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# Mercury in fishes from Augusta Bay (southern Italy): Risk assessment and health implication

M. Bonsignore [a], D. Salvagio Manta [b], E. Oliveri [a], M. Sprovieri [a,*], G. Basilone [a], A. Bonanno [a], F. Falco [a], A. Traina [a], S. Mazzola [a]

[a] Institute for Coastal and Marine Environment (IAMC) – CNR, Via del Mare, 3, 91021 Torretta Granitola, Campobello di Mazara (TP), Italy
[b] Institute for Coastal and Marine Environment (IAMC) – CNR, Calata Porta di Massa, 80100 Naples, Italy

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ABSTRACT

Our study reports on the total mercury (HgT) concentrations measured in the muscles and livers of several benthic, demersal and pelagic fish species caught inside and outside of Augusta Bay (southern Italy), a semi-enclosed marine area, highly contaminated by the uncontrolled (since the 1950s to 1978s) discharge of the largest European petrochemical plant. Mercury levels in fish tissues are discussed with regard to specific habitat, size and/or age of the specimens and HgT distribution in the bottom sediments. Results suggest a still active Hg release mechanism from the polluted sediments to the marine environment. Also, the high HgT concentrations measured in fishes caught in the external area of the bay imply a potential role of Augusta Bay as a pollutant source for the Mediterranean ecosystem. Finally, values of hazard target quotient (THQ) and estimated weekly intake (EWI) demonstrate that consumption of fishes caught inside the bay represents a serious risk for human health. Also, data indicate that intake of fishes caught from the external area of the bay, especially for that concern demersal and benthic species, could be represent a significant component of risk for the local population.



## 1. Introduction

The city of Augusta, located in the SE of Sicily (southern Italy), has experienced an important industrialisation phase since the early 60s. This has led to the creation of several chemical and petrochemical plants and oil refineries resulting in a severe pollution of the surrounding environment. In particular, the petrochemical industry in Augusta Bay is one of the largest in Europe with the most important chlor-alkali plant in Italy (Le Donne and Ciafani, 2008). Its activity started in 1958 and stopped in 2005, with production of chlorine and caustic soda by electrolysis of sodium chloride aqueous solution in electrolytic cells with a graphite anode and metallic mercury cathode. Uncontrolled chemical discharge of Hg occurred in the Augusta Bay until 1978, when restrictions were imposed by the Italian legislation.

In the last decade, several studies have provided detailed information on the pollution levels and risks for human health of resident populations of Augusta Bay (ICRAM, 2005; Ausili et al., 2008; Di Leonardo et al., 2007, 2008; ENVIRON International Team, 2008; Ficco et al., 2009; Sprovieri et al., 2011). Sprovieri et al. (2011) reported high-resolution maps of HgT distribution from superficial sediments collected in 2005, highlighting extremely high concentrations (ranging between 0.1 and 527.3 mg $kg^{-1}$) and speculating on the key role that Augusta Bay could play in exporting Hg to the Mediterranean Sea, as an effect of the outflow intercepted by the Levantine Intermediate Waters (LIWs). Also, data recently collected by ICRAM (2008), ENVIRON International Team (2008) and Ausili et al. (2008), demonstrated HgT transfer from the abiotic system (sediments and seawater) to fishes (top predators and filter-feeders) and documented significant health risks associated with the consumption of fish caught in the area. Toxicological Hg effects were also evaluated on mussels and red mullet by micronuclei (MN) studies, which documented DNA damage (Ausili et al., 2008 and ICRAM, 2008). Finally, Tomasello et al. (2012) report on DNA genotoxic and oxidative damages in *Coris julis* specimens from Augusta Bay.

Fish food seems to constitute the main route of Hg uptake for humans (Holsbeek et al., 1996; Nakagawa et al., 1997).

Renzoni et al. (1998) demonstrated that long-term and frequent intake of fish with high Hg levels is statistically associated with a toxic risk, especially in pregnant women. A sad, famous poisoning episode occurred in the 1950s among people living around Minamata Bay (Japan), showing the irreversible neurological damage and teratogenic effects due to consummation of Hg-contaminated fish (De Flora et al., 1994). Methylmercury (MeHg) is the most toxic form, able to interfere with thiol metabolism, causing inhibition or inactivation of proteins containing thiol ligands and ultimately

* Corresponding author. Tel.: +39 092440670; fax: +39 092440445.
*E-mail address:* mario.sprovieri@iamc.cnr.it (M. Sprovieri).

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leading to mitotic disturbances (Das et al., 1982; Elhassani, 1983). Numerous recent studies indeed have concluded that the majority, if not all, of the Hg that is bioaccumulated through the food chain is as MeHg (Winfrey and Rudd, 1990; Mason and Fitzgerald, 1990, 1991; Gilmour and Henry, 1991; Horvat et al., 1999; Carbonell et al., 2009).

High mortality rates, statistical high frequency of neonatal malformations and cancerous diseases reported for resident populations around Augusta Bay (Martuzzi et al., 2006; Bianchi et al., 2004, 2006; Fano et al., 2005, 2006; Madeddu et al., 2006) definitively calls for more detailed exploration and definitive assessment of the role played by the intake of Hg-contaminated fish on the health of the consumers.

In this work, we aim to explore the effects of HgT pollution in Augusta Bay on the fish compartment, inside and outside the semi-enclosed area, and to assess the potential health risks associated with the consumption of contaminated fish.

## 2. Materials and methods

### 2.1. Sampling

Four different sampling sites were selected: two inside, and two outside of Augusta Bay (Fig. 1). Sampling outside the bay was performed during May 2001, on board of the N/O "Dallaporta", by means of a mid-water trawl-net at 50–100 m of depth in two sampling areas, in front of the Scirocco inlet (300-m wide and 13-m deep), and the Levante inlet (400-m wide and 40-m deep) (Fig. 1: C1, C2). Mainly pelagic fish specimens were caught (Table 1). Sampling inside the bay was performed during May 2012 by means of a fishing boat equipped with a gillnet wall, positioned at the bottom (mean depth = 20–25 m) (Fig. 1: C3, C4). Several specimens of benthic and demersal fishes were collected. From the two sampling activities, a total of 227 fish specimens were collected: 107 from mid-water sampling (outside the bay) and 120 from bottom-water sampling (inside the bay). Moreover, specimens of *Engraulis encrasicolus* ($n = 38$) were caught from the unpolluted marine area of Marsala (western Sicily) (Fig. 1), during July 2001, on board of a fishing boat equipped with a purse seine net. After collection, fishes were stored at −20 °C until biological and chemical analyses were performed in the laboratories of biology and biogeochemistry at the Institute for Coastal and Marine Environment (CNR) of Capo Granitola.

### 2.2. Biological data and tissue collection

The total length (TL) of each specimen was measured. Muscle and liver tissues were collected from each organism, using plastic materials cleaned with $HNO_3$ (10%) and MilliQ water, in order to avoid Hg contamination. Tissues were stored at −20 °C until analysis. Otoliths were extracted from anchovy and sardine specimens for age determination. Readings and interpretation of otolith increment growths were carried out by transmitted visible lights based on higher-resolution microscopy (20–25× magnification) (Campana et al., 1987; Nielsen, 1992). The procedure adopted for European anchovy age determination follow Uriarte et al. (2007) and La Mesa et al. (2009).

### 2.3. Chemical analyses

Total mercury concentrations (HgT) in tissues were measured using a direct mercury analyser (Milestone_DMA-80), atomic absorption spectrophotometer, according to analytical procedures reported in EPA 7473. Briefly, approximately 0.1 g of fresh tissue was loaded in nickel boats and transferred to the DMA-80 system. In order to minimise contamination risks, acid-cleaned laboratory materials were used during sample preparation and analyses. A Reference Standard Material (TORT-2; HgT certificate value = 0.27 ± 0.06 $\mu g\ g^{-1}$) was analysed to assess analytical accuracy (estimated to be ~3%) and precision (routinely better than 4%; RSD%, $n = 3$). Finally, duplicated samples (about 20% of the total number of samples) were measured to estimate reproducibility, which resulted in better than 7%.

### 2.4. THQ and EWI calculation

Target hazard quotient (THQ) and estimated weekly intake (EWI) were calculated for muscles of fishes caught inside and outside the bay.

The target hazard quotient was calculated according to the US EPA (1989) method and it is described by the following equation:

$$THQ = \left(\frac{EF \times ED \times FIR \times C}{RFD \times WAB \times TA}\right) \times 10^{-3}$$

where EF is exposure frequency (365 days/year); ED is the exposure duration (70 years), equivalent to the average lifetime; FIR is the food ingestion rate (36 g/person/day) (FAO, 2005); $C$ is the metal concentration in seafood ($\mu g\ g^{-1}$); RFD is the USEPA's reference dose (0.1 $\mu g$ Hg kg $bw^{-1}\ d^{-1}$) (http://cfpub.epa.gov) or acceptable daily intake determined by WHO (0.23 $\mu g$ Hg kg $bw^{-1}\ d^{-1}$) (http://apps.who.int); WAB is the average body weight (60 kg), and TA is the average exposure time for no carcinogens (365 days/year × ED).

The THQ was calculated for all the studied species in the Augusta Bay using the US-EPA's reference dose (THQa) and the acceptable daily intake determined by the WHO (THQb). In particular, we assumed that the measured mercury is integrally in its methylated form (Winfrey and Rudd, 1990; Mason and Fitzgerald, 1990, 1991; Gilmour and Henry, 1991; Horvat et al., 1999; Carbonell et al., 2009).

The estimated weekly intake (EWI) was calculated by multiplying the HgT concentration (C) times by the weekly dietary intakes (FIR × 7) and reporting to the average body weight (WAB).

Finally, mean THQ and EWI values were calculated for each studied species. Also, considering that fishing activity within the bay has been interdicted since 2007 (Order No. 73/07), data relative to fishes from inside and outside the bay were processed separately.

## 3. Results

### 3.1. Biological features

The fish caught from bottom-water sampling (inside the bay) consisted of 2 pelagic, 106 demersal and 16 benthic specimens, while specimens from mid-water sampling (outside the bay), consisted of 103 pelagic, 3 demersal and 1 benthic (Table 1). A total of 21 different species were recognised. The number of specimens per species and total length ranges are shown in Table 2. Almost all the caught species, in particular, *E. encrasicolus, Sardina pilchardus, Boops boops, Mullus barbatus* and *Illex coindetii*, are typical of the Mediterranean Sea and are commercially relevant to Italian fishing (Irepa, 2010). Only one specimen was found to belong to a so-called alien species, specifically *Sphyraena sphyraena*. This is a typical species of the tropical seas, today present also in the Mediterranean Sea (Streftaris and Zenetos, 2006).

### 3.2. Total mercury concentrations (HgT)

Total mercury concentrations measured in tissues from pelagic, demersal and benthic fishes, caught inside and outside of Augusta Bay, are graphically summarised in Fig. 2a and b. Mercury mean values calculated for each species, together with available comparative data from the literature and HgT content measured in anchovies from Marsala, are presented in Table 2.

Mercury concentrations ranged between 0.021 and 2.709 $\mu g\ g^{-1}$ in muscles (Fig. 2a) and between 0.029 and 9.720 $\mu g\ g^{-1}$ in livers (Fig. 2b). The HgT content in liver is from 1.5 to 6 times higher than that measured in muscles from the same specimens (Table 2). The highest HgT values were found in species caught inside the bay: 2 demersal specimens, a specimen of *Diplodus vulgaris* (HgT in liver = 4.979 $\mu g\ g^{-1}$) (extreme point in Fig. 2b) and a specimen of *Serranus scriba* (HgT in muscle = 2.709 $\mu g\ g^{-1}$) (extreme point in Fig. 2a), a large pelagic specimen of *S. sphyraena* (HgT = 9.720 and 2.269 $\mu g\ g^{-1}$ in liver and muscle, respectively) (Table 2) and a benthic specimen of *Murena helena* (HgT = 2.638 $\mu g\ g^{-1}$ in muscle) (Table 2). However, these very high levels represent outliers of the whole dataset (Fig. 2a and b). The highest non-outlier values refer once again to specimens caught inside the bay and specifically to benthic species (Fig. 2a and b). In particular, *Scorpanea scrofa* and *Scorpanea notata* show the highest HgT mean concentrations for both liver (1.638 and 2.339 $\mu g\ g^{-1}$, respectively) and muscle (1.082 and 1.341 $\mu g\ g^{-1}$, respectively) (Table 2). The lowest non-outlier ranges were found in pelagic specimens caught outside the bay (0.021–0.167 $\mu g\ g^{-1}$ for muscles and 0.029–0.5708 for livers) (Fig. 2a and b), and the HgT mean values measured in the different studied species are substantially comparable (Table 2). Finally, data for demersal species from the