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Investigation of Hg Content by a Rapid Analytical Technique in Mediterranean Pelagic Fishes

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Abstract: Mercury (Hg) fish and seafood contamination is a global concern and needs worldwide sea investigations in order to protect consumers. The aim of this study was to investigate the Hg concentration by means of a rapid and simple analytical technique with a direct Mercury Analyzer (DMA-80) in pelagic fish species, *Tetrapturus belone* (spearfish), *Thunnus thynnus* (tuna) and *Xiphias gladius* (swordfish) caught in the Mediterranean Sea. Hg contents were evaluated also in *Salmo salar* (salmon) as pelagic fish not belonging to the Mediterranean area. The results obtained were variable, ranging between 0.015–2.562 mg kg^{-1} for *T. thynnus* species, 0.477–3.182 mg kg^{-1} for *X. gladius*, 0.434–1.730 mg kg^{-1} for *T. belone* and 0.004–0.019 mg kg^{-1} for *S. salar*, respectively. The total Hg tolerable weekly intake (TWI) and tolerable weekly intake % (TWI%) values according to the European Food Safety Authority (EFSA) were calculated. The results highlighted that the pelagic species caught in the Mediterranean Sea should be constantly monitored due to their high Hg contents as well as their TWI and TWI% with respect to *S. salar* samples.

Keywords: mercury; pelagic fish; direct mercury analyzer; Mediterranean Sea; tolerable weekly intake

1. Introduction

The Mediterranean diet with its constituents is nowadays recognised to be one of the most healthy diets worldwide [1,2]. In addition to the several plant-based foods promoted by this diet, a moderate consumption of fish is important for its contribution of healthy nutrients such as Omega 3, or "n-3 long-chain polyunsaturated" fatty acids but with low levels of saturated fatty acids [3–5]. However, according to the European Food Safety Authority (EFSA), the consumption of seafood and fish is relevant for the assessment of dietary exposure to mercury from food. Fish meat is considered among the most important contributors of mercury intake for people, from children to adults [3].

Mercury (Hg) commonly known also as quicksilver, is a heavy, silvery-white liquid metal chemical element, highly toxic to the environment and living creatures. Indeed, contamination of fish species is a potential health hazard for humans as the last component of the food chain. In this context, since the Mediterranean Sea is a closed sea, with a limited exchange of water mainly coming from the Atlantic Ocean and with a minor contribution from the Black Sea, it results to be one of the geographic areas of concern for Hg concentrations due to the ever-increasing industrialization processes [6–11]. People may be exposed to inorganic Hg by their employment or pollution, and exposed to organic Hg (methylmercury, CH_3Hg^+) predominantly through the consumption of seafood [9,10]. The pelagic species represent a significant fishing resource and a widely consumed food in the human diet because of the traditional recipes of the Mediterranean diet and also as sushi and sashimi, typical of Japanese cuisine, have rapidly spread through Western countries during the last few decades [4,5,12]. In the light of all the above, the purpose of this study was to carry out an investigation using a rapid analytical technique on the Hg content in some of the most representative pelagic fish species, in particular, *Tetrapturus belone*, *Thunnus thynnus* and *Xiphias gladius* (spearfish, tuna and swordfish, respectively) caught in the Mediterranean Sea and *Salmo salar* (salmon) as a commercial pelagic fish not belonging to the Mediterranean area.

The Hg contents were also used to calculate the total Hg Tolerable Weekly Intake (TWI) and Tolerable Weekly Intake % (TWI%) values for each species according to the EFSA guidelines [3] to underline the potential health risk for consumers.

2. Materials and Methods

A total of 48 fishes from the Mediterranean Sea were kindly provided by the Italian Institute for Environmental Protection and Research. *Thunnus thynnus*, *Xiphias gladius* and *Tetrapturus belone* samples were collected from Cape d'Orlando, Cape Rasocolmo, Patti Gulf, Tyrrenian Sea and Strait of Messina (Italy). The commercial *Salmo salar* samples were produced in the North Sea (Norway and Scotland, UK). The specimens had lengths of 15 to 240 cm and weights of 150 g to 220 kg. From each sample, an approximate amount of about 50–100 g of muscle tissue from the region around abdominal cavity was taken. Once in the laboratory, samples were frozen at −20 °C and stored until analysis.

For Hg level determination, the analyser used was a Milestone DMA-80 Direct Mercury Analyser (Milestone GmbH, Leutkirch im Allgäu, Germany). It is an innovative analytical instrument that allows for the elimination of the use of reagents, and to simultaneously obtain validated results with no digestion and chemical pre-treatment steps.

The DMA-80 has been used according to the US EPA method 7473 (mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry). Furthermore, it was compliant with the ASTM method D-6722-01 (total mercury in coal and coal combustion residues) and ASTM method D-7623-10 (total mercury in crude oil).

About 0.1 ± 0.001 g of the samples were weighted, put onto nickel vessels and introduced to the direct analyser, dried at 200 °C for 3 min, then chemically and thermally decomposed at 650 °C for 2 min. Hg content of samples was determined by measuring absorbance at 253.7 nm.

The calibration curve was constructed using standards with known concentrations of mercury, associating a value of absorbance to each known Hg concentration. The standard solutions were prepared in glass flasks (5 concentration points from 0.050 to 10 mg kg^{-1}) from the 1000 mg/L certified standard (CZECH Metrology Institute Analytika). The evaluation of the linearity was based on six injections of the standard solution. Good linearity was observed, achieving a correlation coefficient $r = 0.9996$. The detection limit (DL) and quantification limit (QL) were counted as 3 times and 6 times the standard deviation for blanks and were 0.25 μg kg^{-1} and 0.51 μg kg^{-1}, respectively.

Hg content for a certified reference material TB149, a spearfish sample, is shown in Table 1. Analyses of the certified reference material TB 149 showed satisfactory recoveries of about 95% for Hg content both for total Hg and methylmercury (CH_3Hg^+). The data obtained were shown to agree with those of the certified value, thus the procedure was confirmed to be adequate for measuring Hg contents in the muscle sample collected.

The software of the instrumentation automatically calculates the total Hg levels of the sample by interpolating the absorbance values directly with the calibration curve [4].

The risk exposure to CH_3Hg^+ expressed as TWI (mg kg^{-1} b.w.) was calculated for an average serving portion of 200 g of fish for a 60 kg adult according to the following formula (Equation (1)).

$$\text{Tolerable Weekly Intake Hg (TWI)} = [\text{Hg}] \times \text{serving portion/body weight} \quad (1)$$

the corresponding weekly risk exposure to CH_3Hg^+ was calculated as TWI% of 4 mg kg^{-1} b.w. recommended by EFSA (2012).

3. Results

Table 1 shows the reported Hg content obtained for *T. thynnus*, *X. gladius*, *T. belone* and *S. salar* samples expressed in mg kg^{-1}.

Table 1. Sample information: Site of collection, length (cm) and weight (kg), concentration levels of Hg expressed as mg kg^{-1}.

Sample	Site	Length (cm)	Weight (kg)	Hg (mg kg^{-1}) [a]
TT0032	Rasocolmo Cape	26.80	0.32	0.0562 ± 0.0032
TT0033	Rasocolmo Cape	27.00	0.36	0.0833 ± 0.0035
TT005	d'Orlando Cape	20.00	0.22	0.0192 ± 0.0007
TT094	Patti Gulf	195.00	150.00	0.5570 ± 0.0413
TT0951	Patti Gulf	115.00	28.00	0.7057 ± 0.0275
TT0952	Patti Gulf	240.00	180.00	0.9057 ± 0.0514
TT0953	Patti Gulf	90.00	16.00	0.7697 ± 0.0265
TT096	Southern Tyrrhenian sea	15.40	0.15	0.0146 ± 0.0028
TT097	Southern Tyrrhenian sea	24.80	0.30	0.0348 ± 0.0140
TT158	Strait of Messina	128.00	36.00	1.3689 ± 0.0631
TT159	Strait of Messina	165.00	66.00	2.5618 ± 0.4609
XG321	Strait of Messina	173.00	74.00	2.0623 ± 0.2434
XG330	Strait of Messina	52.00	5.00	0.4775 ± 0.0287
XG343	Strait of Messina	171.00	58.00	0.8837 ± 0.0279
XG344	Strait of Messina	170.00	61.00	2.1336 ± 0.0908
XG352	Strait of Messina	161.00	55.00	2.2072 ± 0.0310
XG365	Strait of Messina	181.00	48.00	2.5557 ± 0.2700
XG367	Strait of Messina	133.50	35.00	2.0135 ± 0.1949
XG372	Strait of Messina	180.00	70.00	1.7924 ± 0.0598
XG376	Strait of Messina	180.00	65.00	1.6910 ± 0.0302
XG377	Strait of Messina	140.00	30.00	2.1496 ± 0.1989
XG379	Strait of Messina	164.00	54.00	2.9100 ± 0.0171
XG380	Strait of Messina	196.00	83.00	1.5631 ± 0.1708
XG381	Strait of Messina	180.00	75.00	1.2367 ± 0.2219
XG384	Strait of Messina	183.00	49.00	3.1819 ± 0.1949
TB 103	Strait of Messina	155.00	13.00	0.4344 ± 0.0335
TB 106	Strait of Messina	151.00	11.00	0.7986 ± 0.0889
TB 108	Strait of Messina	156.00	16.00	1.6499 ± 0.2199
TB 113	Strait of Messina	150.00	11.00	0.6695 ± 0.0563
TB 117	Strait of Messina	170.00	16.00	0.8687 ± 0.0230
TB 120	Strait of Messina	179.00	22.00	1.7303 ± 0.0268
TB 121	Strait of Messina	151.00	6.00	0.6021 ± 0.0642
TB 122	Strait of Messina	178.00	21.00	1.6086 ± 0.1290
TB 126	Strait of Messina	157.00	16.00	0.5199 ± 0.0209
TB 131	Strait of Messina	155.00	16.00	0.6017 ± 0.0433
TB 132	Strait of Messina	142.00	13.00	0.5306 ± 0.0525
TB 144	Strait of Messina	151.00	11.00	0.5693 ± 0.0534
TB 145	Strait of Messina	155.00	14.00	1.0583 ± 0.2837
TB 149	Strait of Messina	148.00	12.00	0.5559 ± 0.0228
SS1	Scotland	n.d.	n.d.	0.0188 ± 0.0007
SS2	Norway	n.d.	n.d.	0.0089 ± 0.0013
SS3	Norway	n.d.	n.d.	0.0114 ± 0.0004
SS4	Norway	n.d.	n.d.	0.0193 ± 0.0014
SS5	Norway	n.d.	n.d.	0.0091 ± 0.0003
SS6	Norway	n.d.	n.d.	0.0117 ± 0.0004
SS7	Norway	n.d.	n.d.	0.0037 ± 0.0006
SS8	Norway	n.d.	n.d.	0.0159 ± 0.0004
SS9	Norway	n.d.	n.d.	0.0087 ± 0.0005

n.d.: Not determined. [a] Mean value (n = 3).

The results were variable, ranging from 0.015–2.562 mg kg^{-1} for *T. thynnus* species, 0.477–3.182 mg kg^{-1} for *X. gladius*, and 0.434–1.730 mg kg^{-1} for *T. belone*. For *S. salar* the Hg content obtained ranged from 0.004–0.019 mg kg^{-1}. The following decreasing order among the examined pelagic species could be defined: *X. gladius* (mean 1.829 mg kg^{-1}) > *T. thynnus* (mean 1.288 mg kg^{-1}) > *T. belone* (mean 1.082 mg kg^{-1}) > *S. salar* (mean 0.012 mg kg^{-1}). The significant *p*-level below 0.05 obtained by a Kruskal-Wallis test, confirmed that the samples of *T. thynnus* and of *T. belone* have a Hg level not significantly different between them (Figure 1).

In Table 2, the ranges of Hg contents and their relative TWI value calculated in this study are shown. The weekly consumption of a portion of 200 g of the most examined pelagic fish species by a 60 kg adult body weight, point out a remarkable health risk of exposure to the toxic action of CH_3Hg^+ for *T. thynnus*, *X. gladius* and *T. belone*, as the TWI value was exceeded in many samples caught in the Mediterranean Sea. Meanwhile, no risk were observed for *S. salar* samples from the North Sea.

Table 2. Ranges and mean values of tolerable weekly intake (TWI) and relative % (TWI%) for Hg contents in analyzed fishes.

Species	N Samples	Hg				
		mg/kg	mg/kg	TWI	TWI%	TWI%
		Range Min–Max	Mean Value	Mean Value ± Dev.st	Range Min–Max	Mean Value
S. salar	9	0.004–0.002	0.012	0.0003 ± 0.001	5.10–11.28	6.97
T. belone	14	0.434–1.730	1.082	0.0200 ± 0.011	253.41–1009.35	508.23
T. thynnus	11	0.015–2.562	1.288	0.0150 ± 0.018	8.52–1494.38	375.29
X. gladius	14	0.477–3.182	1.829	0.0400 ± 0.017	278.53–1856.08	1119.08

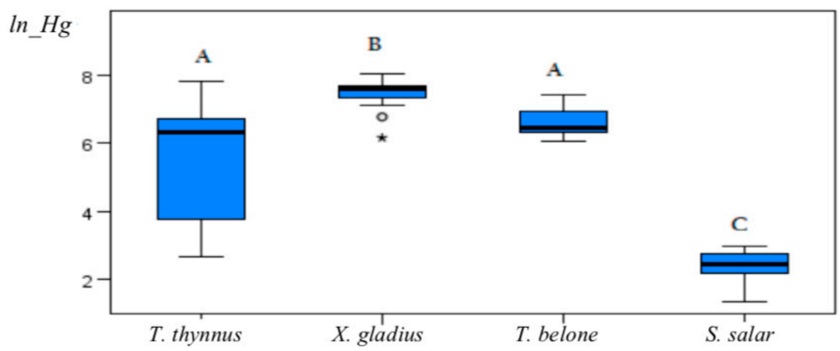

Figure 1. Kruskall-Wallis test for analyzed samples.

4. Discussion

The use of the DMA-80 permitted fast and reliable results. In fact, its unique processing of the sample by thermal decomposition, amalgamation, and atomic absorption spectrometry allowed the direct analysis of the fish samples.

The Hg values showed a noteworthy variability among the examined samples. As expected, these differences could be due to different biological and ecological aspects of the species, including different territory characteristics between the collection sites around Sicily (Italy) and especially between the Mediterranean Sea and the North Sea. In regard to the collection sites, the samples caught in the Strait of Messina were highly rich in Hg with respect to the other Mediterranean Sea collection sites and the North Sea.

Furthermore, focusing on *T. thynnus* samples, it was possible to notice that the highest Hg contents were found in the samples collected from the Strait of Messina and the Patti Gulf, followed by the samples collected in the other sites. Fish size correlations with Hg contents were difficult to establish and further studies should investigate this aspect.

A comparison of the Hg contents with the literature data on pelagic fish species previously investigated, pointed out that the results obtained in this study were in agreement, with some minor differences, with Hg levels in muscle tissue previously reported in this geographic area and also with fishes from oceans around the world [4,5,11,13–15].

It is important to also compare the results obtained with the EC (European Commission) [16] maximum levels for certain contaminants in foodstuffs, including seafood. Unfortunately, it was seen that except for the *S. salar* species, all other samples fell outside the EC maximum level established. For the *T. thynnus* species, two samples exceeded the maximum limit, while for *T. belone* and *X. gladius*, most samples were above the limit values.

In light of the those results, it is interesting to note that an adult who consumes 200 g of *T. thynnus*, *X. gladius* and *T. belone* fish weekly, the limit of mercury exposure, which corresponds to one or two serving portions of those fishes, is highly exceed. There is also a minor risk for the weekly consumption of *S. salar* fish muscle.

The ingestion of Hg from the analysed fish samples could present health risks for the average consumer even at 200 g portions, which is a regular serving size according to the Mediterranean diet. Indeed, it should be kept in mind that regular or excessive consumption of such pelagic fish species might exceed the recommended weekly intake (TWI).

In light of all the above, pelagic fishes may contribute significantly to the intake of hazardous elements from the environment especially, in this case, the Mediterranean Sea.

5. Conclusions

The results established that the three pelagic species *T. thynnus*, *X. gladius* and *T. belone* caught in the Mediterranean Sea need to be constantly monitored due to their high Hg content. In most cases, the content level was higher or very close to the European legislative limit [3]; while, *S. salar* Hg concentrations were far from the maximum limits.

Further studies should address the monitoring of Hg levels in Mediterranean fish specimens, in order to ensure the population's safety.

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