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Cadmium and lead in selected tissues of two commercially important fish species from the Adriatic Sea

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Abstract

Baseline levels of cadmium and lead were determined in muscle tissue and liver of hake (*Merluccius merluccius*) and red mullet (*Mullus barbatus*), two commercially important fish species from the eastern Adriatic. Concentrations of trace metals in liver (Cd: 6–183 $\mu g\,kg^{-1}$ w. wt. ; Pb: 39–970 $\mu g\,kg^{-1}$ w. wt.) were within the range of recently published data for the Mediterranean. In the muscle tissue, cadmium concentrations (4.1–29 $\mu g\,kg^{-1}$ w. wt.) were among the lowest reported values for the Mediterranean, whereas lead levels (49–158 $\mu g\,kg^{-1}$ w. wt.) were within the range of values reported for various coastal areas of the Mediterranean. Presented data on cadmium and lead content in the studied fish species provide no proof of the general pollution of the Adriatic. Obtained data were tested in relation to fish length. Metal concentrations in liver decreased with the increase in fish size, whereas no significant correlation was found between trace metal levels in the muscle tissue and the length of both species. Relationships between metal concentrations and sex were also tested, but they gave no significant results. A comparison of contaminant concentrations in the edible tissue of hake and red mullet with the Croatian legislation shows that the consumption of their meat is not harmful for humans, not even for the most endangered population from the coastal region.

Keywords: Adriatic Sea; Cadmium; Lead; Fish; Muscle tissue; Liver

1. Introduction

The discharge of potentially toxic trace metals into the marine environment has become a global problem. Non-essential metals (e.g. Pb, Cd, Hg) are held to be the most dangerous, since continuous exposure of marine organisms to their low concentrations may result in bioaccumulation, and subsequent transfer to man through the food web. As a result of the global concern over the impact of metals on human health, the muscle tissue of fish has been investigated more than other organs [1]. Nowadays, different fish tissues (muscle tissue, liver, gills, etc.) are commonly used as indicators of the degree of contamination of marine environment with trace metals [2].

Trace metal levels in different compartments of the eastern Adriatic ecosystem are in the normal range of values and offer no evidence of appreciable pollution, although increased levels can be found locally [3]. Unfortunately, since the pioneering work of Viviani et al. [4] and Ozretić et al. [5] there have been no follow-up studies on trace metals in commercial fish species from the Adriatic, even though fish meat is an important component of the diet in the coastal region.

The aim of this study was to determine ranges and variations of potentially toxic trace metals (Cd and Pb) in the muscle tissue and liver of two commercially important demersal fish species from the eastern Adriatic in order to estimate the health risk for seafood consumers. Concentrations in the two species were compared, and the effects of age and sex on trace metal levels examined.

2. Materials and methods

Samples of hake (*Merluccius merluccius* (L.)) and red mullet (*Mullus barbatus* L.) were collected by the bottom

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trawl at 47 locations in coastal and open waters of the eastern Adriatic (Fig. 1). Sampling was carried out during June and July of 1995 as a part of MEDITS Program. Details on the survey methodology can be found elsewhere [6]. Both species were not available at all locations.

Sampling and pre-treatment of samples were performed in laboratory according to UNEP/FAO/IAEA/IOC [7]. Since we wanted to investigate the relationship between the contaminant concentration and size of organism, individuals of different length were selected at each sampling site, whenever possible. Upon measuring fork length and weight, sex of each specimen was determined, and then fish were wrapped in plastic bags and stored frozen. Biometric parameters of the investigated species are presented in Table 1.

The decomposition procedure of organic matter consisted of wet digestion with a mixture of concentrated acids (3.5 mL HNO_3 and 1.0 mL $HClO_4$) in a kitchen-type microwave, and subsequent evaporation on a hot plate, according to the laboratory standard operating procedure developed using certified reference materials.

The reagents for the cleaning treatments and wet digestion were of ultra-pure quality. The water used for cleaning of glass and plastic ware, preparation of stock solutions and dilution of samples was deionized with a Milli-Q Water Purification System (Millipore). Glass- and plastic-ware used for tissue analysis were cleaned with 5% solution of nitric acid for 48 h, and rinsed with deionized water to minimize the possibility of contamination.

The analyses of Cd and Pb were performed by graphite furnace atomic absorption spectrophotometry (GF-AAS), using a Perkin Elmer 1100 B instrument. All results are expressed in terms of $\mu g\,kg^{-1}$ wet weight

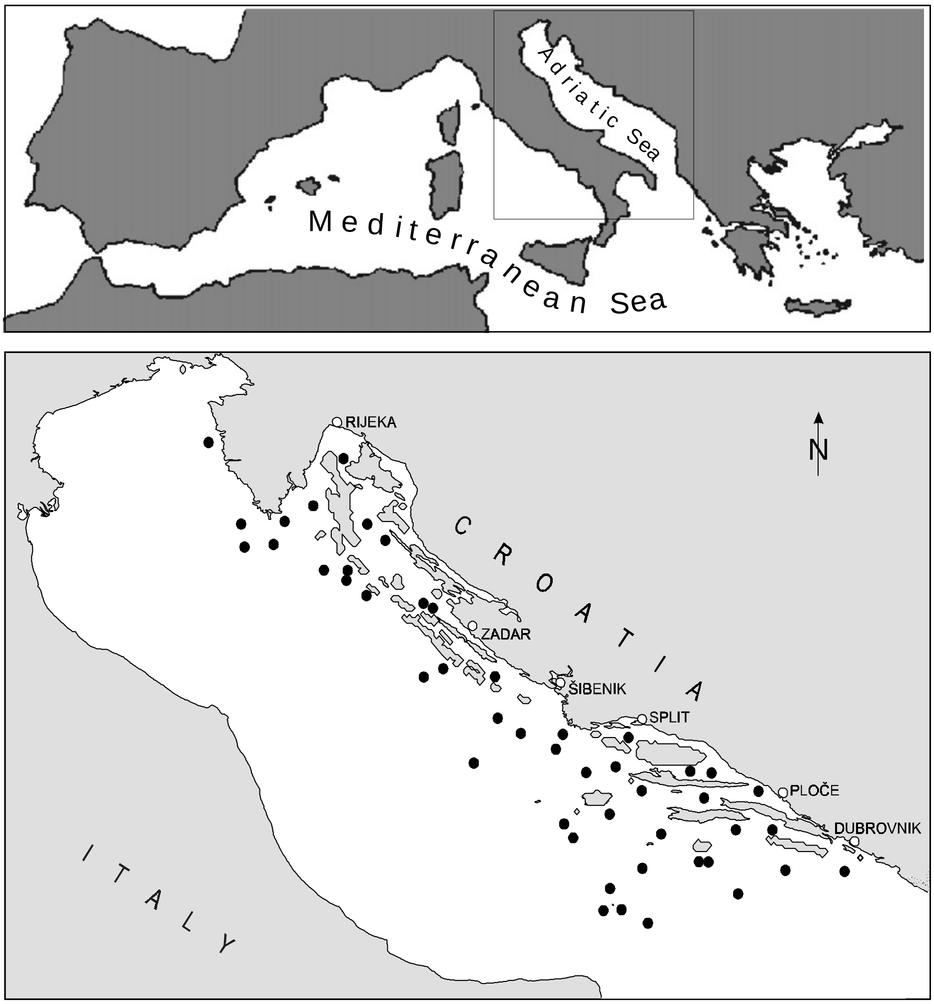


Fig. 1. The Adriatic Sea with geographical locations of sampling points.

Table 1
Mean length, total body weight and sex of studied species (length and weight ranges are given in parentheses)

Species	Number of individuals	Length (mm)	Total body weight (g)	Sex		
				M	F	?
Hake	138	243.6 (115–715)	175.9 (9–3000)	69	56	13
Red mullet	99	156.5 (103–255)	44.6 (10–193)	48	45	6

M–males; F–females; ?–not determined.

(w. wt.) to enable comparison with the maximum permissible concentrations under Croatian legislative provisions for trace metals content in fresh seafood. The accuracy of the applied analytical procedure was tested using certified reference materials (DORM-1, TUNA FISH, DOLT-1 and TORT-1) provided by the International Atomic Energy Agency (Marine Environmental Laboratory, Monaco). Concentrations measured in reference materials were within −5% (DOLT-1, Pb) to +22% (IAEA 350, Pb) of the certified values.

Normality of the data was tested using χ^2 test. Statistical differences between species, organs and sex were evaluated using non-parametric Sign test. The regression analysis (Pearson's Product–Moment Correlation) was used to examine the effects of length on trace metal content in tissues. In all cases, the level of significance was set at $P<0.05$.

3. Results and discussion

3.1. Element levels

Cadmium concentrations in the muscle tissue of hake ranged from 4.1 to 14.3 μg kg^{-1} w. wt., while concentrations in liver were in the range of 6.5–153 μg kg^{-1} w. wt. (Fig. 2a). Muscle tissue of red mullet contained from 7.6 to 28.9 μg Cd kg^{-1} w. wt., while values in the liver were in the range of 11.2–183 μg Cd kg^{-1} w. wt. (Fig. 2a).

Concentrations of lead in the muscle tissue and liver of hake ranged from 49 to 141 μg kg^{-1} w. wt., and from 39 to 298 μg kg^{-1} w. wt., respectively (Fig. 2b). Lead concentrations in the muscle tissue of red mullet varied from 57 to 158 μg kg^{-1} w. wt., while concentrations in liver were in the range of 99–970 μg kg^{-1} w. wt. (Fig. 2b).

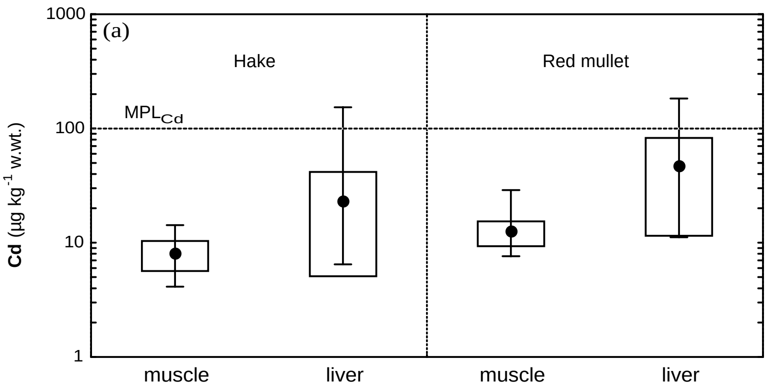
Obtained values on cadmium content in the muscle tissue of the monitored species are lower than most values previously reported for the Mediterranean [8–10]. Only the recent study by Kucuksezgin et al. [11] revealed lower values of cadmium in the muscle tissue of red mullet from Aegean Sea than those reported in the present study. Lead concentrations in the muscle tissue fall within the range of values reported for various coastal areas of the Mediterranean [11,10].

Lack of comparable data related to the hepatic trace metal content of hake and red mullet prevents a comprehensive evaluation of the presented results. Nevertheless, the obtained mean values for cadmium and lead content in liver match well with the results of Focardi et al. [12].

Although the complexity of interaction between different compartments of an ecosystem makes it difficult to draw firm conclusions, the presented results are within the range of normal values reported for the Mediterranean, offering no evidence of appreciable pollution of the Croatian coastal area.

When considering the trace metal content in organisms suitable for human consumption, the most important aspect is their toxicity to humans. Generally, the overall cadmium levels in the edible tissue of hake and red mullet from the eastern Adriatic are 6–18 times lower than the maximum permissible value (100 μg Cd kg^{-1} fresh fish) under Croatian legislative provisions for trace metals content in fresh seafood [13], while the mean lead content is 9–16 times lower in comparison to the maximum permissible value of 1000 μg Pb kg^{-1} fresh fish [13] (Fig. 2). We have calculated that, in the worst case, adults (60 kg) can consume more than 45 meals (1 meal ~350 g) of fish per week before exceeding the provisionally tolerable weekly intake for cadmium and lead [14].

A general examination of the statistical summary (Fig. 2) shows that processes of uptake and distribution of trace metals within a fish body are species specific. According to the results of multiple non-parametric comparisons, red mullet, with the exception of lead in the muscle tissue, accumulated higher levels of trace metals than hake. Differences between the two analysed species were particularly evident ($P<10^{-6}$) in the metal content of their liver. Thus, the mean content of cadmium and lead in liver of red mullet was



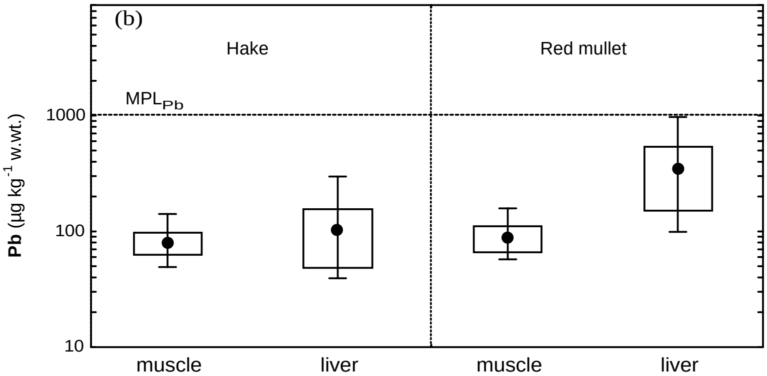


Fig. 2. The summary statistics of cadmium (a) and lead (b) content in the muscle tissue and liver of hake and red mullet from the Eastern Adriatic. Dots represent mean values; lower and upper box edges represent mean ± 1 SD; outlying bars are minimum and maximum values. MPL represents maximum permissible concentration under Croatian legislative provisions for trace metals content in fresh seafood.

approximately 2.1 and 3.4 times higher, respectively, in comparison with hake liver. Our results are consistent with findings of other researchers [1,15], whose field studies showed that bottom-dwelling species usually contain substantially higher concentrations of trace metals than the pelagic ones. This is largely related to the organism mobility, food preferences, or to other characteristics of behaviour with respect to the environment. The European hake is a semi-pelagic active predator, whose diet consists of pelagic and bottom-dwelling organisms, whereas red mullet is typical demersal fish, feeding mainly on epi- and endo- benthic organisms [16]. Since the later species may often contain substantially elevated concentrations of trace metals [17], it could be expected that red mullet will accumulate higher levels of trace metals than hake.

Generally, liver of both species accumulated higher levels of trace metals than the muscle tissue. Observed patterns of trace metal distribution between tissues match well with the results of numerous field and laboratory studies [17–19]. Differences between trace metal concentrations in analysed tissues originate from differences in physiological functions of muscles and liver [20,2].

Nevertheless, the calculated ratios of trace metals between liver and muscles (L/M) indicated that the overall concentration of metal in tissues, and the relative distribution between tissues depends on both the fish species and the trace metal properties. It was previously reported that, for most fish species, the differences between lead content in liver and muscle tissue are minor with respect to cadmium [17,19]. We obtained similar results only for hake ($L/M_{\mathrm{Pb}} = 1.36$; $L/M_{\mathrm{Cd}} = 2.72$). On the contrary, there were no significant differences related to Cd and Pb distribution between the analysed tissues of red mullet ($L/M_{\mathrm{Cd}} = 3.8; L/M_{\mathrm{Pb}} = 3.95$). The differences between lead and cadmium distributions in liver and muscles originate from different mechanisms involved in cadmium and lead sequestration in fish organism. Once absorbed, cadmium accumulates in liver [19] due to the formation of soluble metallothioneins (Cd-MT) [21,2]. Unfortunately, there is no specific low molecular weight protein in liver cytosol with high affinity for lead [22]. Thus, absorbed lead is quickly

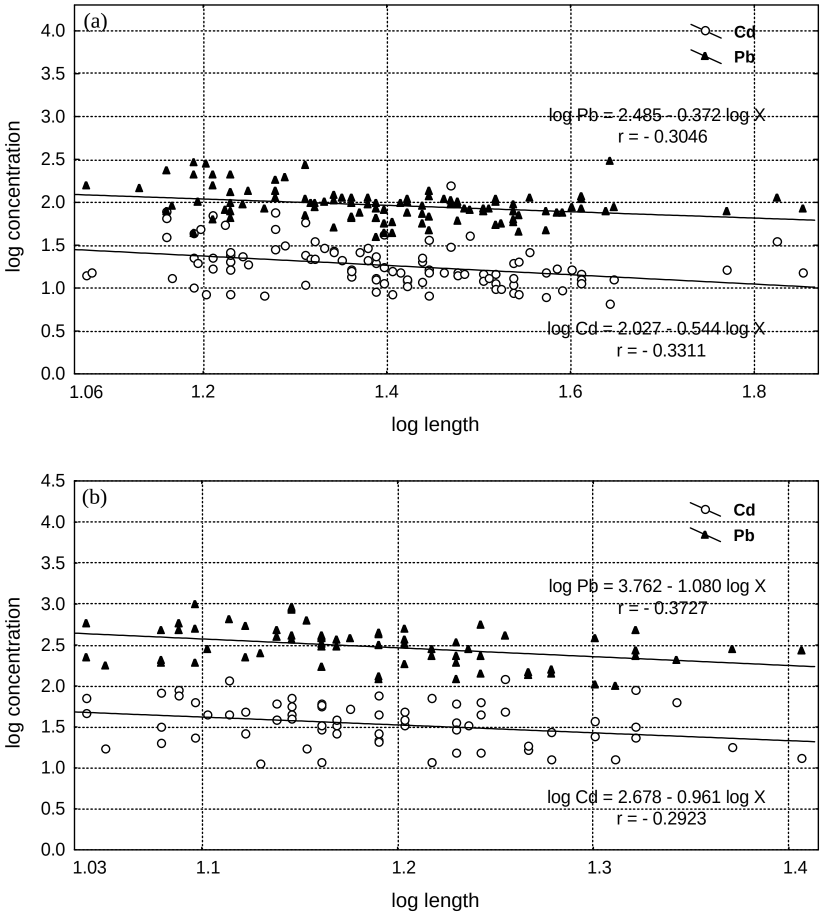


Fig. 3. Significant log–log relationships between metal concentrations ($\mu g\,kg^{-1}$ w. wt.) in liver of hake (a) and red mullet (b), and fish length (cm).

distributed to other tissues and organs (e.g. bones, kidneys, muscle), rather than accumulating in the liver [19].

4. Concentration–size relationships

Trace metal content in aquatic organisms can vary with their size (age). The slope values of the concentration–size correlations depend on several factors, such as: availability of the trace metal in the environment, the specific turnover rate of the trace metal in the animal, tissue type, and the competition between the opposing effects of ageing and tissue growth on metal accumulation within tissues. As a result, concentrations of most trace metals in tissues of small- and medium-sized fish species usually decrease or remain unchanged with the fish length increase [23,8,2]. Our results match well with these findings; based on the relationships between metal concentrations in tissues and fish size, we have found statistically significant correlations only between metal content in liver and fish length (Fig. 3). In all the cases, cadmium and lead concentrations in liver decreased with the increasing fish length. In contrast to the liver, no significant correlation was found between trace metal levels in the muscle tissue and the length of both species. Cossa et al. [23] believe that higher metal concentrations in juvenile specimens are related to the higher metabolic rates and insufficiently developed mechanisms for the neutralization of toxic trace metals. Further, if the growth of organism is faster than the metal accumulation, the observed trace metal concentration will decrease with age and weight, even though the overall metal content may be increasing.

5. Conclusions

The data presented on cadmium and lead content in Adriatic fish provide no proof of general pollution.

Results of the comparison of contaminant concentrations in the edible tissue of hake and red mullet with the Croatian legislation imply that fish meat is a minor source of cadmium and lead intake, even for the most endangered population in the coastal region.

In conclusion, we may remark that current efforts to monitor trace metal levels by means of biological indicators are fully justified and essential to a better understanding of the fate and cycles of trace metals in the marine environment, especially for the areas where baseline data are sparse. Presented results can be used as a base for comparison with possible future changes in the Adriatic ecosystem regarding trace metal contamination.

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