Bull. Environ. Contam. Toxicol. (2006) 77:43–50

DOI: 10.1007/s00128-006-1030-y

Bulletin of Environmental Contamination and Toxicology

Trace Metals in Tissues of Mugilids (*Mugil auratus, Mugil capito,* and *Mugil labrosus*) from the Mediterranean Sea

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Received: 20 January 2006/Accepted: 17 May 2006

Metals have long been recognized as the most deleterious contaminants to biota in the world's marine waters. Some of these elements are toxic to living organisms even at quite low concentrations, whereas others are biologically essential and natural constituents of the aquatic ecosystems and become toxic only at very high concentrations. In fish, the toxic effects of metals may influence physiological functions, individual growth rates, reproduction and mortality (Woodward et al., 1995; Farag et al., 1995). Therefore, numerous reports describe metal residues in marine fish (Abou-Arab et al., 1996; Usero et al., 2003), even if most of these studies concentrate on muscle tissue only, without any broader picture of the accumulation and distribution of the metals among the other tissues. According to the mechanisms of absorption, regulation, storage and excretion of metals, the various fish tissues present varying bioaccumulation rates and due to their different roles in the above processes, their analysis leads to results with special interest and interpretation (Catsiki et al., 1999).

Mullets are among the most common species of tropical and temperate marine coastal waters in the world. Being limno-benthofagous species, they are particularly exposed to possibly sediment-associated contamination. In addition, these organisms seems to be appropriate for contaminant-monitoring purposes in estuarine environments and in coastal waters (Chen and Chen, 1999; Licata et al., 2003; Marcovecchio, 2004). The Adriatic Sea, one of the Eastern Mediterranean sub-basins is located between the Italian peninsula and the Balkans. It is connected with the Ionian Sea through the Otranto channel, width only 45 miles. The semi-enclosed nature of this water body and the increasing population density along its coastline are conducive to water pollution. In spite of this, of all the studies carried out up to now on the concentration of metals in the Mediterranean area using monitoring species, only a few have been specifically dedicated to Adriatic Sea, and especially to the southern end of this sea (Giordano et al., 1991; Storelli et al., 2001).

The aim of this study was to evaluate the bioaccumulation of essential and non-essential elements (Hg, Pb, Cr, Ni, Cu and Zn) in various tissues, including muscle tissue, liver, gills and skin of different mullet species (*Mugil auratus, Mugil capito* and *Mugil labrosus*) to gain insight into the distribution of metals in

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these fish and by comparison with data from same organisms living in other water bodies, to assess indirectly the level of pollution of this sub basin of Mediterranean Sea. In addition, as these species are of economic importance, metals concentrations in muscle tissue were compared with the respective maximum permissible limits established by law in order to ascertain whether this food could be considered suitable for human consumption.

MATERIALS AND METHODS

A total of 120 specimens of three species *Mugil auratus* (golden grey mullet), *Mugil capito* (grey mullet) and *Mugil labrosus* (lesser grey mullet) were collected in coastal waters of the western Adriatic Sea (Fig. 1) during June-July 2003. Total fish length were measured (golden grey mullet, length: 25.0-42.0 cm, mean 33.8±5.8; grey mullet, length: 27.8-45.0 cm, mean 37.8±6.1; lesser grey mullet, 25.0-42.0 cm, mean 36.8±5.8), and no significant difference regarding this parameter among various species was observed ($p>0.05$). For each species, from the total number of specimens were formed pools (golden grey mullet: n° 8; grey mullet: n° 7; lesser grey mullet: n° 10) within which individual fish were collected as a function of their similar length. From each composite sample gills, liver, skin and muscle tissue were dissected and preserved at -25 °C until analysis. The analytical methods for metals have been previously described (Storelli et al., 1998). Briefly, homogenised sub-samples (about 2 g) were digested in a HNO_3-$HClO_4$ mixture conc. for Pb, Cr, Cu Zn and Ni determination, and in a H_2SO_4-HNO_3 mixture conc. for Hg (Sigma-Aldrich, Germany). Quantitative determinations of Pb, Cr, Cu, Zn and Ni were made using an atomic absorption spectrophotometer (Analyst 800 P.E.) equipped with a heated graphite furnace system (THGA-800 P.E.), while Hg was determined by the cold vapour technique after reduction by $SnCl_2$ (FIMS 100 P.E.). The instrumental limits of detection were: Hg: 1.0 ng g^{-1}; Pb = 10 ng g^{-1}; Cr = 5 ng g^{-1}; Ni = 25 ng g^{-1}; Cu = 25 ng g^{-1}; Zn = 25 ng g^{-1}. The accuracy of the analytical procedure was checked by analyzing the standard reference material (National Research Council of Canada; DORM-2 dogfish muscle). Recovery ranged from 95% to 100% for all investigated elements. Non-parametric statistics (Kruskal-Wallis and Mann-Withney tests) were used in testing differences among samples. All data were expressed in μg g^{-1} wet weight.

RESULTS AND DISCUSSION

Mean concentrations of metals in muscle tissue, skin, gills and liver from different fish species are presented in Table 1. Among the three studied species no significant variation in metal concentrations was detected ($p>0.05$). This comparability in body metal load might be a result of a life style and feeding behaviour similar among different species examined. All mullets are, in fact, omnivorous species and filter-feeders. They filter plankton and fine suspended particles in water and consume organic matter, such as bacteria, bottom diatom and algae encrusting on mineral grains. Moreover they are known to uptake and utilize sand granules to grind the cell wall of microalgae in digestion. However, apart from concentration homogeneity within different species, the data also revealed that metals were differentially distributed in the various tissues and organs studied (Fig. 2). For Hg, the highest concentrations were always found in

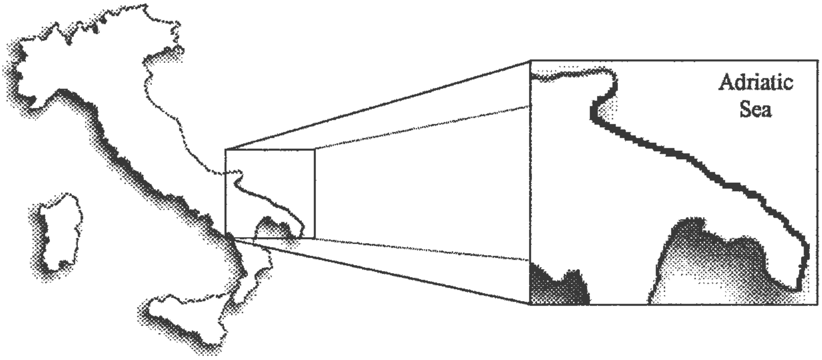

Figure 1. Sampling area.

liver, with levels many times higher than in other tissues, which showed a mercury content of similar magnitude ($p>0.05$). The concentrations of Pb in liver were generally twice as high as in skin, whereas the highest and the lowest levels were found in gills and in muscle tissue, respectively. Cr made up the highest concentrations in liver and the lowest in muscle tissue, while skin and the gills showed comparable levels ($p>0.05$). For Ni the concentrations in liver and gills generally were of similar magnitude ($p>0.05$), and significantly higher than in skin and muscle tissue ($p<0.0001$). The hepatic Cu contents were enormously higher than those observed in the other tissues ($p<0.0001$), while for Zn the highest concentrations were in skin, followed by liver, gills and muscle tissue. With a few exception, present data showed that in all species examined, the concentrations of both essential and non-essential metals were higher in liver and gills than skin, while muscle tissue exhibited always the lowest concentrations. Zinc was the only element that was found in the highest concentrations in skin relative to other tissue contents ($p<0.0001$). It was also interesting to note that for Pb and Ni the accumulation in the tissues showed the following sequence: gills>liver>skin>muscle tissue. Generally, increased metal concentrations in gills reflect the metal concentrations of the water where the fish lives. Regards to Ni, Tankere and Statham (1996) found a metal enrichment of the coastal waters of the Adriatic Sea and explained it in terms of direct inputs of terrestrial material, as a consequence of riverine discharge. In contrast, in the case of Pb, the highest concentrations in gills are not easily explicable because the level of this element is generally in decline in the whole Mediterranean basin (Nicolas et al., 1994). However, in general the observed patterns of metal distribution among tissues match well with the results of numerous field and laboratory study (Romeo et al., 1999; Yilmaz, 2003). Likewise other metal tissue localisation studies have shown either higher levels of Zn in skin than liver or gills, probably related to the metal complexing with the mucus on the surface of the fish body (Kovekovdova and Simokon, 2002; Yilmaz, 2003), or remarkably high copper hepatic concentrations as a result of swimming activity of these species (Kalay et al., 1999; Canli and Atli, 2003).

Metal distribution pattern in the tissues reflect the role covered by them in

Table 1. Mean concentrations (μg g^{-1} wet wt) of Hg, Pb, Cr, Ni, Cu and Zn in the muscle tissue, skin, gills and liver of *M. auratus*, *M. labrosus* and *M. capito*.

Species	Muscle Tissue	Skin	Gills	Liver
		Mercury		
M. auratus	**0.04±0.02**	**0.05±0.02**	**0.05±0.02**	**0.11±0.03**
M. labrosus	**0.05±0.02**	**0.05±0.02**	**0.04±0.02**	**0.14±0.05**
M. capito	**0.05±0.02**	**0.05±0.02**	**0.05±0.02**	**0.11±0.03**
		Lead		
M. auratus	**0.04±0.02**	**0.10±0.06**	**2.33±0.32**	**0.30±0.14**
M. labrosus	**0.04±0.02**	**0.17±0.05**	**2.49±0.40**	**0.30±0.19**
M. capito	**0.05±0.02**	**0.18±0.06**	**2.48±0.44**	**0.28±0.17**
		Chromium		
M. auratus	**0.15±0.06**	**0.20±0.07**	**0.35±0.14**	**0.68±0.29**
M. labrosus	**0.16±0.05**	**0.21±0.05**	**0.32±0.18**	**0.64±0.35**
M. capito	**0.15±0.06**	**0.23±0.07**	**0.32±0.06**	**0.90±0.31**
		Nickel		
M. auratus	**1.06±0.12**	**1.54±0.12**	**4.62±0.15**	**3.85±0.11**
M. labrosus	**1.22±0.25**	**1.57±0.26**	**4.67±0.30**	**3.04±0.78**
M. capito	**1.10±0.24**	**1.45±0.34**	**4.48±0.47**	**3.59±0.37**
		Copper		
M. auratus	**0.93±0.14**	**1.09±0.12**	**2.41±0.28**	**154.63±24.31**
M. labrosus	**0.84±0.17**	**0.92±0.17**	**2.17±0.48**	**169.32±13.38**
M. capito	**0.88±0.06**	**1.14±0.31**	**2.43±0.44**	**177.78±10.45**
		Zinc		
M. auratus	**6.59±1.14**	**72.69±14.42**	**33.58±18.05**	**53.19±21.30**
M. labrosus	**6.90±0.16**	**60.65±14.94**	**33.06±17.32**	**54.93±14.06**
M. capito	**6.53±1.34**	**65.87±14.88**	**36.96±19.46**	**49.20±20.72**

bioaccumulation processes. Gills are regarded as the important site for direct metal uptake from the water whereas the body surface is generally assumed to play a minor role in metal uptake of fish (Pourang et al., 1995). From these uptake sites, and via food, the absorbed metals are redistributed throughout the body and accumulate in various organs. Liver, being a metabolically active tissue, has a tendency to accumulate metals to higher degree than muscle tissue, which usually exhibits a low accumulation potential of metals (De Boeck et al., 1997; Romeo et al., 1999). Analyses of tissue concentrations of metals, beside to extend our knowledge about biological role of the various organs and tissues examined, can also provide reliable information on contamination degree of marine environment, as well as constitute a tool to check the quality of marine foodstuffs concerning human health.

In relation to the impact of metals on human health, the muscle tissue of fish has been investigated more than other organs, because it is the main fish part consumed by humans. The European Community established maximum levels only for two of the metals studied here, above which human consumption is not permitted: 0.5 μg g^{-1} wet weight for mercury and 0.2 μg g^{-1} wet weight for lead

(G.U.C.E. 2001). The concentrations of these metals in the muscle tissue of the three species analysed were in all cases well under the proposed limit values and, therefore, the consumption of these fish by humans should be enough safe. For the remained metals the European legislation has not established maximum levels and, therefore, an evaluation of the chemical quality of these fish is possible only utilising dietary standards fixed in other countries. For example, the Western Australian Food and Drug Regulation List limits the levels for Cr and Ni at 5.5 and 40 µg g^{-1} respectively (Usero et al., 2003).

According to the Spanish legislation and UK Food Standard Committee Report, Cu and Zn levels in food should not exceeded 20 and 50 µg g^{-1}, respectively. These limits were not exceeded in the muscle of any of the fish analysed in this study. The highest concentrations found for these elements in muscle were, in fact, seven times lower than the legislated level for Zn, 20 for Cu, 37 for Cr and 40 for Ni. It is possible, thus, to conclude that also these metals present no problem for the consumption of muscle of these fish.

The knowledge of the ranges of metals in different tissues can also help in evaluating environment quality. Target organs, such as liver and gills, as well as the muscle tissue are, in fact, widely recognized as valuable indicators of pollution (Gomaa et al., 1995). On this basis, comparison with values detected in specimens of the same species caught in other sea zones (Table 2) may provide useful information on the pollution degree of this wide area of the eastern Mediterranean. The Pb, Cu and Zn concentrations in muscle tissue in this study generally accorded with those reported in literature (Perez Cid et al., 2001; Usero et al., 2003), whereas for Cr and Ni our levels were noticeable higher. Conversely, Marcovecchio (2004) in *Mugil liza* from Samborombon Bay (Argentina) reported a higher muscular Hg content. In *Mugil cephalus* samples from the Iskenderun Bay, one of the most polluted coastal waters of Turkey, the content of metals were markedly higher (Yilmaz et al., 2003) than those here reported, except for Ni whose values were close to our results. Licata et al., (2003) in mullet muscle tissue from Ionian Sea (Straits of Messina) registered, on the contrary, lower Ni concentrations and a more consistent Pb load. For the remained organs and tissues examined there are almost no data on metal concentrations in these species, with the exception of a few studies. Concerning mullet skin, Yilmaz et al., (2003) registered levels extremely higher than those reported here, especially for Pb, whereas in gills of *Mugil cephalus* from Ionian Sea, Licata et al., (2003) reported lower levels, relatively to Ni and Pb. For liver Pb, Zn and Cu concentrations obtained here were not substantially different from those reported for comparable species sampled along Atlantic coast of Spain, whereas our Hg, Cr and Ni levels were higher (Usero et al., 2003). Moreover, Hg concentrations reported by Marcovecchio, (2004) for *Mugil liza* from Argentina coast were lower than those here obtained.

The results of our study reveal no species-specific patterns of heavy metal accumulation probably due to similar ecological needs and feeding behaviours among the three fish species. In addition the distribution pattern of the metals

Table 2. Comparison with previous data on metal concentration (μg g^{-1} wet weight) in various organs and tissues of different mullet species.

Species	Provenience	Mercury	Lead	Chromium	Nickel	Copper	Zinc	References
				Muscle Tissue				
M. auratus	Mediterranean Sea	0.04	0.04	0.15	1.06	0.93	6.59	This study
M. labrosus	"	0.05	0.04	0.16	1.22	0.84	6.90	This study
M. capito	"	0.05	0.05	0.15	1.10	0.88	6.53	This study
L. aurata	Atlantic Ocean	-----	0.09	-----	0.10	0.79	11.90	Pérez Cid et al., 2001
C. labrosus	"	-----	0.06	-----	0.09	0.85	10.14	Pérez Cid et al., 2001
M. cephalus	"	-----	0.01-0.04	-----	0.06-0.10	0.52-0.58	4.71-7.79	Pérez Cid et al., 2001
L. aurata	"	0.010-0.013	0.03-0.05	0.03-0.04	0.02-0.07	0.20-0.60	3.10-8.41	Usero et al., 2003
M. cephalus	Mediterranean Sea	-----	7.45	1.46	1.22	1.45	38.23	Yilmaz, 2003
L. aurata	"	-----	0.39	-----	0.29	-----	-----	Licata et al., 2003
M. liza	Atlantic Ocean	0.40	-----	-----	-----	-----	48.8	Marcovecchio, 2004
				Skin				
M. auratus	Mediterranean Sea	0.05	0.10	0.20	1.54	1.09	72.69	This study
M. labrosus	"	0.05	0.17	0.21	1.57	0.92	60.65	This study
M. capito	"	0.05	0.18	0.23	1.45	1.14	65.87	This study
M. cephalus	"	----	37.39	3.22	2.72	5.36	100.56	Yilmaz, 2003
				Gills				
M. auratus	Mediterranean Sea	0.0.5	2.33	0.35	4.62	2.41	33.58	This study
M. labrosus	"	0.04	2.49	0.32	4.67	2.17	33.06	This study
M. capito	"	0.05	2.48	0.32	4.48	2.43	36.96	This study
L. aurata	"	-----	0.52	-----	0.36	-----	-----	Licata et al., 2003
				Liver				
M. auratus	Mediterranean Sea	0.11	0.30	0.68	3.85	154.63	53.19	This study
M. labrosus	"	0.14	0.30	0.64	3.04	169.32	54.93	This study
M. capito	"	0.11	0.42	0.90	3.59	177.78	49.20	This study
L. aurata	Atlantic Ocean	0.01-0.04	0.25-0.48	0.01-0.03	0.13-0.39	13.7-164.0	30.6-81.8	Usero et al., 2003
M. liza	"	0.53	-----	-----	-----	-----	52.0	Marcovecchio, 2004

among the tissues and organs of fish examined agree with that generally described for marine fish. However, a particularly interesting result of our investigation is the elevated presence of Ni and Pb in the fish gills that might be indicative of an anthropogenic input to the study area. On the other hand, the comparison with published data from other Mediterranean coastal areas shows that Ni values in the present study are similar to those measured in fish from waters strongly affected by anthropogenic activities. Relatively to remained metals the low levels found in different organs and tissues of these species suggest a negligible contamination of the studied area. Finally, in terms of food safety, the muscle tissue of the species studied may be considered suitable for human consumption, as the metal concentration levels observed are below the legal limits, even if it must be pointed out that in the European and national ambit, such limits are totally lacking for some metals.

Further investigations of the residual levels of metals particularly of Ni and Pb are necessary both to monitor their presence and to identify the possible pollution sources.

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