UDC 546.49:546.655.3:637.56
ISSN 0352-9193

original scientific paper

Content of Total Mercury and Methylmercury in some Commercial Fish Species of the Middle Adriatic Area

Udjel žive i metilžive u nekim komercijalnim vrstama riba na području Srednjeg Jadrana

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Received: June 15, 1994
Accepted: December 6, 1995

Summary

An attempt was made to determine mercury in some commercially important fish species (hake, red mullet and pandora) from the central Adriatic Sea (Kaštela Bay, Split Channel, Bay of Mali Ston and Vis Island – open waters).

The highest mass fractions of both total and methylmercury were recorded in fish from the Kaštela Bay and the Split Channel. Kaštela area used to receive considerable amounts of mercury from the chlor-alkali plant. Mass fractions were lowest in fish from the Bay of Mali Ston.

Pandora showed the highest and hake the lowest mass fractions among fish species studied. As to the sex, male pandora had higher mass fractions, most probably due to protogyny. Red mullet showed the lowest fractions.

Comparison with the maximum permissible mercury level in seafood in Croatia (Official Gazette of the Republic of Croatia, No. 46/1994) shows that mercury concentrations in pandora species from the mercury contaminated areas, highly exceed the limits beyond which no sale and consumption is allowed.

Sažetak

Svrha rada bila je određivanje udjela žive u nekim komercijalnim ribljim vrstama (mol, trlja, arbun), ulovljenih na području Srednjeg Jadrana (Kaštelanski zaljev, Splitski kanal, Malostonski zaljev i područje oko otoka Visa). Poznato je da se anorganska živa lako transformira u vrlo toksičnu metilživu. Jedan od glavnih načina na koji živa može dospjeti do čovjeka je onečišćena morska hrana u kojoj se živa akumulira kao metilživa.

Najveći maseni udjeli ukupne žive i metilžive utvrđeni su u ribama Kaštelanskog zaljeva i Splitskog kanala. To je područje dugo vremena bilo podložno akumulaciji značajnih količina anorganske žive zbog prisutnosti pogona kloralkalne elektrolize bivše tvornice Jugovinil. Najmanje koncentracije zabilježene su u ribama Malostonskog zaljeva.

Kao riblja vrsta arbun je sadržavao najveće, a mol najmanje koncentracije ukupne žive i metilžive. S obzirom na spol, muški su arbuni sadržavali najveće masene udjele žive, vjerojatno zbog proteroginije, a najmanje su vrijednosti imali mužjaci trlje.

Uspoređujući dobivene rezultate s maksimalno dopuštenim koncentracijama propisanim Pravilnikom o količinama pesticida, toksina, mikotoksina, metala i histamina i sličnih tvari koje se mogu nalaziti u namirnicama (Narodne novine 46/94), vidimo da koncentracije nađene u arbunu iz Kaštelanskog zaljeva i Splitskog kanala dvostruko premašuju dopuštene vrijednosti.

Introduction

The increase of human population affects the growth of its demand for food, particularly seafood. Unfortunately, human activities, rapid industrial development and population increase are accompanied with an increase of waste water and sewage inputs and consequently the accumulation of pollutants in the sea, particularly heavy metals. Their concentration in marine organisms is of great concern since seafood is one of the main routes by which mercury can reach man *(1,2)*.

Mercury cycle in the hydrosphere is of particular interest for our study. Elementary mercury, mercury (II) and methylmercury are the most common forms found in sediment and sea water. Inorganic mercury is easily methylated in the surface sediment (to methyl and dimethylmercury) wherefrom it is transported to the water column and reaches marine organisms *(3)*. Mercury is accumulated in marine organisms and may reach the mass fraction of 1.5 mg kg^{-1}. Its levels in marine organisms are dependent on the bioconcentration factors, which range from 10^4 to 10^5 for total mercury in fish and from 10^5 to 10^6 in mammals *(3,4)*.

Methylmercury is absorbed through the gut with a high efficiency. It mainly binds to red blood cells. It shows great affinity for proteins, amino acids and nucleic acids. Linking to sulfhydryl group of proteins by bisulfide bonds, mercury compounds cause selective membrane permeability for ions and nutrients, blocking transport processes through the cell membrane.

The toxic effects of mercury are primarily manifested in the damage of the central nervous system and prenatal poisoning of the faetus *(5)*. Several thousand accute poisonings with a large number of lethal cases were recorded during the second half of this century caused by consuming mercury contaminated fish in Japan (Minamata, Niigata)*(6)*.

So it is quite clear that mercury is a specific pollutant of the biosphere which calls for systematic studies and monitoring of its levels, particularly in areas receiving this metal through waste discharges over long periods.

Materials and Methods

Fish sampling stations (Fig. 1) were located at four sites in the middle Adriatic: a) Kaštela Bay, b) Split Channel, c) Bay of Mali Ston, and d) area of Vis Island. The positions of stations were selected with respect to the presence (a and b) or absence (c and d) of land-based pollution sources. Three fish species, *Merluccius merluccius, Mullus barbatus* and *Pagellus erythrinus* were examined. Samplings were performed in spring 1991 and 1993. Immediately upon sampling fish species were selected, fork length were taken and sex determined. Thereupon specimens were frozen in polyethylene bags and preserved until analyses. Age of fish was determined from the fork length and the age-length curves *(7–9)*. Composite sample was made of two filletes of fish of the same length, species and sex. Numbers of samples are given in Tables 2 through 5.

Total mercury in fish was measured by flameless AAS after digestion in a mixture of concentrated sulphuric and nitric acid in a closed system. Ionic mercury was reduced to elementary mercury with sodium boron hydride and swept by air stream into the quartz chamber where the absorption was measured *(10)*. This method has detection limit of 0.05 mg kg^{-1}.

Methylmercury was determined after the procedure recommended by UNEP/ FAO/ IAEA *(11)*. Methylmercury was isolated from the sample with hydrochloric acid and extracted with toluene. Residue impurities were removed with water solution of cystine by forming thiol-bound methylmercury compounds. Obtained compounds were digested by hydrochloric acid and the released methylmercury was again extracted with toluene the aliquot of which was injected in the gas chromatograph (with ECD detector) *(12)*. This method has detection limit of 0.02 mg kg^{-1}.

Method accuracy was tested by the parallel analysis of reference material and the obtained data are presented in Table 1.

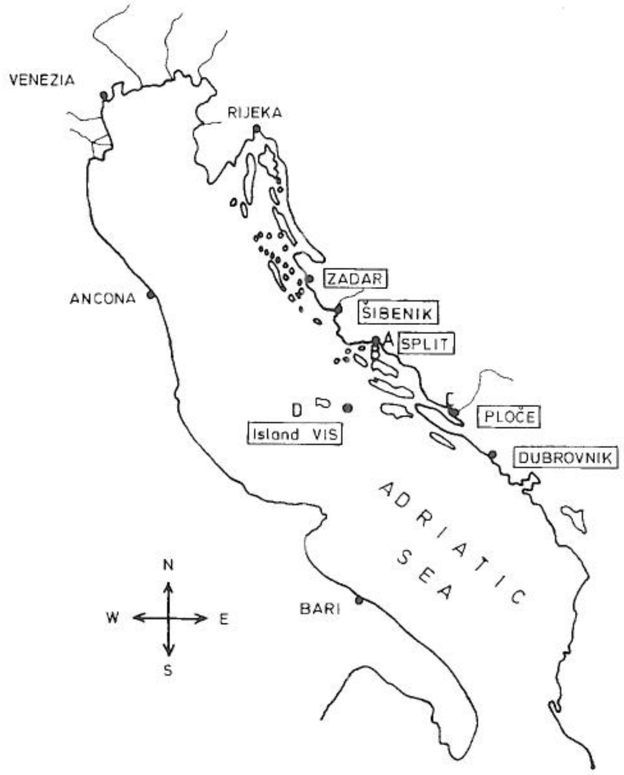

Fig. 1. Study area with sampling stations
Slika 1. Ispitivano područje s lokacijama uzorkovanja

Table 1. Results of analyses of the standard reference material
Tablica 1. Rezultati analize standardnog referentnog materijala

Reference material	*w* (Total mercury) mg kg^{-1}		*w* (Methylmercury) mg kg–1	
	a	b	a	b
Fish tissue (MA-A-2/TM)	470 ± 20	448 ± 18	312 ± 17	337 ± 47
Fish tissue (MA-B-3/TM)	510 ± 70	486 ± 29	439 ± 16	438 ± 36
Sharks (DORM 1)	798 ± 74	714 ± 15	749 ± 35	770 ± 19
Fish tissue (TORT -1)	330 ± 6	363 ± 21	126 ± 4	142 ± 52

a – certified value, b – our value

Results and Discussion

The highest total mercury and methylmercury concentrations were recorded in fish from the Kaštela Bay (Table 2) and the Split Channel (Table 3). This is due to the long-term work of the chlor-alkali plant »Jugovinil« which used to discharge considerable quantities of untreated inorganic mercury in to the Kaštela Bay. Even though mercury emission ceased in 1990, large quantities of mercury deposited in sediment and will be for a long time the main source of this metal in the Kaštela Bay. Similar results were reported by Horvat *et al. (13)* for the study period 1985–1988. Orlov *et al. (14)*, reported considerably lower values for pandora from this area, ranging from 0.2–0.6 mg kg^{-1}. Age and sex of fish were not determined in any of the earlier studies. Mikac *et al. (15)*, analysed pandora and red mullet. They measured the length of specimens which may allow for the determination of age, but they did not analyse either mercury or methylmercury levels with respect to sex. However, the values reported for pandora and red mullet by all the above mentioned authors are, in general, similar to present results (red mullet 0.6–0.7 mg kg^{-1}; pandora 0.8–0.9 mg kg^{-1}).

The lowest values were recorded in fish from the Bay of Mali Ston (Table 4). Similar values were reported by Horvat *et al.* *(13)*. The greatest differences between their results and ours were for pandora from the Vis Island (where Horvat *et al.* found 1.3 mg kg^{-1}). However, morphometric data such as age and sex were not given in that study which makes comparison impossible.

Higher mercury concentrations in fish from the Split Channel may be caused by seawater currents, as well as fish living and feeding habits. Currents may exchange the whole body of the Kaštela Bay water once in a month's period. Therefore it could be expected that mercury would be transported out of the bay in the direction of currents to the Split Channel *(16)*.

The Bay of Mali Ston is only a spawning ground and not the habitat of fish and since there is no bigger pollution source in the vicinity, low mercury data obtained in fish were quite expected.

The highest mercury concentrations were found in pandora, which may be partly attributed to their living and feeding habits. They are migratory and omnivorous fish. In addition, they are hermaphrodites, that is females become males between the third and fourth year of life (protogyny)*(7)*. This throws some more light on the results from the Kaštela Bay and the Split Channel, since the examined males were from three to four years old and their mercury burden was higher than in females. The fish from other locations were younger so the differences cannot be ascribed solely to sex change, but also to their habitat. Pandora mainly feed on small crustaceans, Polychaetes and shellfish, that is on sessile benthic organisms which are most strongly affected by the polluted environment *(17)*.

At all localities except the Bay of Mali Ston, have showed the lowest total mercury and methylmercury levels. This may be related to their biological behaviour. As young fish, up to 16 cm in length (age of about one year), they inhabit the open sea waters. Then they migrate to the coastal area where they remain till maturity (between the second and third year) again returning to the open sea for spawning and finally return to the coastal area. Their nutrition is also important. Younger fish mainly feed on planktonic crustaceans and adults on sardine and sprat *(8)*. Consequently they do not inhabit polluted coastal areas for most of the time, which results in the low mercury level in this species.

The analysis of concentrations of total mercury and methylmercury in red mullet from all four localities showed the highest burden in males from the Split Channel and in females from the Kaštela Bay. It may be partly due to different growing rates, since females grow faster than males *(9)*. So mercury levels from the Kaštela Bay and the Bay of Mali Ston were higher in females than in males although they were of almost the same age. Males from the Split Channel were older, which explains why their mercury levels were higher. The area of the Vis Island was an exception, since total mercury levels were higher in younger females.

With respect to the dynamics, red mullet are slightly less mobile than pandora, their food consisting of ben-

Table 2. Mean mass fractions of total mercury and methylmercury in fish from the Kaštela Bay
Tablica 2. Maseni udio ukupne žive i metilžive u ispitivanim ribljim vrstama u Kaštelanskom zaljevu

Species	Sex	Age/year	No. of fish	$\frac{w(\text{total Hg})}{\text{mg kg}^{-1}}$	$\frac{w(\text{Me Hg})}{\text{mg kg}^{-1}}$	$\frac{w(\text{Me Hg})}{w(\text{total Hg})} \cdot 100$
Merluccius merluccius	Females	3.5	5	0.162(0.005)*	0.154(0.019)*	95.1
Hake	Males	3.5	5	0.175(0.016)*	0.151(0.018)*	86.3
Mullus barbatus	Females	5.5	5	0.416(0.037)*	0.366(0.045)*	88.0
Red mullet	Males	5.5	6	0.329(0.022)*	0.244(0.038)*	74.2
Pagellus erythrinus	Females	2.8	5	0.962(0.013)*	0.792(0.031)*	82.3
Pandora	Males	3.0	5	1.010(0.020)*	0.812(0.037)*	80.4

*Standard deviation

Table 3. Mean mass fractions of total mercury and methylmercury in fish from the Split Channel
Tablica 3. Maseni udio ukupne žive i metilžive u ispitivanim ribljim vrstama u Splitskom kanalu

Species	Sex	Age/year	No. of fish	$\frac{w(\text{total Hg})}{\text{mg kg}^{-1}}$	$\frac{w(\text{Me Hg})}{\text{mg kg}^{-1}}$	$\frac{w(\text{Me Hg})}{w(\text{total Hg})} \cdot 100$
Merluccius merluccius	Females	2.8	5	0.263(0.010)*	0.206(0.031)*	78.3
Hake	Males	2.0	5	0.303(0.012)*	0.229(0.041)*	75.6
Mullus barbatus	Females	4.0	5	0.234(0.008)*	0.195(0.048)*	83.3
Red mullet	Males	5.0	7	0.416(0.024)*	0.339(0.040)*	81.5
Pagellus erythrinus	Females	2.5	5	0.903(0.016)*	0.657(0.029)*	72.8
Pandora	Males	4.0	6	1.230(0.070)*	0.844(0.029)*	68.6

*Standard deviation

Table 4. Mean mass fractions of total mercury and methylmercury in fish from the Bay of Mali Ston
Tablica 4. Maseni udio ukupne žive i metilžive u ispitivanim ribljim vrstama u Malostonskom zaljevu

Species	Sex	Age/year	No. of fish	$\frac{w(\text{total Hg})}{\text{mg kg}^{-1}}$	$\frac{w(\text{Me Hg})}{\text{mg kg}^{-1}}$	$\frac{w(\text{Me Hg})}{w(\text{total Hg})} \cdot 100$
Merluccius merluccius	Females	2.0	8	0.111(0.012)*	0.090(0.005)*	81.1
Hake	Males	2.0	8	0.091(0.007)*	0.081(0.017)*	89.0
Mullus barbatus	Females	1.0	5	0.113(0.005)*	0.050(0.011)*	44.2
Red mullet	Males	1.5	5	0.083(0.006)*	0.030(0.003)*	36.1
Pagellus erythrinus	Females	1.5	5	0.305(0.010)*	0.291(0.012)*	95.4
Pandora	Males	1.5	6	0.272(0.027)*	0.238(0.043)*	87.5

*Standard deviation

Table 5. Mean mass fractions of total mercury and methylmercury in fish from the Vis Island area
Tablica 5. Maseni udio ukupne žive i metilžive u ispitivanim ribljim vrstama u području otoka Visa

Species	Sex	Age/year	No. of fish	$\frac{w(\text{total Hg})}{\text{mg kg}^{-1}}$	$\frac{w(\text{Me Hg})}{\text{mg kg}^{-1}}$	$\frac{w(\text{Me Hg})}{w(\text{total Hg})} \cdot 100$
Merluccius merluccius	Females	3.5	8	0.190(0.008)*	0.152(0.020)*	80.0
Hake	Males	3.0	5	0.226(0.007)*	0.189(0.015)*	83.6
Mullus barbatus	Females	3.0	6	0.380(0.008)*	0.368(0.068)*	96.8
Red mullet	Males	5.5	5	0.210(0.008)*	0.199(0.027)*	94.8
Pagellus erythrinus	Females	1.5	6	0.251(0.012)*	0.220(0.046)*	87.6
Pandora	Males	1.5	5	0.262(0.008)*	0.235(0.036)*	89.7

*Standard deviation

thic organisms, small crustaceans, polychaetes and shellfish *(17)*. The demersal habitat of red mullet (as well as of pandora) and particularly their dependence on the sea bottom, brings them in close contact with mercury polluted sediment in the Kaštela Bay. This results in higher levels of total mercury and methylmercury in the tissue of red mullet from the Kaštela Bay and the Split Channel.

The results of this paper confirmed that concentrations of the total mercury and methylmercury are increased in some fish species. They were lowest in hake, slightly higher in red mullet and the highest in pandora. These results generally agree with the results published in 1992 for fish species sampled from the middle Adriatic *(18)*. Total mercury concentrations reported for the Mediterranean fish were much lower *(19)*. However, since neither the sampling sites nor basic morphometric parameters were taken into consideration in these reports our results could not be adequatly compared with the earlier published data.

Most of the total mercury in fish is in the methylmercury form, which is in agreement with the literature data. Commercial fish species from uncontaminated areas contain from 0.01 to 0.4 mg kg^{-1} of mercury. In the cases of the extreme environmental mercury contamination (Minamata, Niigata) the values amounted to 50 mg kg^{-1} *(1,3)*.

If our results are compared with recommended FAO/WHO *(20)*, PTWI values of 0.3 mg of the total mercury and 0.2 mg of methylmercury for a man of about 70 kg and with the Croatian maximally permissible level *(21)* which allows not more than 0.5 mg kg^{-1} of the total mercury and 0.4 mg kg^{-1} of methylmercury, the values in pandora from the Split Channel and the Kaštela Bay are almost twice the maximum permissible limits. This is, by no means, encouraging but it is an example of how irrational use of a water ecosystem as a recipient of waste discharges (as was the case of the Kaštela Bay) may have long-term enviromental implications.

Conclusions

The highest levels of total mercury and methylmercury were found in fish from the Kaštela Bay and the Split Channel and the lowest in fish from the Bay of Mali Ston.

Pandora showed the highest total mercury and methylmercury levels and hake the lowest.

The percentage of methylmercury was quite balanced in all fish species except in red mullet from the Bay of Mali Ston ranging between 70 and 97 %.

With respect to sex, pandora males contained higher levels of total mercury and methylmercury than females (because of protogyny, between the third and fourth year of age females become males). Red mulett females from the Kaštela Bay and the Split Channel showed higher levels of total mercury and methylmercury than males of the same age.

This suggests that no data of total mercury and methylmercury will be comparable if age and sex of fish are not determined.

The obtained results indicate that some fish species from the coastal areas under the influence of anthropogenic mercury pollution contain mercury concentrations higher than maximum permissible level in Croatia.

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