 doi:10.20944/preprints201807.0550.v1

Peer-reviewed version available at *Separations* **2018**, *5*, 51; doi:10.3390/separations5040051

Article

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

Giuseppa Di Bella [1], Roberta Tardugno [1]* and Nicola Cicero [1]

[1] Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy

* Correspondence: Dr. Roberta Tardugno; roberta.tardugno@gmail.com

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 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* specie, 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 healthful eating habit worldwide (Metro et al., 2018; Metro et al., 2017). Besides the several plant-based foods promoted by this diet, a moderate consumption of fish is important for its contribution in healthy nutrients such as Omega 3, or 'n-3 long-chain polyunsaturated' fatty acids but with low levels of saturated fatty acids (EFSA, 2012; Camilleri et al., 2017; Di Bella et al., 2015). However, according to the European Food Safety Authority, the consumption of seafood and fish is relevant for 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 (EFSA, 2012).

Mercury (Hg) commonly known also as *hydrargyrum* or quicksilver is a heavy, silvery-white liquid metal chemical element, highly toxic for 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 (Di Bella et al., 2018; 2017; Graci et al., 2017; Salvo et al., 2014; Lo Turco et al., 2013; Eisler 2010). People may be exposed to inorganic Hg for their employment and pollution, while to organic Hg (methylmercury, CH_3Hg+) predominantly through the consumption of seafood (Salvo et al., 2014; Lo Turco et al., 2013). The pelagic species represent a significant fishing resource and a widely consumed food in the human diet, according to the traditional recipes of the Mediterranean diet and also as sushi and sashimi, typical of Japanese cuisine, rapidly spreading also in Western countries during the last decades (Camilleri et al., 2017; Di Bella et al., 2015; 2006). In the light of all the above, the purpose of this study, was to carry out an investigation by means of a rapid analytical technique on the Hg content in some of the most representative pelagic fish species, in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 July 2018 doi:10.20944/preprints201807.0550.v1

Peer-reviewed version available at *Separations* **2018**, *5*, 51; doi:10.3390/separations5040051

particular, *Tetrapturus belone, Thunnus thynnus* and *Xiphias gladius* (spearfish, tuna and swordfish, respectively) caught in the Mediterranean Sea and *Salmo salar* (salmon) as 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 specie according to the EFSA guidelines (EFSA, 2012) to underline the potential health risk for consumers.

2. Materials and Methods

A number 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 collected from the North Sea (Norway and Scotland, UK). The specimens had length from 15 to 240 cm and weight from 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 levels determination the analyser used for this work was a Milestone DMA-80 Direct Mercury Analyser (Milestone GmbH, Germany), an innovative analytical instrument which allowed to eliminates the use of reagents and simultaneously to obtain validated results, with no digestion and chemical pre-treatment steps.

The DMA-80 has been used to develop the US EPA method 7473. It is furthermore compliant with ASTM method D-6722-01 and ASTM method D-7623-10.

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. In glass flasks the standard solutions were prepared (5 concentration points form 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, with a correlation coefficient r = 0.9996 achieved. Detection limit (DL) and quantification limit (QL) were counted as 3 times and 6 times of standard deviation for blank and were 0.0025 ng and 0.0051 ng, respectively.

The software of the instrumentation calculates automatically the total Hg levels of the sample by interpolating the absorbance values directly with the calibration curve (Camilleri et al., 2018; Bua et al., 2016; Han et al., 2003).

The risk exposure to CH_3Hg^+ (methylmercury) 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 (1).

Tolerable Weekly Intake Hg (TWI) = [Hg] x serving portion /body weight (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

In Graphics 1,2 and Table 1 are reported the 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}, Tolerable Weekly Intake (TWI) and Tolerable Weekly Intake % (TWI%).

Sample	**Site**	**Length**	**Weight**	**Hg**	**TWI**	**TWI %**

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 July 2018 doi:10.20944/preprints201807.0550.v1

Peer-reviewed version available at Separations 2018, 5, 51; doi:10.3390/separations5040051

		(cm)	(kg)	(mg kg^{-1})		
TT0032	Rasocolmo Cape	26,80	0,32	0,0562 ± 0,0032	0,0013	32,78
TT0033	Rasocolmo Cape	27,00	0,36	0,0833 ± 0,0035	0,0019	48,59
TT005	d'Orlando Cape	20,00	220,00	0,0192 ± 0,0007	0,0004	11,19
TT094	Patti Gulf	195,00	150,00	0,5570 ± 0,0413	0,0130	324,91
TT0951	Patti Gulf	115,00	28,00	0,7057 ± 0,0275	0,0165	411,66
TT0952	Patti Gulf	240,00	180,00	0,9057 ± 0,0514	0,0211	528,30
TT0953	Patti Gulf	90,00	16,00	0,7697 ± 0,0265	0,0180	449,02
TT096	southern Tyrrhenian sea	15,40	0,15	0,0146 ± 0,0028	0,0003	8,52
TT097	southern Tyrrhenian sea	24,80	0,30	0,0348 ± 0,0140	0,0008	20,29
TT158	Strait of Messina	128,00	36,00	1,3689 ± 0,0631	0,0319	798,54
TT159	Strait of Messina	165,00	66,00	2,5618 ± 0,4609	0,0598	1494,38
XG321	Strait of Messina	173,00	74,00	2,0623 ± 0,2434	0,0481	1202,98
XG330	Strait of Messina	52,00	5,00	0,4775 ± 0,0287	0,0111	278,52
XG343	Strait of Messina	171,00	58,00	0,8837 ± 0,0279	0,0206	515,49
XG344	Strait of Messina	170,00	61,00	2,1336 ± 0,0908	0,0498	1244,60
XG352	Strait of Messina	161,00	55,00	2,2072 ± 0,0310	0,0515	1287,53
XG365	Strait of Messina	181,00	48,00	2,5557 ± 0,2700	0,0596	1490,82
XG367	Strait of Messina	133,50	35,00	2,0135 ± 0,1949	0,0470	1174,52
XG372	Strait of Messina	180,00	70,00	1,7924 ± 0,0598	0,0418	1045,58
XG376	Strait of Messina	180,00	65,00	1,6910 ± 0,0302	0,0395	986,39
XG377	Strait of Messina	140,00	30,00	2,1496 ± 0,1989	0,0502	1253,95
XG379	Strait of Messina	164,00	54,00	2,9100 ± 0,0171	0,0679	1697,52
XG380	Strait of Messina	196,00	83,00	1,5631 ± 0,1708	0,0365	911,80
XG381	Strait of Messina	180,00	75,00	1,2367 ± 0,2219	0,0289	721,38
XG384	Strait of Messina	183,00	49,00	3,1819 ± 0,1949	0,0742	1856,09
TB 103	Strait of Messina	155,00	13,00	0,4344 ± 0,0335	0,0101	253,41
TB 106	Strait of Messina	151,00	11,00	0,7986 ± 0,0889	0,0186	465,84
TB 108	Strait of Messina	156,00	16,00	1,6499 ± 0,2199	0,0385	962,43
TB 113	Strait of Messina	150,00	11,00	0,6695 ± 0,0563	0,0156	390,55
TB 117	Strait of Messina	170,00	16,00	0,8687 ± 0,0230	0,0203	506,71
TB 120	Strait of Messina	179,00	22,00	1,7303 ± 0,0268	0,0404	1009,35
TB 121	Strait of Messina	151,00	6,00	0,6021 ± 0,0642	0,0140	351,25
TB 122	Strait of Messina	178,00	21,00	1,6086 ± 0,1290	0,0375	938,33
TB 126	Strait of Messina	157,00	16,00	0,5199 ± 0,0209	0,0121	303,25
TB 131	Strait of Messina	155,00	16,00	0,6017 ± 0,0433	0,0140	350,96
TB 132	Strait of Messina	142,00	13,00	0,5306 ± 0,0525	0,0124	309,52
TB 144	Strait of Messina	151,00	11,00	0,5693 ± 0,0534	0,0133	332,07
TB 145	Strait of Messina	155,00	14,00	1,0583 ± 0,2837	0,0247	617,31

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 July 2018 doi:10.20944/preprints201807.0550.v1

Peer-reviewed version available at Separations 2018, 5, 51; doi:10.3390/separations5040051

TB 149	Strait of Messina	148,00	12,00	0,5559 ± 0,0228	0,0130	324,29
SS1	Scotland	n.d.	n.d.	0,0188 ± 0,0007	0,0004	10,99
SS2	Norway	n.d.	n.d.	0,0089 ± 0,0013	0,0002	5,16
SS3	Norway	n.d.	n.d,	0,0114 ± 0,0004	0,0003	6,64
SS4	Norway	n.d.	n.d.	0,0193 ± 0,0014	0,0005	11,28
SS5	Norway	n.d.	n,d.	0,0091 ± 0,0003	0,0002	5,33
SS6	Norway	n.d.	n.d.	0,0117 ± 0,0004	0,0003	6,81
SS7	Norway	n.d.	n.d.	0,0037 ± 0,0006	0,0001	2,18
SS8	Norway	n.d.	n.d.	0,0159 ± 0,0004	0,0004	9,26
SS9	Norway	n.d.	n.d.	0,0087 ± 0,0005	0,0002	5,10

n.d.: not determined.

[a]mean value (n=3).

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

Specie	**N° Samples**	**Hg** **mg/kg** *range min-max*	**mg/kg** *mean value*	**TWI** *mean value*	**TWI%** *range min-max*	**TWI %** *mean value*
S. salar	9	0,004-0,002	0,012	0,0003	5,10-11,28	6,97
T. belone	14	0,434-1,730	1,082	0,0200	253,41-1009,35	508,23
T. thynnus	11	0,015-2,562	1,288	0,0150	8,52-1494,38	375,29
X. gladius	14	0,477-3,182	1,829	0,0400	278,53-1856,08	1119,08

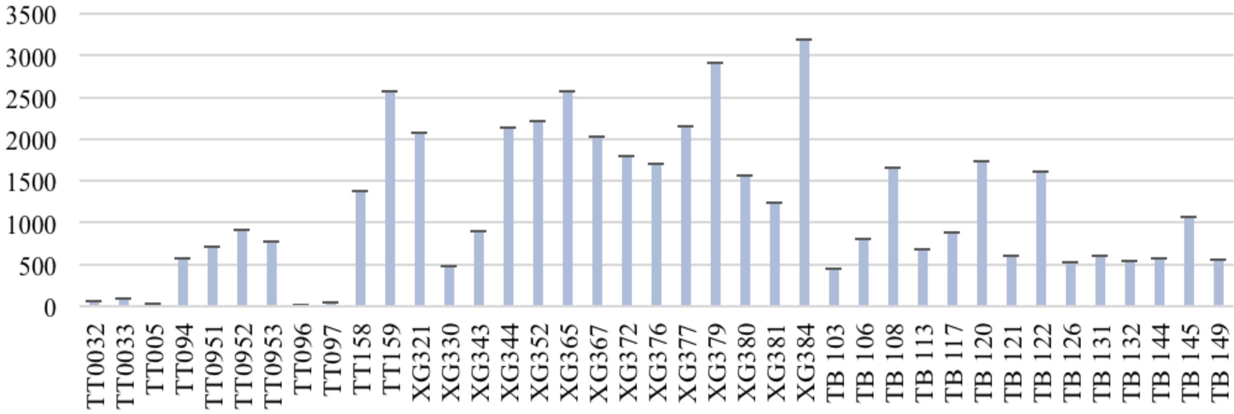
The results were variable ranging between 0,015-2,562 mg kg^{-1} for *T. thynnus* specie, 0,477-3,182 mg kg^{-1} for *X. gladius*, 0,434-1,730 mg kg^{-1} for *T. belone*, respectively. For *S. salar* the Hg content obtained ranged between 0,004-0,019 mg kg^{-1}. The following decreasing order among the 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 by Kruskal-Wallis test, confirmed that samples of *T. thynnus* and of *T. belone* have Hg amount not significantly different between them (Graphic 3).

In Table 2 are reported the ranges of Hg contents and their relative TWI value calculated in this study. The weekly consumption of a portion of 200 g of most examined pelagic fish species by a 60 kg adult body weight, point out remarkable health risk of exposure to the toxic action of CH_3Hg+ for *T. thynnus*, *X. gladius* and *T. belone,* since the TWI value was exceeded in many samples caught in the Mediterranean Sea. In the meanwhile, any risk was observed for *S. salar* samples from the North Sea.

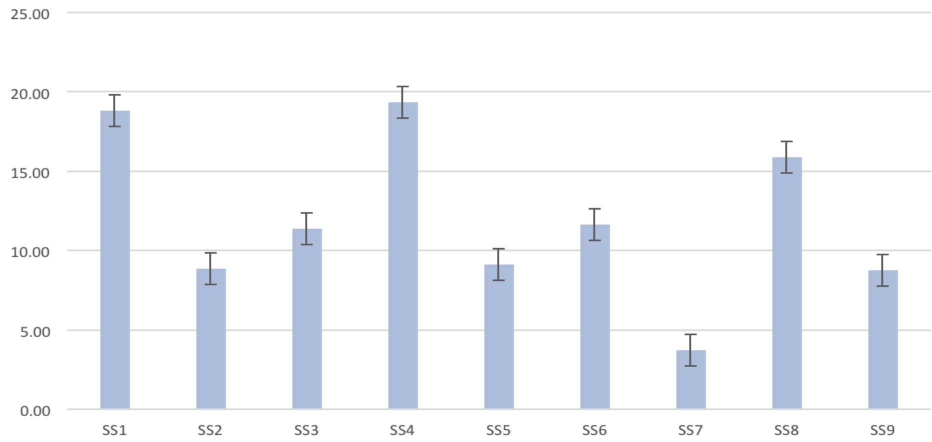
Graphic 1. Hg content expressed as μg kg^{-1} in *T. thynnus* (TT), *X. gladius* (XG) and *T. belone* (TB) samples. See supplementary for samples details.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 July 2018 doi:10.20944/preprints201807.0550.v1

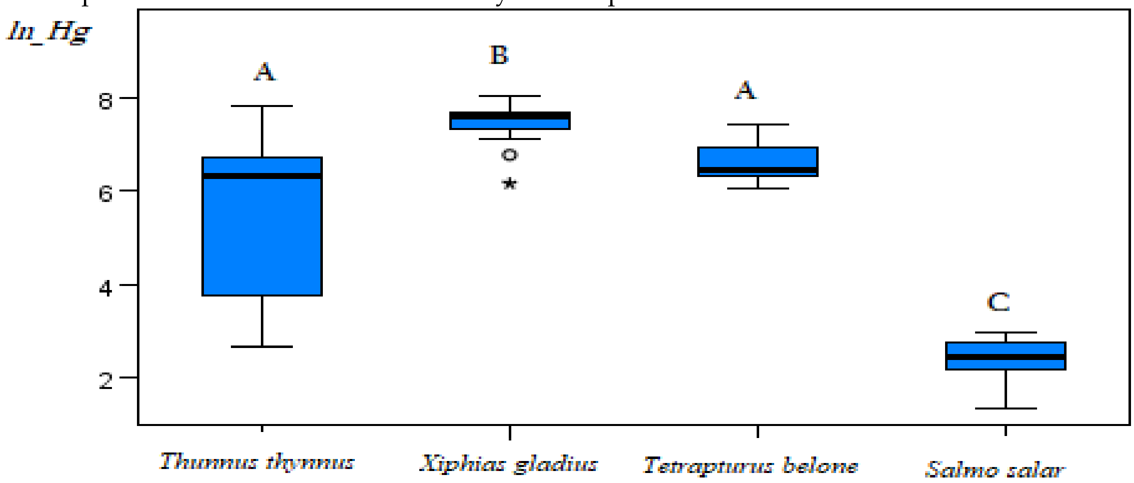
Peer-reviewed version available at Separations 2018, 5, 51; doi:10.3390/separations5040051



Graphic 2. Hg content expressed as µg kg^{-1} in *S.salar* (SS) samples.



Graphic 3. Kruskall-Wallis test for analyzed samples.



4. Discussion

The use of DMA- 80 known for its versatility, *in situ* applications, allowed to obtain fast and reliable results. In fact, its unique processing of the sample by thermal decomposition, amalgamation, and atomic absorption spectrometry allows for direct analysis of fish samples.

The Hg values showed a noteworthy variability among examined samples, as expected, these differences could be due to different biological and ecological aspects of the species; different territory characteristics among the collection sites around Sicily (Italy) and especially between the Mediterranean Sea and the North Sea. Considering the collection sites, the samples caught in the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 July 2018 doi:10.20944/preprints201807.0550.v1

Peer-reviewed version available at *Separations* **2018**, *5*, 51; doi:10.3390/separations5040051

Strait of Messina showed to be the highly rich in Hg amount with respect to the other Mediterranean Sea collection sites and the North Sea.

A comparison of the Hg contents with available literature data about pelagic fish species previously investigated pointed out that the results obtained in this study were in good 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 (Bodin et al., 2017; Camilleri et al., 2017; Covaci et al., 2017; Di Bella et al., 2015, Eisler, 2010).

It is important to compare the results obtained also with the EC (European Commission) (2006) maximum levels for certain contaminants in foodstuffs, including seafood. Unfortunately, it was possible to notice that only for *S. salar* specie all samples fall within the EC maximum level established, for *T. thynnus* specie 2 samples exceeded the maximum limit, while, for *T. belone* and *X. gladius* most samples were above the limit values.

In the light of the above mentioned results, it is interesting to note that an adult by consuming 200 g of *T. thynnus*, *X. gladius* and *T. belone* fishes weekly, highly exceed the risk of exposure from mercury, which corresponds to 1 or 2 serving portions of those fish. The situation is definitely with minor risk for consuming *S. salmon* fish muscle weekly.

The ingestion of Hg from analysed fish samples could present health risks for the average consumer even at 200 g portion of fish, which is a regular size serving portion 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 the light of all the above, pelagic fishes may contribute significantly to 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 needs to be constantly monitored due to their high Hg content since, in most cases, it results to be higher or very close to the European legislation (EFSA, 2012); while, *S. salar* Hg concentrations were far from the maximum limits.

Further studies should be addressed monitoring Hg levels in Mediterranean fish specimens, in order to ensure people safety.

Acknowledgments: This research was fully supported by the *Leo Club International District 108 ITALY* through the *Leo Hunting Mercury Project* grant.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "X.X. and Y.Y. conceived and designed the experiments; X.X. performed the experiments; X.X. and Y.Y. analyzed the data; W.W. contributed reagents/materials/analysis tools; Y.Y. wrote the paper." Authorship must be limited to those who have contributed substantially to the work reported.

Conflicts of Interest: The authors declare no conflict of interest and the founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results".

References

Bodin N, Lesperance D, Albert R, Holland S, Michaud P. 2017. Trace elements in oceanic pelagic communities in the western Indian Ocean. Chemosphere, 174: 354-362.

Cammilleri, G.; Vazzana M, Arizza V, Giunta F, Vella A, Lo Dico G, Giaccone V, Giofrè SV, Giangrosso G, Cicero N, Ferrantelli V. 2017. Mercury in fish products: what's the best for consumers between blue tuna and yellow tuna? Nat Prod Res. doi:10.1080/14786419.2017.1309538.

Covaci E, Senila M, Ponta M, Darvasi E, Frentiu M, Frentiu T. 2017. Mercury speciation in seafood using non-chromatographic chemical vapor generation capacitively coupled plasma microtorch optical emission spectrometry method e Evaluation of methylmercury exposure. Food Control 82: 266-273.

Di Bella G, Licata P, Bruzzese A, Naccari C, Trombetta D, Lo Turco V, Dugo G., Richetti A, Naccari F 2006.Levels and congener pattern of polychlorinated biphenyl and organochlorine pesticide residues in bluefin tuna (*Thunnus thynnus*) from the Straits of Messina (Sicily-Italy).Environment International, 32, 705-710.

Di Bella G, Potortì AG, Lo Turco V, Bua D, Licata P, Cicero N, Dugo G. 2015. Trace elements in *Thunnus thynnus* from Mediterranean Sea and benefit–risk assessment for consumers. Food Additives and Contaminants: Part B Surveillance, 8 (3): 175-181.

Di Bella G, Russo E, Dugo G. 2017. Heavy metals and Persistent Organic Pollutants in marine organisms from two Sicilian protected areas: Strait of Messina and Cape Peloro lakes. Current Organic Chemistry, 21 (5), 387-394.

Di Bella G, Pizzullo G, Bua GD, Potortì AG, Santini A, Giacobbe S. 2018 Mapping toxic mineral contamination: the southern oyster drill, S. haemastoma (L., 1767), as evaluable sentinel species Environmental Monitoring and Assessment, 190 (1)

Eisler R, Chapter 3 - Fishes, In Compendium of Trace Metals and Marine Biota, Elsevier, Amsterdam, 2010, Pages 39-220, ISBN 9780444534392, https://doi.org/10.1016/B978-0-444-53439-2.00016-3.

European Food Safety Authority 2012. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012, 10(12):2985.

Graci S, Collura R, Cammilleri G, Buscemi MD, Giangrosso G, Principato D, Gervasi T, Cicero N, Ferrantelli V. 2017. Mercury accumulation in mediterranean fish and cephalopods species of sicilian coasts: Correlation between pollution and the presence of anisakis parasites. Natural Product Research. 31(10):1156-1162.

Lo Turco V, Di Bella G, Furci P, Cicero N, Pollicino G, Dugo G. 2013. Heavy metals content by ICP-OES in *Sarda sarda*, *Sardinella aurita* and *Lepidopus caudatus* from the Strait of Messina (Sicily, Italy). Natural Product Research. 27 (6):518-523.

Metro D, Tardugno R, Papa M, Bisignano C, Manasseri L, Calabrese G, Gervasi T, Dugo G, Cicero N. 2017. Adherence to the Mediterranean diet in a Sicilian student population. Nat Prod Res doi. 10(1080/14786419):2017.

Metro D, Papa M, Manasseri L, Gervasi T, Campone L, Pellizzeri V, Tardugno R, Dugo G. 2018. Mediterranean diet in a Sicilian student population. Second part: breakfast and its nutritional profile, Natural Product Research, DOI: 10.1080/14786419.2018.1452016.

Naccari C, Cicero N, Ferrantelli V, Giangrosso G, Vella A, Macaluso A, Naccari F, Dugo G. 2015. Toxic Metals in Pelagic, Benthic and Demersal Fish Species from Mediterranean FAO Zone 37. Bulletin of Environmental Contamination and Toxicology. 95 (5): 567-573.

Salvo A, Potortì AG, Cicero N, Bruno M, Lo Turco V, Di Bella G, Dugo, G. 2014. Statistical characterisation of heavy metal contents in *Paracentrotus lividus* from Mediterranean Sea. Nat Prod Res. 28(10):718-726.

EC (European Commission) (2006) Commission Regulation No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance). Available at: http://faolex.fao.org/docs/pdf/eur68134.pdf