SUPPLEMENTARY MATERIAL

Mercury accumulation in Mediterranean Fish and Cephalopods Species of Sicilian coasts: correlation between pollution and the presence of Anisakis parasites

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Abstract

The aim of this study was to investigate mercury accumulation in some species, caught in Mediterranean Sea, in the period between may-december 2015, and to compare it to Anisakis parasites presence. The samples were examined by direct mercury analyzer (DMA-80) for their Hg levels. The metal concentration was compared to the presence or the absence of Anisakis parasites. Significant differences in Hg concentration in analyzed samples were observed. The low infested fishes contained from 1 to 6 larvae of parasites instead the high-infested one had from 7 to 83 larvae.

Keywords Anisakidae, fish product, Hg, pollution, heavy metals

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Sampling area and storage

In may-december 2015 total of 80 samples of fish and cephalopod were taken from 2 different fishing areas: Sciacca (FAO zone 37.2.2) and Palermo (FAO zone 37.1.3) were caught during several trawl surveys. Fishes belonged to the following species: *Mullus barbatus*, *Loligo vulgaris*, *Trachurus trachurus*, *Scomber scombrus*, *Sardina pilchardus*, *Conger conger*, *Todarodes sagittatus*, *Merluccius merluccius*. Samples were measured and muscle tissue was removed from the organisms in each pool and frozen at -25°C until the analysis for the determination of mercury was carried out.

Anisakis family assessemet.

The larvae were isolated and washed in physiological saline. They were examined microscopically and identified on the basis of the morphological characteristics described previously (Smith, 1983).

Chemical analyses

About $0,09 \pm 0,01$ g (w/w) of each sample was added into nickel boats and introduced in a DMA-80[®] analyzer (Milestone, Bergamo, Italy), where it was incinerated. The DMA-80 is based on the principles of sample thermal decomposition, mercury amalgamation and atomic absorption detection.

The lamp emits a light beam at a specific wavelength which passes through the fumes produced by the decomposed samples and it is detected by an UV detector photodiodes. The amount of mercury present in the samples was detected and quantified according the calibration curve previously created. Standards for the instrument calibration were prepared on the basis of mono-element certified reference solution ICP standard (VWR, Milan, Italy). Mercury was selectively trapped, in a separate furnace, through gold amalgamation. Combusted by-products were flushed off. The amalgamation furnace was heated and mercury was rapidly released. Mercury was flowed out through the carrier gas into a unique block with a dual-cell positioned along the optical path of the spectrophotometer, where it was quantitatively measured by atomic absorption at 253,65 nm.

Instrumental conditions and Calibration curves

After the drying, conducted in two steps the first for 10" and the second for 60" at 200°C, the sample decomposition was performed for 60" at high temperature (> 600° C) thanks to the total mercury release. Another step at 650°C for 60" ensured the complete decomposition. In order to obtain a calibration curves the reference solutions of known concentration were used (Tab.1S). The instrument was equipped with dual-cells for different ranges of concentration: the first with a longer optical path for low concentrations and the second shorter for high concentrations.

Table S1. Values obtained during the construction of the calibration curve.

Hg [ng]	Peak	Cell 1
0.0000	0.0015	
0.5080	0.0963	
0.9960	0.1778	
2.0252	0.3188	
3.2403	0.4723	
5.1643	0.3053	Cell 2
10.2273	0.5394	
13.1200	0.6371	
15.1890	0.7548

Figure S1.

A. Box plot shows the distribution of concentrations of mercury in the three different groups of samples grouped by their degree of infestation. (N=non-infested, L= low infested, H= high infested).

B. Box plot shows the distribution of concentrations of mercury in the three different groups of samples, according to lifestyle. (P=pelagic, B=benthic, D=demersal);

Reference

Smith JW. 1983. *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878) (*Nematoda: Ascaridoidea*): morphology and morphometry of larvae from euphausiids and fish, and a review of the life-history and ecology. J Helminthol. 57:205–224.