still receives a heavy influx of sewage and effluents from ports, industry and shipping area, as well as domestic and agricultural wastes, all of which contain various hazardous chemicals. Although some of the sampling sites changed their purpose over time and luxury resorts replaced some heavy industry and shipyard spots, the repercussions of that pollution are still visible (Joksimovic *et al.*, 2019). Additionally, tourism and recreational activities in the coastal area further pollute the Montenegrin coastal waters (Joksimovic *et al.*, 2011).

Fish are widely used as bio-indicators of marine pollution by metals (Evans *et al.*, 1993). *Mullus barbatus*, being bottom dwellers to a certain extent, is species that tend to concentrate contaminants to a higher degree than other species which are characterized by higher mobility. For this reason, it was recommended as the monitoring species by FAO/UNEP (1993). The number of red mullet samples in each season were 15. The concentrations of heavy metals (Hg, Cu and Zn) were higher during the spring than fall period, except for the Pb, for which the concentration was higher during the fall, Fig. 2. The average concentrations of the heavy metals found in red mullet samples were: 0.045 ± 0.011 mg/kg for Pb, 0.65 ± 0.14 mg/kg for Hg, 0.26 ± 0.04 mg/kg for Cu and 2.39 ± 0.21 mg/kg for Zn, wet-weight.

Heavy metal concentrations in red mullet samples found in this study were similar and/or lower than those found in the polluted areas of the Mediterranean and the Adriatic Sea (Storelli & Marcotrigisno, 2005; Tepe *et al.*, 2008; Bilandzic *et al.*, 2011; Kucuksezgin *et al.*, 2011; Naccari *et al.*, 2015; Türkmen & Pınar, 2018). The European legislation (EC 2006; 2008) and the Official Gazette of Montenegro (81/2009) set the maximum levels for Pb, Cd and Hg in fishery products and fish muscle. For Pb, the actual concentration limit is set to 0.3 mg/kg, for Cd to 0.05 mg/kg and for Hg to 1.0 mg/kg wet-weight for the red mullet. Metal concentrations in red mullet samples in both seasons were lower than the maximum allowable concentrations, defined by the National and the European legislation (Official Gazette of Montenegro (81/2009); EC 2006; EC 2008).

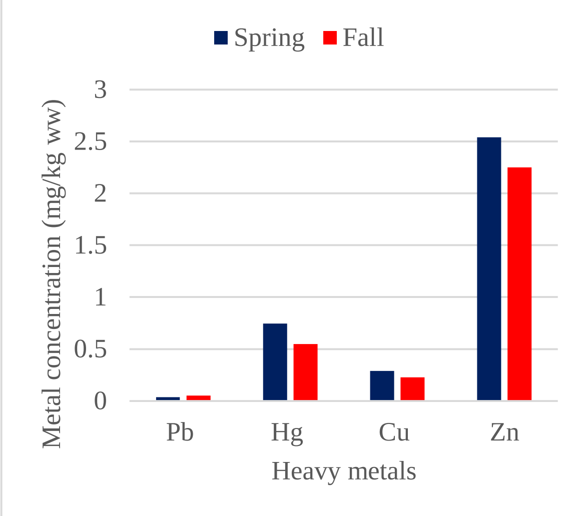

Figure 2. The metal concentrations in the red mullet muscle from Boka Kotorska Bay

Many authors showed that, in comparison with pelagic species, demersal or bottom-dwelling fish species accumulate higher proportions of heavy metals, often exceeding the maximum permissible levels (Kljaković Gašpić *et al.*, 2002; Saei-Dehkordi *et al.*, 2010; Storelli & Barone, 2013). Benthic fish, living right above the surface of the seafloor, can accumulate contaminants from sediments.Marine sediments are an important source of trace metals, from where these elements can be released into the water column due to the environmental changes (pH, redox potential or presence of organic chelators) and, consequently, absorbed by fish as free metal ions or through the ingestion (Oronsaye *et al.*, 2010). The lowest residual levels of heavy metals found in benthic fish species, according to other authors (Bustamantea *et al.*, 2003), could be considered as an expression of a not significant sediment pollution.

CONCLUSION

Environmental pollution is a worldwide problem and heavy metals belong to the group of the most important pollutants, because they tend to accumulate and, when present above threshold concentrations, they can be toxic. The threshold concentration depends on the metal, the animal species, and also on the environment, which determines the availability. Some metals are harmful to humans, some of them are necessary for sustaining metabolism of the human body, even though high concentrations of all metals can pose a threat to human health. Within this study heavy metal levels were determined in both surface sediments and fish species living above the surface of the seafloor in Boka Kotorska Bay. High concentrations of the trace metals (Pb, Hg, Cu and Zn) at some locations were largely contributed by respective pollution sources (urban areas, harbours, marinas). This paper represents one of the first studies in the area of metal determinations in fish species from Montenegrin coast. The fish contamination levels should be carefully monitored on a regular basis, to detect any changes in their patterns that could become a hazard to human safety. Also, the research should be extended to other sampling stations, with other pelagic as well as benthic species.

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Koncentracija teških metala u sedimentu i ribama u Bokokotorskom zalivu

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SAŽETAK

Koncentracije olova, žive, bakra i cinka određene su u sedimentu i mišićnom tkivu barbuna, uzorkovanih u Bokokotorskom zalivu na crnogorskoj obali. Prosječne koncentracije teških metala izmjerene u uzorcima sedimenata bile su: 31.8 ± 7.04 mg/kg za Pb, 0.388 ± 0.056 mg/kg za Hg, 57.7 ± 27.4 mg/kg za Cu i 111 ± 31.1 mg/kg za Zn, suve mase, dok su prosječne koncentracije teških metala u barbunu iznosile: 0.045 ± 0.011 mg/kg za Pb, 0.65 ± 0.14 mg/kg za Hg, 0.26 ± 0.04 mg/kg za Cu i 2.39 ± 0.21 mg/kg za Zn, vlažne mase. Prema Smjernicama za kvalitet sedimenta (SQS) uključene u kanadski kriterijum za kvalitet sedimenta, samo koncentracije Cu i Hg premašuju niži nivo dozvoljenih vrijednosti, označen kao TEL (engl. threshold effect level). Takođe, prema Smjernicama za kvalitet sedimenta US EPA, sedimenti su klasifikovani kao nezagađeni za Pb i umjereno zagađeni za Cu i Zn. U obje sezone (proljeće, jesen) koncentracije metala u barbunu bile su ispod maksimalno dozvoljenih vrijednosti prema nacionalnom i evropskom pravilniku.

Ključne riječi: teški metali, sediment, *Mullus barbatus*, Bokokotorski zaliv