



Total and Methylmercury in Sardines *Sardinella aurita* and *Sardina pilchardus* from Tunisia

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This paper reports concentrations of total mercury and methylmercury in muscle, liver and gonads of two sardines (*Sardinella aurita*, $n=184$ and *Sardina pilchardus*, $n=87$) from three coastal areas off Tunisia. Mercury concentration in muscle and liver showed significant positive correlation with fish length (i.e. with age). The relative methylmercury concentration was high and constant in muscle (>85% MeHg), and was decreasing with age in liver (from 50% to 20%), which might reflect the existence of a slow demethylation process. From the geographic point of view, Hg concentration was similar in the northern and eastern areas ($0.32 \mu\text{g g}^{-1}$ dw in muscle for *Sardinella aurita*, 0.41 for *Sardina pilchardus*), but significantly lower in the southern zone (0.19 and 0.26 , respectively, median values). Mercury concentration in the Tunisian sardines was twice to four times lower than in the northern Mediterranean Sea. © 1999 Elsevier Science Ltd. All rights reserved

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Metals, particularly heavy metals such as mercury, are toxic to most marine organisms as they are to man. They can reach us through our food, mainly seafood. It is, therefore, most important to determine the concentration of such metals in fish, shellfish and others edible marine organisms. Many studies have been done to describe the level of mercury along the northern and eastern coasts of the Mediterranean Sea, but few along the southern coasts: most available results cannot be used to describe the whole Mediterranean Sea.

Two pelagic fish: sardine (*Sardinella aurita*) and pilchard (*Sardina pilchardus*), were used in order to study the mercury levels in Tunisian marine ecosystems. These two species are widely distributed and found all over the Tunisian coast, are numerically dominant in most catches and the most consumed ones, both fresh and

canned. Other studies showed the existence of different stocks and/or populations of sardines in the Mediterranean Sea (Kortas, 1981; Ben Hassine *et al.*, 1990): this is why possible geographic variations must be taken into account as well.

Materials and Methods

Sampling took place in August and September 1994, using small and large purse-seiners at the locations shown in Fig. 1. Fish muscle was collected at all locations, liver at two stations and gonads at one location only. The sampling period corresponded to the spawning period for *Sardinella aurita*, but not for *Sardina pilchardus*. Samples were immediately deep-frozen and stored at -20°C until analysis in Brussels.

Total mercury (ΣHg) was determined with atomic absorption spectrophotometer (MAS-50 Mercury analyser, Perkin-Elmer) after mineralisation of samples with sulfuric acid (10 ml of 97% acid per g wet weight) and oxidizing the mercury to Hg^{2+} . After reducing the Hg^{2+} to Hg^0 with stannous chloride, the volatile Hg^0 is bubbled into the closed system of the MAS-50 analyser (wavelength: 253.7 nm). The method used (Hatch and Ott, 1968) was already described in detail by Joiris *et al.* (1991).

Organic mercury (mainly monomethylmercury) was determined by gas liquid chromatography with capillary column and electron capture detector (Packard, model 437) (Uthe *et al.*, 1972). In order to extract all the organic Hg, lyophilised samples were treated with NaBr and CuSO_4 in sulfuric acid, which facilitates the liberation of CH_3 bound to thiol groups; the bromine forms a stable compound in a sulfuric medium with CH_3 . This compound was extracted with toluene. The organic Hg was extracted with a thiosulfate hydroalcoholic solution which makes up a specific thio-organic mercury compound. This compound is reconverted in bromide and re-extracted with toluene.

The accuracy of the Hg analyses (total and methylmercury) was tested by analysis of certified dogfish

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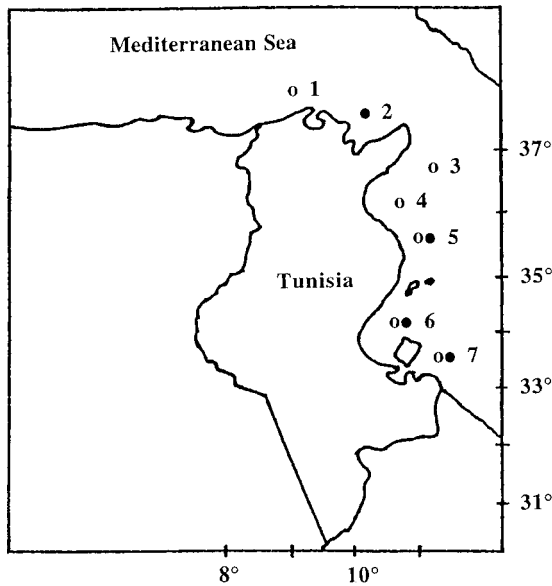


Fig. 1 Sampling location. Stations 1, 2: northern zone; stations 3, 4 & 5: eastern zone; stations 6 & 7: southern zone; circles: *Sardinella aurita*; dots: *Sardina pilchardus*.

muscle (DORM-1, Marine Analytical Chemistry Standards Program, Ottawa, Canada). Our measurements ($0.77 \pm 0.05 \mu\text{g } \Sigma \text{Hg g}^{-1} \text{ dw}$ and $0.73 \pm 0.03 \mu\text{g MeHg g}^{-1} \text{ dw}$) were in the range of the certified material: $0.789 \pm 0.074 \mu\text{g } \Sigma \text{Hg g}^{-1} \text{ dw}$ and $0.731 \pm 0.06 \mu\text{g MeHg g}^{-1} \text{ dw}$.

Data treatment was mainly based on the analysis of covariance (ANCOVA), using fish length as the co-variant. The principal reason for not using a method depending on mean values is the existence of a correlation between the age/size of fish and the concentration of contaminants that bioaccumulate, leading to a non-normal distribution of data. So, to reduce (or eliminate) the effect of body size, ANCOVA was used to compare mercury concentration in sardines from different zones. This is also why median values were used instead of means.

Results and Discussion

Total mercury (ΣHg) concentration in different organs of *Sardinella aurita* and *Sardina pilchardus* is summarised in Table 1. A positive correlation between length and total mercury concentration in muscle and liver was observed for the different regions for both species (Figs. 2 and 3, Table 2), confirming results reported for other marine fish (e.g. Thompson, 1990; Joiris *et al.*, 1995). This relationship, however, was not found for the gonads. The total mercury concentrations in the muscle of *Sardinella aurita* and *Sardina pilchardus* from the northern and eastern zones were similar: 0.32 and $0.41 \mu\text{g g}^{-1} \text{ dw}$, respectively ($p > 0.05$), but significantly lower in the southern zone: 0.19 and 0.30 , respectively ($p < 0.01$); this difference was not due to

TABLE 1

Total mercury concentration in different tissues ($\mu\text{g g}^{-1} \text{ dw}$), and total body length (cm) of Tunisian sardines (median values; n : number of samples).

Tissue >	Species	Zone	Muscle		Liver		Gonads		
			n	Length	ΣHg	n	ΣHg	n	ΣHg
<i>Sardinella aurita</i>	North		31	17.3	0.32				
	East		84	20.1	0.32	40	0.50	21	0.24
	South		32	18.5	0.19				
<i>Sardina pilchardus</i>	North		27	16.9	0.41	14	0.93		
	East		22	17.5	0.42				
	South		38	17.4	0.26				

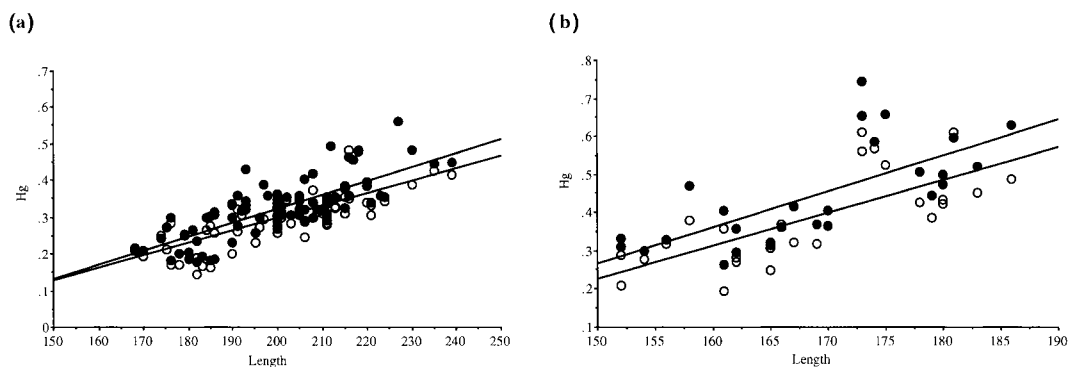


Fig. 2 Correlation between Hg concentration ($\mu\text{g g}^{-1} \text{ dw}$) in muscle of Tunisian sardines and total body length (mm); dots: total Hg; circles: methylHg (a) *Sardinella aurita*, eastern zone; (b) *Sardina pilchardus*, northern zone.

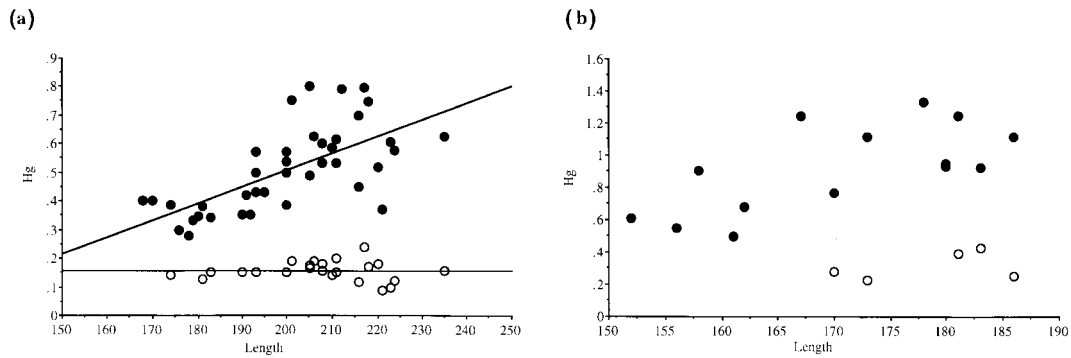


Fig. 3 Correlation between Hg concentration ($\mu\text{g g}^{-1}$ dw) in liver of Tunisian sardines and total body length (mm); dots: total Hg; circles: methylHg. (a) *Sardinella aurita*, eastern zone; (b) *Sardina pilchardus*, northern zone.

TABLE 2

Regression between total mercury concentration ($\mu\text{g g}^{-1}$ dw) and length (cm) in Tunisian sardines ($y = ax + b$).

Species	Zone	Tissue	<i>n</i>	<i>a</i>	<i>b</i>	Rsqr	<i>p</i>
<i>Sardinella aurita</i>	North	Muscle	31	0.04	-0.44	0.67	< 0.01
	East	Muscle	84	0.04	-0.44	0.89	< 0.01
		Liver	40	0.1	-0.66	0.44	< 0.01
	South	Muscle	32	0.03	-0.41	0.41	< 0.01
<i>Sardina pilchardus</i>	North	Muscle	27	0.1	-1.16	0.49	< 0.01
		Liver	14	0.2	-1.87	0.46	< 0.01
	East	Muscle	22	0.1	-1.23	0.29	< 0.01
	South	Muscle	38	0.1	-0.92	0.61	< 0.01

differences in size distribution of the fish, and thus reflects an actual geographic variation. A closer examination of these results, however, shows that they do not reflect a spatial variation of Hg level in the environment, since the rate of MeHg accumulation (the slope of the accumulation kinetic, Fig. 4) is similar in all zones. The differences of Hg concentration were in fact due to differences in background inorganic Hg concentration, as reflected by different *b* values in the $y = ax + b$ equations (Fig. 4, Table 2) and by the lower %MeHg content in the sardines with the highest ΣHg concentration, namely

pilchards from the northern zone: 86% MeHg instead of 91 – 95% in the other zones.

Total Hg concentration was higher in liver than in other tissues (Table 1). The ratio of Hg in liver to that in muscle was 1.4 for *Sardinella aurita* and 1.7 for *Sardina pilchardus*, similar to the ratio found in other fish species (see Thompson, 1990).

As for total mercury, an increase in MeHg concentration in muscle with increasing length was noted for both species (Fig. 2), in good agreement with earlier results for other fish species (Capelli *et al.*, 1987;

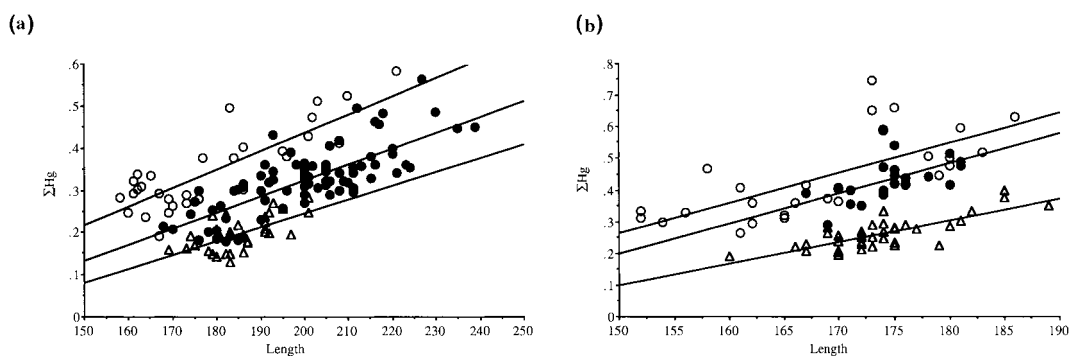


Fig. 4 Correlation between total Hg in muscle of Tunisian sardines ($\mu\text{g g}^{-1}$ dw) and total body length (mm). (a) *Sardinella aurita*, (b) *Sardina pilchardus*; circles: northern zone; dots: eastern zone; triangles: southern zone.

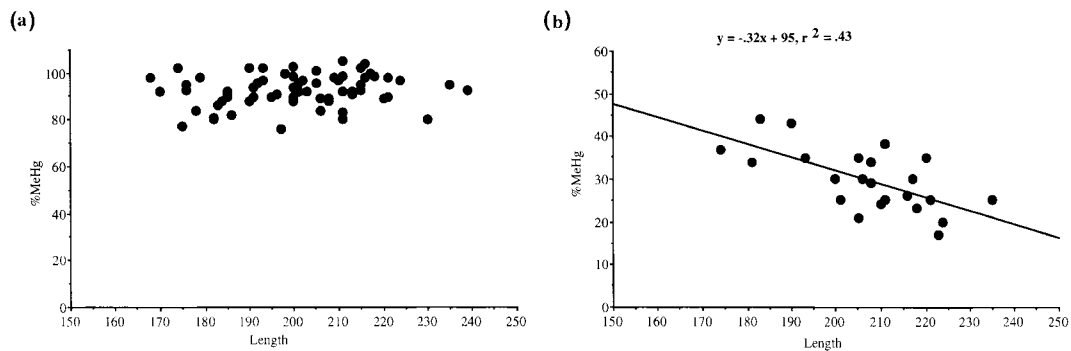


Fig. 5 Correlation between relative MeHg concentration and total body length (mm) of Tunisian sardines. (a) muscle; (b) liver (*Sardinella aurita*, eastern zone).

TABLE 3

Total mercury concentration ($\mu\text{g g}^{-1}$ dw) in *Sardina pilchardus* from Tunisia and other mediterranean regions.

Zone	n	Body weight (g)			ΣHg			Ref.
		Mean	Median	Range	Mean	Median	Range	
North of Tunisia	27	37	37	26–53	0.44	0.41	0.27–0.75	a
East of Tunisia	22	40	40	33–48	0.43	0.42	0.29–0.59	a
South of Tunisia	38	33	33	30–45	0.26	0.26	0.19–0.40	a
Thyrrhenian Sea	53		40	30–55		1.06	0.55–2.05	b
La Spezia	26	35		26–50	0.96		0.48–1.43	c
Maddalena	9	47		42–57	1.3		0.70–2.07	c
Strait of Gibraltar ^a	27		35	30–55		0.22	0.09–0.47	b

^a Atlantic part.

(a) This study, (b) Bernhard (1985), (c) Baldi and Renzoni (1980).

Thibaud and Noel, 1989; Lansens *et al.*, 1991; Holsbeek *et al.*, 1997; Joiris *et al.*, 1997). The percentage of MeHg was high and constant in muscle, between 85% and 97% for all regions and both species (Fig. 5a). In contrast, the MeHg concentration in liver was almost not increasing with length (Fig. 3), resulting in relative MeHg concentrations decreasing with length, from 50% to 20% (Fig. 5b). This might reflect the existence of a slow mineralisation process in the liver. The same observation was made for the liver of *Sardina pilchardus* by Aboul-Dahab *et al.* (1986). The percentage of MeHg in the gonads for *Sardinella aurita* varied strongly (22–80% with a mean value of 50%), and was relatively high compared with liver, so that spawning could be considered a potential excretion route of mercury for female fish. The same observation was made for organochlorines in other fish (Cubit *et al.*, 1976).

The mercury concentration found in this study was twice to four times lower than in other areas of the northern Mediterranean (Table 3); these differences were not due to important differences in size nor weight distribution, and thus reflect actual geographic variations.

Conclusions

Total mercury concentration in *Sardinella aurita* and *Sardina pilchardus* was positively correlated with length,

reflecting an accumulation of Hg with time (considering length as directly depending on age). Mercury is mostly in the organic form in the muscle (>85% MeHg), and mainly inorganic in liver (30% MeHg). The decrease of liver relative MeHg concentration with age (i.e. with length) might reflect the existence of a slow methylmercury mineralization process.

A similar geographical trend of total mercury is observed for both species, the northern and the eastern specimens containing more mercury than the southern ones, confirming the existence of different sardine stocks (Kortas, 1981; Ben Hassine *et al.*, 1990).

Mercury levels in the Tunisian sardines were twice to four times lower than those from the northern Mediterranean regions. Within the Tunisian zone, one could have expected the eastern region to be less contaminated, taking into account the anthropogenic input. Besides the anthropogenic input, however, the natural sources should not be forgotten: the northern and eastern areas are situated near mercuryiferous belts and previous mining areas. Another source of mercury in the eastern zone could be an atmospheric input from Mount Etna volcano and high industrial activities along the Italian coast (Decadt, 1985).

The maximum Hg content was $0.17 \mu\text{g } \Sigma\text{Hg g}^{-1}$ wet weight (1.7 times the mean), corresponding to $0.15 \mu\text{g MeHg g}^{-1}$ wet weight. For a person eating 100–150 g of sardines daily, the maximum amount of MeHg ingested

is of 15–22 µg daily (105–153 µg weekly), a value of the same order of magnitude as the 200 µg permissible tolerable weekly intake (PTWI) for methylmercury proposed by World Health Organisation (WHO, 1978; 1989).

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