

## Inorganic mercury and methylmercury in surface sediments and mussel tissues from a microtidal lagoon (Bizerte, Tunisia)

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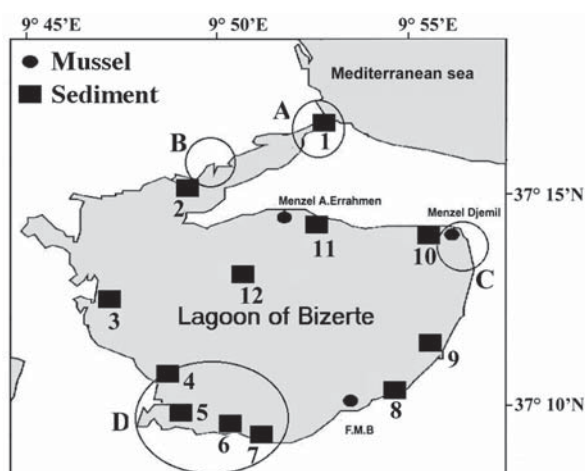
**Abstract.** The aim of this study was to investigate the distribution of mercury compounds in marine sediments and mussel tissues collected in the lagoon of Bizerte, Tunisia, during two seasons (summer and winter). Inorganic mercury ( $\text{Hg}^{2+}$ ) concentrations in sediments were found to be highly variable, ranging from 0.04  $\text{nmol.g}^{-1}$  to 3.22  $\text{nmol.g}^{-1}$  (dry weight) with a mean value of 0.52  $\text{nmol.g}^{-1}$ . Anthropogenic sources of  $\text{Hg}^{2+}$ , most probably metallurgy or tire production industries, have been evidenced. The mean concentration of monomethylmercury ( $\text{MeHg}^+$ ) in the surface sediments is 2.32  $\text{pmol.g}^{-1}$  ranging from below the detection limit (0.45  $\text{pmol.g}^{-1}$ ) to 14.6  $\text{pmol.g}^{-1}$ . No significant variation was observed between winter and summer seasons for both mercury species concentration in the sediments. The  $\text{Hg}^{2+}$  concentrations in mussel tissues are also variable, ranging from 0.007 to 1.347  $\text{nmol.g}^{-1}$  (dry weight). The mean concentration is 0.70  $\text{nmol.g}^{-1}$ . In these tissues,  $\text{Hg}^{2+}$  is generally the major compound, making up ca. 88% of total mercury concentrations. However, methylmercury concentrations are significant and homogeneous, ranging from 62 to 121  $\text{pmol.g}^{-1}$  (mean 96  $\text{pmol.g}^{-1}$ ). The results suggest that a fraction of the inorganic mercury load in the sediments of the lagoon undergoes methylation pathways.  $\text{MeHg}^+$  produced is assimilated in the mussels more readily than  $\text{Hg}^{2+}$ .

**Keywords:** Bio-indicator; Mediterranean Sea; *Mytilus*, Pollution; Toxicity.

### Introduction

Mercury contamination and toxicity has long been of concern due to the occurrence of large-scale poisoning in the last decades. Mercury is a well known toxic element, especially in the form of monomethylmercury ( $\text{MeHg}^+$ ) compounds, which are considerably more toxic than inorganic mercury ( $\text{Hg}^{2+}$ ) (Hempel 1995; Abreu 2000). The investigation of mercury behaviour in the environment is a field of particular concern due to its wide distribution in the different environmental compartments (Craig 1986; Fitzgerald 1991). In natural conditions, mercury undergoes transformations among different chemical forms, such as elemental mercury ( $\text{Hg}^0$ ), inorganic mercury ( $\text{Hg}^{2+}$ ), monomethylmercury ( $\text{MeHg}^+$ ), dimethylmercury ( $\text{Me}_2\text{Hg}$ ) and other organomercury species. Transformation and bio-availability of mercury in the environment as well as its physico-chemical and toxicological properties are highly dependent on its chemical form. (Moore 1984; Hempel 1995). The sediments retain about 98% of the mercury compounds in aquatic ecosystems (Stein 1996). Mussels are able to filtrate large volumes of water and to concentrate chemical pollutants in their tissues (Anon. 1993; Cossa 1999). For this reason mussels are considered as good bio-indicator organisms to monitor contamination of the coastal marine environment (i.e. 'mussel watch').

The coastal zones are important from an ecological and economical point of view. However, the speciation of mercury in microtidal lagoons is still less studied. In this work the first data for  $\text{Hg}^{2+}$  and  $\text{MeHg}^+$  concentrations in surface sediments and mussel tissues from a microtidal lagoon (Bizerte, Tunisia) are reported. Attention is given to the possible anthropogenic mercury sources to the lagoon. The seasonal variation of mercury compound concentrations is also investigated.



**Fig. 1.** Map of the lagoon of Bizerte with location of the sampling stations. Different type of industrial zones are also indicated (see Text).

## Material and Methods

### Study area

The lagoon of Bizerte is the second largest lagoon in Tunisia. Its geographical situation with the sampling points is presented in Fig. 1. The surface area is 128 km<sup>2</sup> and the depth is between 3 and 12 m. The lagoon is connected to the Mediterranean Sea and to Lake Ichkeul by straight channels. The exchanges of water between the Mediterranean and the lake determines the salinity of the lagoon of Bizerte, which varies between 32.5 and 38.5 practical salinity units (psu). The water temperature is between 10 °C during winter (wet season) and 29 °C during summer (dry season).

The lagoon of Bizerte is the location of several important industrial activities. There are four main zones of anthropogenic influence, also presented in Fig. 1. In zone A oil refineries, food and ceramic industries are found; in zone B factories for cement production, treatment of metals (coppercraft, asbestos) and sprinkling beverages; in zone C ceramics and metallurgy; in zone D metallurgy (Fe, Zn, Cd, Sn, Hg), naval construction and tire production.

### Sampling and storage

Surface sediments and mussel samples (*Mytilus galloprovincialis*) were taken during two sampling campaigns (August 1999 and January 2000) on board of a research vessel. The sediments and the organic part of the mussels were frozen on board and later freeze-dried for 48 h, ground and stored in double Ziplock plastic bags until analysis.

### Analytical procedures

The methods for the determination of mercury compounds were adapted from Tseng (1997a, b) and Stoichev (2002).

#### Analysis of sediments

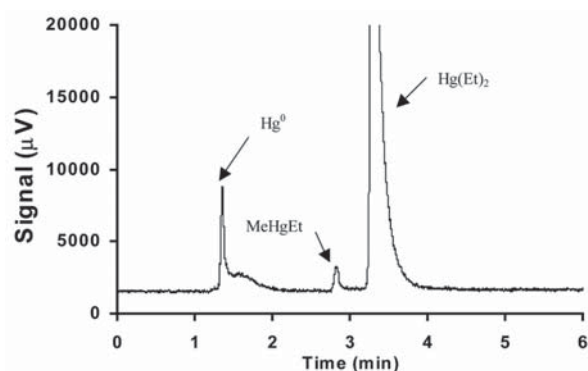
**Mercury compound determination.** A sample of ca. 0.5 g of homogenized dry sediment and 10 ml of 6N HNO<sub>3</sub> solution were exposed to microwave irradiation at 60 W for 3 min. After extraction, ca. 90% of the total Hg<sup>2+</sup> can be recovered (Tseng 2001). The analysis of the extracts was performed by hyphenated system combining ethylation on-line with cryogenic trapping, gas chromatography and atomic fluorescence spectrometry. Blanks were run after each three analyses to check for 'memory effects'.

A certified reference sediment for MeHg<sup>+</sup> from the International Atomic Energy Agency, IAEA 356, was used for quality control procedure. The results (MeHg<sup>+</sup> 27.86 ± 0.33 picomol per gram (pmol.g<sup>-1</sup>); *n* = 3) showed good agreement with the certified value (MeHg<sup>+</sup> 27.30 ± 1.9 pmol.g<sup>-1</sup>). The detection limits of the Eth-CT-GC-AFS procedure are 0.5 pmol.g<sup>-1</sup> for MeHg<sup>+</sup> and 1.9 pmol.g<sup>-1</sup> for Hg<sup>2+</sup>. The error of the determination is estimated as relative percent deviation (RPD), based on duplicate analysis of estuarine sediments. For concentration of Hg<sup>2+</sup> in the samples (*n* = 8) from 0.5 to 3.2 nanomol per gram (nmol.g<sup>-1</sup>) the average RPD is 5%. For MeHg<sup>+</sup> with concentration in the samples (*n* = 5) from 0.9 to 12 pmol.g<sup>-1</sup> mean RPD was 16%.

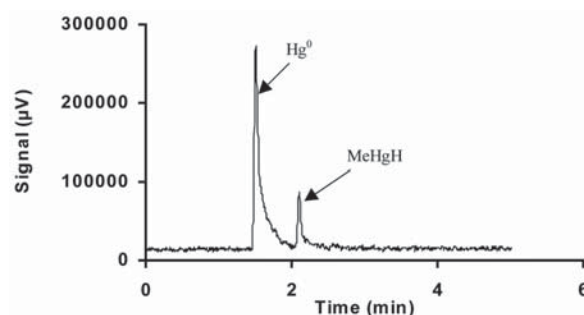
**Determination of the grain-size distribution.** One aliquot (not frozen) of the wet sediment samples was used in the laboratory for the determination of the grain size distribution by wet sieving with a series of sieves (500 and 100 μm). Each fraction was taken separately and dried until constant weight. In this study we will only consider the fraction smaller than 100 μm, which would represent the silt and clay particles in the sample.

#### Analysis of biological tissues

A sample of 0.2 g of freeze-dried tissue and 5 ml of 25% tetramethylammonium hydroxide (TMAH) solution were exposed to microwave irradiation at 45 W for 2.5 min. The extracts were analysed by the same hyphenated system as for the sediment but with hydride generation (HG) as derivatization reaction. The accuracy was checked in an intercomparison exercise, organized by the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium). In T-38 (oyster tissue) the concentration for MeHg<sup>+</sup> obtained, 0.255 ± 0.015 nmol.g<sup>-1</sup>, agrees with the recommended value of 0.284 ± 0.030 nmol.g<sup>-1</sup>. The concentration of total



**Fig. 2.** Chromatogram of a sediment extract (Sample 3 in winter, Table 1).



**Fig. 3.** Chromatogram of a mussel extract (MA in winter, Table 2).

mercury ( $\text{Hg}^{2+} + \text{MeHg}^+$ ) obtained for T-38 is  $0.50 \pm 0.04 \text{ nmol.g}^{-1}$ , comparable with the indicative value of  $0.60 \pm 0.05 \text{ nmol.g}^{-1}$ . The detection limits of the HG-CT-GC-AFS procedure are  $0.1 \text{ pmol.g}^{-1}$  for  $\text{MeHg}^+$  and  $2.5 \text{ pmol.g}^{-1}$  for  $\text{Hg}^{2+}$  (Stoichev 2002).

## Results and Discussion

### Mercury compound concentrations in surface sediments

The chromatogram of sediment extract is presented in Fig. 2 which shows the different mercury compounds as  $\text{Hg}^0$ ,  $\text{MeHgEt}$  and  $\text{Hg(Et)}_2$ . The results for the mercury compound concentrations, as well as the percentage of the fine fraction ( $F < 100 \mu\text{m}$ ) are presented in Table 1.

The mean concentration of  $\text{Hg}^{2+}$  in the winter sample was  $0.64 \pm 0.07 \text{ nmol.g}^{-1}$  and in the summer sample  $0.40 \pm 0.01 \text{ nmol.g}^{-1}$ , with no significant difference between the seasons (two-tailed  $t$ -test;  $p < 0.1$ ). The standard errors of the population are not equal ( $F$ -test) therefore they should be considered in the  $t$ -test for comparison of the mean values. The concentrations are highly variable ranging from  $0.04$  to  $3.22 \text{ nmol.g}^{-1}$  and do not depend on the percentage of the fine fraction ( $F < 100 \mu\text{m}$ ). This result is surprising, since the concentrations of the micropollutants in the sediments are usually higher, when the fine fraction increases. It is therefore possible that point sources of  $\text{Hg}^{2+}$  occur in the lagoon of Bizerte. During winter, the most polluted stations were 4, 3 and 2, with  $3.22$ ,  $1.12$  and  $0.76 \text{ nmol.g}^{-1}$ , respectively; the highest summer values were found for stations 11 and 4, with  $1.54$  and  $0.73 \text{ nmol.g}^{-1}$ , respectively. The sediments from these stations can be consid-

**Table 1.** Mercury compound concentrations and fine fraction ( $< 100\mu\text{m}$ ) content in surface sediments from the lagoon of Bizerte in winter and summer ( $\pm$  Standard Error).

Sample	$F < 100 \mu\text{m}$ (%)	$\text{Hg}^{2+}$	$\text{MeHg}^+$	$\frac{\text{MeHg}^+}{\sum \text{Hg}} (\%)$ winter	$\text{Hg}^{2+}$	$\text{MeHg}^+$	$\frac{\text{MeHg}^+}{\sum \text{Hg}} (\%)$ summer
		( $\text{nmol g}^{-1}$ ) winter	( $\text{pmol g}^{-1}$ ) winter		( $\text{nmol g}^{-1}$ ) summer	( $\text{pmol g}^{-1}$ ) summer	
1	22.0	0.11	4.76	4.1	0.26	1.83	0.7
2	5.3	0.76	2.02	0.3	0.27	4.06	1.5
3	13.9	1.12	14.60	1.3	0.35	3.54	1.0
4	16.6	3.22	< DL*	0	0.73	< DL	0.0
5	17.6	0.33	< DL	0	0.44	< DL	0.0
6	4.8	0.32	3.72	1.1	0.34	2.72	0.8
7	23.1	0.28	< DL	0	0.25	1.23	0.5
8	29.7	0.20	< DL	0	0.06	3.45	5.7
9	27.5	0.42	3.54	0.8	0.04	2.30	5.8
10	16.0	0.32	< DL	0	0.26	< DL	0.0
11	15.7	0.07	< DL	0	1.54	7.19	0.5
12	19.2	0.48	0.63	0.1	0.30	< DL	0.0
<b>Mean</b>	<b><math>17.6 \pm 5.2</math></b>	<b><math>0.64 \pm 0.07</math></b>	<b><math>2.44 \pm 1.61</math></b>	<b><math>0.6 \pm 0.13</math></b>	<b><math>0.40 \pm 0.01</math></b>	<b><math>2.19 \pm 0.43</math></b>	<b><math>1.4 \pm 0.4</math></b>

**Table 2.** Concentrations of the mercury compounds in mussel tissues (dry weight) from the lagoon of Bizerte in winter and summer ( $\pm$  Standard Error). MA = Menzel Abderrahmen site; MJ = Menzen Jemil site; FMB = Farm of Mollusk Bivalves.

Sample	IHg(II)	MMHg	$\frac{\text{MMHg}}{\sum \text{Hg}} (\%)$	IHg(II)	MMHg	$\frac{\text{MMHg}}{\sum \text{Hg}} (\%)$
	(nmol g <sup>-1</sup> )	(pmol g <sup>-1</sup> )		(nmol g <sup>-1</sup> )	(pmol g <sup>-1</sup> )	
	winter	winter	winter	summer	summer	summer
MA	1.194	121.1	9.2	0.613	61.9	9.2
MJ	0.210	93.0	30.7	0.007	87.0	92.6
FMB	0.835	115.6	12.2	1.347	94.8	6.6
<b>Mean</b>	<b>0.75 <math>\pm</math> 0.12</b>	<b>110 <math>\pm</math> 15</b>	<b>-</b>	<b>0.70 <math>\pm</math> 0.23</b>	<b>81.2 <math>\pm</math> 17.2</b>	<b>-</b>

ered as moderately polluted, possibly due to some of the activities in the industrial zone D; the concentrations for the remainder of the samples are close to the geochemical background value, generally considered to be 0.2  $\mu\text{g/g}$ .

The mean concentrations of MeHg<sup>+</sup> are statistically the same – 2.44  $\pm$  1.61 pmol.g<sup>-1</sup> for the winter and 2.19  $\pm$  0.43 pmol.g<sup>-1</sup> for the summer (two-tailed *t*-test; *p* < 0.1). Therefore, the higher temperature during summer does not lead to a significant increase of the MeHg<sup>+</sup> in the surface sediments. However, the mean of the percentage of MeHg<sup>+</sup> with respect to total mercury is 0.6  $\pm$  0.13% during winter and 1.4  $\pm$  0.4% during summer, possibly due to the enhanced microbial activity during the warmer season. The concentrations of MeHg<sup>+</sup> are variable, ranging from below the detection limit to 14.60 pmol.g<sup>-1</sup>. They are correlated neither with Hg<sup>2+</sup> nor with the percentage of the fine fraction (*F* < 100  $\mu\text{m}$ ).

#### Mercury compound concentrations in mussels

A chromatogram of a mussel extract is shown in Fig. 3. The mercury compound concentrations in mussel tissue sampled from Menzel Abderrahmen (MA), Menzel Jemil (MJ) and the Farm of Mollusk Bivalves (FMB) are presented in Table 2.

**Inorganic mercury.** The mean concentration of Hg<sup>2+</sup> in the mussel tissue is 0.70  $\pm$  0.17 nmol.g<sup>-1</sup>, similar to Hg<sup>2+</sup> content in the sediments. There is not clear seasonal trend and the concentrations are spatially highly variable ranging between 0.007 and 1.347 nmol.g<sup>-1</sup>. The lowest concentrations, irrespective of the season, are measured for MJ samples.

**Monomethylmercury.** The mean concentration of MeHg<sup>+</sup> in the mussels is 95.56  $\pm$  16.04 pmol.g<sup>-1</sup> or ca. 40 $\times$  higher than the average MeHg<sup>+</sup> content of the surface sediments. The percentage of MeHg<sup>+</sup> with respect to the total Hg is variable, ranging from 6.6 to 92.6%, comparable to previous model experiments per-

formed with mussels (Gagnon et al. 1997). The same authors have measured that the assimilation efficiency of MeHg<sup>+</sup> in particle-feeding mussels is higher than that of Hg<sup>2+</sup>. However, mussels usually accumulate less methylmercury than predatory fish, which are situated in the upper part of the food chain. The concentration limit established by the World Health Organization (WHO) is 0.5  $\mu\text{g/g}$  (Anon. 1999). Consequently, for filter-feeding organisms, such as mussels, the concentrations of Hg species determined in the Bizerte Lagoon do not present a significant toxicological risk for human consumption.

We conclude that the surface sediments and mussel tissues from Bizerte lagoon show a relatively low level of mercury contamination. The comparison of the results in this study with other similar microtidal lagoons is difficult due to the lack of available data. The concentrations of mercury compounds in the surface sediments from the lagoon of Bizerte are comparable to those found in the lagoon of Varna in Bulgaria (Stoichev 2002) and much smaller than the values reported for the Ravenna lagoon in Italy with its high concentration of anthropogenically derived mercury in sediments (Fabbri et al. 2001).

However, anthropogenic sources of Hg<sup>2+</sup>, most probably from metallurgy or tire production industries have been evidenced. One part of Hg<sup>2+</sup> is methylated in the sediments and is assimilated by the mussels from the lagoon of Bizerte more readily than Hg<sup>2+</sup>. Finally, MeHg<sup>+</sup> contamination of biological organisms is probably limited because concentrations observed in mussel tissues are rather low when compared to other coastal environments (Cossa 1999).

**Acknowledgements.** T. Stoichev acknowledges the scholarship of the French government for his Ph.D. study. N. Mzoughi and T. Stoichev wish to thank all the members of the Laboratory of Bioinorganic Chemistry and Environment at the University of Pau (France) for their help.

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Received 28 May 2002;

Accepted 7 December 2002;

Final revision received 20 December 2002;

Coordinating Editor: Chr. Pergent-Martini.